WORLD VETERINARY CANCER CONGRESS BRAZIL

Foz do Iguaçu, Brazil

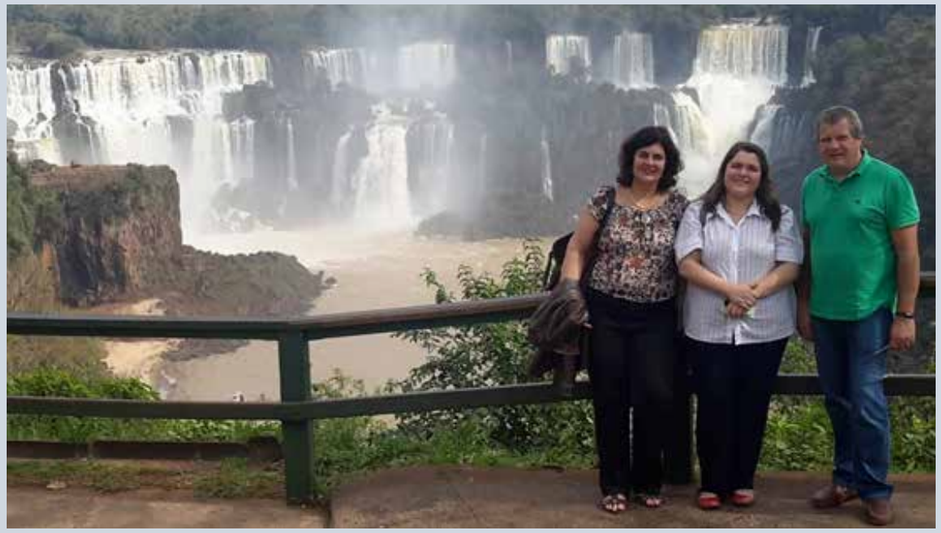
26-29 May 2016

Message from ABROVET President

Welcome to the 3rd World Veterinary Cancer Congress!

The Associação Brasileira de Oncologia Veterinária, ABROVET, welcomes the speakers and delegates from all over the World that landed in Foz do Iguassu, Brazil, to attend the 3rd WVCC. ABROVET was created in 2004, by a Brazilian group of veterinarians and students interested in Veterinary Oncology. In 2009, ABROVET joined the WVCC Organizing Committee, and, since then, has endeavoured to produce a scientifically relevant and socially and touristically attractive meeting. The enthusiastic and motivating cooperation with ESVONC, VCS and JVCS contributed to make WVCC an even more interesting Congress. The shaped Program includes 16 invited international speakers, 43 oral abstract presentations and 97 posters, showing the most recent scientific discoveries in Veterinary Cancer Science and Practice. In addition, 3 receptions and 2 sponsored lunches will be offered. We hope you all enjoy the comprehensive Program and the brazilian hospitality. Do not miss the opportunity to visit the touristic points at this spectacular venue!

Maria Lucia Zaidan Dagli, DVM, MSc, PhD, Full Professor
President, ABROVET



Dr. Maria Dagli, ABROVET President, Dr. Carolina Scarpa Carneiro, ABROVET treasurer, and Marcelo Acquaviva, from Acquaviva Company, during the Iguassu site visit.

ABROVET
Associação Brasileira de Oncologia Veterinária

3rd WVCC 2016

Message from ESVONC President

As founding member of WVCC, ESVONC has hosted the first 2 editions of this World congress (2008, Kopenhagen & 2012, Paris). Among organizers and participants, we have always felt a strong willingness to cooperate in our field beyond oceans and continents. We feel that we share the same problems and challenges. We hope that WVCC is more than just a 4-year event, but a continuum for clinicians and researchers to hold the lines open in between the congresses. We need each other to help make progress in the diagnosis and treatment of the devastating disease called cancer.
Furthermore, we hope that you will enjoy the program, which was a joint effort of the organizing committee and the worldwide scientific committee. Thank you for the many submitted abstracts.
Finally, we wish to thank our colleagues in Brazil for taking up the organization of this event. It's always more work than expected, but the reward for all participants is so valuable.

Frédérique Ponce DVM, PhD, HDR, DipECVIM-CA (Oncology)
President, ESVONC
(by Tom Hendrix, ESVONC Treasurer)

ESVONC

Message from VCS President

VCS began its collaboration with members of ESVONC and ABROVET in 2008 when members of each group met in Copenhagen, Denmark to discuss a collaboration amongst the groups with the goal of organizing an international World Veterinary Cancer Congress every four years. That meeting in Copenhagen was considered to be the first World Congress. VCS soon decided to forgo our typical mid-year conference every fourth year and, instead, collaborate with our partners on what became the World Veterinary Cancer Congress. The 2nd World Veterinary Cancer Congress was held in 2012 in Paris.

Today, not only are VCS, ESVONC and ABROVET involved in the World Veterinary Cancer Congress, the Japanese Veterinary Cancer Society (JCVS) has also joined the collaboration. The 4th World Veterinary Cancer Congress will be held in Tokyo, Japan in 2020.

The VCS is truly excited to participate with our global partners in this energizing collaborative endeavour and we look forward to sharing in the informative cross-talk with investigators from around the world.

Best Regards,

David Vail, DVM, DACVIM (Oncology)
President, Veterinary Cancer Society

VCS VETERINARY CANCER SOCIETY

3rd WVCC 2016

Message from JVCS President

While the VCS meetings held annually in the U. S. are mainly designed for oncology specialists and those who are seeking for this speciality, the quadrennial WVCC is held somewhere in the world and is intended for all the practitioners and scholars who are interested in veterinary oncology. Therefore, the WVCC, by providing comprehensive educational opportunities, is an oncology meeting more open to every practitioner who sees oncology patients and is an excellent meeting for them to learn the recent advancement and the standard practices in veterinary oncology. As progress in veterinary science has lengthened the life span of companion animals, and as the human-animal bond concepts are widely accepted by the society, we veterinarians see more oncology patients and have greater opportunities to treat them. The Japanese VCS (JVCS) was established in 1994 and has been serving the veterinary society by offering quality and targeted continuing educations and by producing oncology specialists through its qualification examination and accreditation system. We are very proud that this organization is the first and only one of its kind in Asian countries. We are very pleased to announce that the next WVCC will be held in Tokyo, Japan in 2020, and we would very much like to invite everyone from all over the world.

Takuo Ishida, DVM, PhD, DJCVP.
President, JVCS

Japan Veterinary Cancer Society
日本獣医がん学会

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The organizing committee wishes to thank all those persons who helped to make WVCC a successful meeting!
Thanks to the Organizing Companies, Acquaviva and Brix.

Special thanks to the Sponsors.

World Veterinary Cancer Congress Committee members meet in Austin, in 2009. Maria Dagli, Malcolm Brearley, Barb Kitchell, Johan de Vos and Barb McGehee

The World Veterinary Cancer Congress Committee meets in San Diego 2010: Barb Kitchell, Johan de Vos, Maria Dagli, Malcolm Brearley, Sandi Strother and Ruthanne Chun

These were the 2 first meetings to discuss the 2nd WVCC in Paris.
Many other WVCC Organizing meetings followed, either during VCS in the USA or ESVONC in Europe. These meetings involving many other people, like Julia Maria Matera, Carolina Scarpa Carneiro, David Vail, Laura Garrett, Kim Selting, Lucas Rodrigues, Rodrigo Ubukata, Frederique Ponce, Tom Hendrix, Takuo Ishida.
To all these people, that helped the WVCC in Brazil come true, in special to Sandi Strother, and the pioneer Barb Mc Gehee, our most sincere thanks!

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Without our sponsors it would be impossible to organise a conference like this!
On behalf of the 3rd WVCC, the Congress Committee wishes to express sincere gratitude.

To all Delegates and Speakers: – Please visit our sponsors and thank them personally!!

Realization

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PROGRAM

Wednesday, MAY 25

12:00	Registration opens
13:30 – 14:00	***Opening ceremony***

Afternoon Session: Historical Aspects of Veterinary Oncology and Cancer Epidemiologyw
Chair: Maria Lucia Zaidan Dagli

Time	Conferences	
14:00 - 15:00	Opening Conference **One Medicine-War on Cancer**	**Gordon Theilen**
15:00 – 16:00	**Historical aspects of Veterinary Oncology in Europe**	**Andre-L. Parodi**
16:00-16:20	***Coffee break***	
16:20 – 17:10	**Human Cancer Epidemiology**	**Luisa Lina Villa**
17:10 – 18:00	**Veterinary Cancer Epidemiology: Experiences with The Danish Veterinary Cancer Registry**	**Annemarie T. Kristensen**
19:30 – 21:30	**WELCOME RECEPTION**	

Thursday, MAY 26

Morning Session: Contemporary aspects of Veterinary Oncology
Chair: Julia Maria Matera

Time	Conferences	
8:00 – 9:00	**Defining the Value of Comparative Oncology Clinical Trials: The North American Experience**	**David Vail** ***VCS President***
9:00-9:50	**ESVONC and veterinary oncology in Europe: past, present and future**	**Johan P. de Vos** ***ESVONC representative***
9:50-10:10	***Coffee Break***	
10:10 – 11:00	**Veterinary oncology, medical, surgical and radiation, practiced everywhere in Japan**	**Takuo Ishida** ***JVCS President***
11:00 – 12:00	**Veterinary Oncology in Brazil and the role of ABROVET**	**Carlos Daleck** **Maria Dagli** ***ABROVET President***
12:00-14:00	**Sponsored lunch and talk (Qbiotics)**	

Afternoon Session: Abstract Presentations		
Time	**Oral Presentations** *Chair: Geovanni Dantas Cassali*	
14:00 – 14:12	Accuracy evaluation of frozen sections compared to paraffin histological sections - retrospective study of 142 cases	Krishna Oliveira
14:12 – 14:24	Both membranous and cytoplasmic cyclooxygenase-2 (COX-2) expression modulates the prognosis of feline invasive mammary carcinoma	Frederique Nguyen
14:24 – 14:36	Impact of luminal phenotypes on outcomes of cats with invasive mammary carcinoma	Frederique Nguyen
14:36 – 14:48	The use of biomarkers and molecular classification as prognostic indicators in patients with canine mammary tumors in Mexico	Alejandro Cervantes
14:48 – 15:00	Global changes in microRNA levels during epithelial to mesenchymal transition in canine mammary carcinoma	Alejandro Cervantes
Time	**Oral presentations** *Chair: Luiz Roberto Biondi*	
15:00 – 15:12	Melatonin as pro-apoptotic and anti-angiogenic therapy in canine mammary tumor cells	Debora Zuccari
15:12 – 15:24	IL-25/siIL-17B modulates apoptosis, angiogenesis and NF-kB signaling in the control of canine mammary tumor cells	Debora Zuccari
15:24 -15:36	FAK and mTOR inhibitors, promising therapeutic agents, modulate cancer cell stemness in both canine and human mammary cancer	Isabelle Tancioni
15:36 – 15:48	Inflammatory response following thalidomide treatment in the 4T1 murine mammary carcinoma	Cecilia Bonolo
15:48 – 16:00	Claudin Gene Expression Analysis of Canine Mammary Non-Neoplastic and Neoplastic Tissue Derived Cell Cultures	Susanne Hammer

16:00-16:30 ***Coffee Break***

Time	**Oral presentations** *Chair: Thais Andrade Costa Casagrande*	
16:30 – 16:42	Surgical approach of mammary tumors in small animals: a survey involving Brazillian veterinary physicians	Nazilton Reis
16:42 – 16:54	Enhanced importance of mammary tumour lymph nodes in radical treatment and prognosis in companion animals	Julianna Thuroczy
16:54 – 17:06	Use of adjuvant chemotherapy in canine mammary gland carcinosarcomas	Cecilia Bonolo

17:06 – 17:18	Mycobacterium Cell Wall-DNA complex (MCW-DNA) as an aid in the treatment of chemotherapy- induced neutropenia in healthy dogs	Aleksander Masic.
17:18 – 17:30	Serum Cytokine Induction After a Single Mycobacterium Cell Wall Fraction (MCWF) treatment in Tumor Bearing Dogs	Lucas Rodrigues

18:30 – 21:30 **POSTER PRESENTATIONS AND RECEPTION**

Friday, MAY 27

Morning Session –Cancer Genetics, Cancer Etiology and Pathology
Chair: Renée Laufer Amorim

Time	**Conferences**	
8:30 – 9:30	Towards the delivery of precision medicine to Veterinary Oncology	**Chand Khanna**
9:30-10:30	Anticancer mechanisms in long-lived mammals	**Andrei Seluanov**

10:30-11:00 ***Coffee Break***

11:00-12:00	Genetic analysis of transmissible cancers in dogs and Tasmanian devils	**ElizabethMurchison**

12:00-14:00 **Sponsored lunch and presentation – MERIAL**

Afternoon Session: Abstract Presentations		
Time	**Oral presentations** *Chair: Bruno Cogliati*	
14:00 – 14:12	Genomic copy number variation in canine cutaneous mast cell tumors	Paulo Jark
14:12 – 14:24	Association between frequency and intensity of gastroduodenal lesions and plasma histamine levels in dogs with cutaneous mast cell tumors	Luciana Oliveira
14:24 – 14:36	Retrospective evaluation of masitinib and prednisolone compared to masitinib alone in canine mast cell tumours	Antonio Giuliano
14:36 – 14:48	New insights in dose-escalation of vinblastine in dogs with biologically aggressive mast cell tumours	Johan P.de Vos
14:48 – 15:00	Heterogeneity in c-kit profile and expression in canine mast cell tumour and its metastasis	Rodrigo Horta

Time	**Oral presentations** *Chair: Lucas Campos de Sá Rodrigues*	
15:00 – 15:12	Prognostic value of Ki67 and mitotic index in dogs with diffuse large B cell lymphoma	Oscar Sierra
15:12 – 15:24	Comparative genomic sequencing of the canine B-cell lymphoma cell lines CLBL-1 and CLBL-1M	Hugo Escobar
15:24 -15:36	Immunophenotypic quantification of T regulatory cells in dogs with multicentric lymphoma submitted to chop chemotherapy protocol associated or not to Firocoxib	Leticia Anai
15:36 – 15:48	L-asparaginase activity and plasma amino acid profile in healthy cats after a single injection of PEG-L-asparaginase or native E. Coli L-asparaginase: a pilot study with potential therapeutic consequences	Ada Krupa
15:48 – 16:00	Canine Histiocytic Sarcoma: toward diagnostic and therapeutic options.	Hedan Benoit

16h00-16h30 ***Coffee Break***

Time	**Oral presentations** *Chair: Cristina Oliveira Massoco de Salles Gomes*	
16:30 – 16:42	Combination of etoposide and piroxicam: A potential treatment for canine osteosarcoma	Ong Siew Mei
16:42 – 16:54	Investigation of sodium dichloroacetate for the treatment of canine osteosarcoma: in vitro preclinical assays.	Vanessa Barraza
16:54 – 17:06	The effect of Dichloroacetate in canine cancer cell lines	Tatjana Harting
17:06 – 17:18	Autologous vaccine for dog osteosarcomas	Patrick Frayssinet
17:18 – 17:30	CDH1 hypermethylation is responsible to E-cadherin plasticity and down-regulation in metastatic process of canine prostate	Carlos E. F Alves
17:30 – 17:42	Characterization of the mediated effects of two aryl indolyl maleimides on canine and human prostate carcinoma cell lines	Jan Torben Schille
17:42-17:54	Serum levels of urokinase-type plasminogen activator in canine patients with cancer	Sofia Ramos

18:00 **FREE EVENING**

Saturday, MAY 28

Morning Session: Light, electricity and Cancer
Chair: Johan P. de Vos

Time	**Conferences**	
8:30 – 9:30	Electrochemotherapy	**Lluis Mir**
9:30-10:30	Can we cure cancer? New imaging tools and therapies and translation to the clinic.	**Clemens Lowik**

10:30-11:00 ***Coffee Break***

Time	**Oral presentations** *Chair: Adriana Tomoko NIshiya*	
11:00 – 11:12	Calculation of body surface area using computed-tomography-guided modeling in dogs.	Kim Selting
11:12 – 11:24	Frameless stereotactic radiotherapy alone and combined with temozolomide in canine gliomas	Mario Dolera
11:24 – 11:36	Tolerability of postoperative standardized definitive-intent conformal radiotherapy for dogs with anal sac apocrine gland adenocarcinoma: a pilot study.	Juan Carlos Serra
11:36 – 11:48	Targeted fluorescence-guided cancer surgery: The use of Prosense 750, a cathepsine targeted probe and the SOLARIS® camera system.	Arno Roos
11:48 – 12:00	Multi-wavelength fluorescence-guided cancer surgery: first experiences with the SOLARIS® camera system	Arno Roos

Time	**Oral presentations** *Chair: Rodrigo Ubukata*	
12:00 – 12:12	Bremachlorin photodynamic therapy in dogs with transitional cell carcinomas of the bladder and/or urethra	Kelly van Vliet
12:12 – 12:24	Electrochemotherapy for the treatment of perianal adenocarcinoma in dogs: 16 cases	Karine Cadrobbi
12:24 – 12:36	In Silico Study of Electrochemotherapy Treatment of Tumoral Tissue near Bone	Daniela Suzuki
12:36 – 12:48	A pilot, uncontrolled study of postsurgical treatment with autologous dendritic cell-based immunologic therapy in 10 dogs with splenic hemangiosarcoma	Thomas Grammel
12:48 – 13:00	Canine oral malignant melanoma: therapeutic anti-cancer pool vaccine and surgical treatment	Claudia Felizzola

13:00 – 13:12	Evaluation of the efficacy of 5-azacytidine and SAHA used alone or in combination with doxorubicin in canine hemangio-sarcoma cell lines	Karen Batschinski

13:12-15:00	**Lunch on your own**

15:00	Boarding to Iguassu Falls at the Recanto das Cataratas Resort
15:30	Buses departure
16:00	Arriving at the Iguassu Park´s gate
17:00	Viewing point – in front of the Hotel das Cataratas
	Group Photo
18:00 – 22:00	**GALA DINNER- Porto Canoas Restaurant – Iguassu Falls** **Buses will leave at 10 pm**

Sunday, MAY 29

Morning Session: New achievements in cancer treatments, and the future
Chair: Heidge Fukumasu

Time	Conferences	
8:30 – 9:30	Where the future lies: Innovations in cancer treatment and prevention	**Jaime Modiano**
9:30-10:30	Advances in targeted therapy: Toceranib and beyond	**Cheryl London**
10:30-11:00	***Coffee Break***	
11:00 – 12:00	**Closing Ceremony** *General Discussion, Awards Information on the 4th WVCC*	
12:00	Meeting adjourned	

WORLD VETERINARY CANCER CONGRESS
FOZ DO IGUASSU / BRAZIL

SPEAKERS

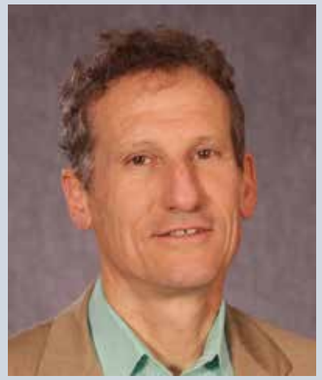
The 3rd WVCC Speakers

Gordon Theilen
Dr. Gordon Theilen is one among a handful of internationally renowned veterinary scientists who founded the discipline of veterinary oncology in the 1960s. He authored and coauthored the first textbooks in veterinary oncology and was noted for his stubborn refusal to concede to the ravages of cancer in animals.
Considered **The Father of Modern Veterinary Oncology**, Dr Theilen wrote his autobiography stressing establishment of Veterinary Cancer Medicine. Importance of One Medicine War on Cancer is emphasized. The Boy with the Wounded Thumb should be of interest to most comparative oncologists.

Andre-Laurent Parodi
Veterinary, academic and researcher, Andre-Laurent Parodi graduated from the National Veterinary School of Alfort (ENVA), of the Faculty of Paris VI and the Institut Pasteur. He published numerous articles, in particular on animal leukemia. He led the 1998 the publication of the book The Alfort National Veterinary School in the Twentieth Century. He led the ENVA from 1992 to 1998. He chaired the Scientific Council of the National Veterinary and Food Research Centre (1987-1997), the National Commission for authorization to market veterinary medicinal products (1987 to 1993), the European College in Veterinary Pathology (1999-2001), the veterinary Academy of France (2000), the National Ethics Committee Reflection on Animal Experimentation (2006-09) and the National Academy of Medicine (2012). He was a member of the Scientific Veterinary Council for Animal Health and Welfare of Animals of the European Commission from 1997 to 2003). He is a member of the National Pharmacy Academy and the Academy of Veterinary France. He is an Officer of the Legion of Honour, Knight of the National Order of Merit, Commander of the Academic Palms and Officer of Agricultural Merit.

Luisa Lina Villa
Dr. Villa holds a degree in Biological Sciences from the University of São Paulo (1972) and PhD in Sciences (Biochemistry) at the Chemistry Institute of USP (1978). It was a researcher at the São Paulo branch of the Ludwig Institute for Cancer Research from 1983 to 2011 and was its director from 2006 to 2010. She has experience in Microbiology, with emphasis in Virology, acting on the following topics: Papillomavirus human (HPV), cervical cancer, anogenital cancer, epidemiological studies of HPV in women and men, immunology of HPV infections. Since May 2011 is professor of the Department. Radiology and Oncology, Faculty of Medicine, USP, and head of the Molecular Biology Laboratory of Translational Research Centre ICESP Oncology. She is a member of the Brazilian Academy of Sciences and Commander of the National Order of Scientific Merit.

Annemarie Thuri Kristensen
Dr. Annemarie Kristensen is a Professor at the University of Copenhagen, Denmark. She is involved in Master, Companion Animal Clinical Sciences (continuing professional development for veterinarians), Director of Studies, Supervision of students, and Course director for specialisation in companion animal oncology, Dr. Kristensen is involved with the Danish Veterinary Cancer Registry, and hosted the 1st World Veterinary Cancer Congress in 2008, in Copenhagen.



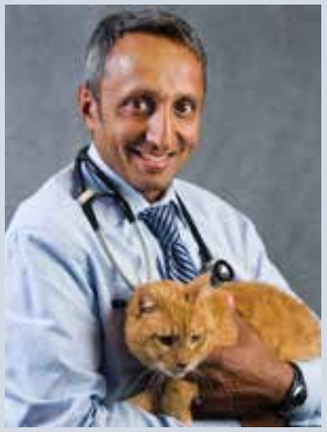
David Vail, President of the Veterinary Cancer Society, VCS
Dr. Vail is board certified in oncology and is a professor of Medical Oncology at the University of Wisconsin- School of Veterinary Medicine. Dr. Vail's research involves the design and implementation of comparative oncology clinical trials through the inclusion of companion animal dogs and cats with spontaneous tumors. These spontaneous tumors have similar biological behavior as their counterparts in humans. David is co-editor of the textbook Small Animal Clinical Oncology, past North American Journal Editor for Veterinary and Comparative Oncology, President of the Canine Comparative Oncology and Genomics Consortium (CCOGC) and a founding member of the Comparative Oncology Trials Consortium (COTC). He is the past Chairman of the Scientific Advisory Boards for both the Morris Animal Foundation and the American College of Veterinary Internal Medicine Foundation. He has been honored as the recipient of both the Mark L. Morris Sr. Distinguished Research Award and the Pfizer Award for Veterinary Research Excellence.

Johan P. de Vos
Johan de Vos studied veterinary medicine at Utrecht University, and graduated (cum laude) in 1978. In 1978 he founded a small animal practice in Terneuzen, The Netherlands, which developed to “De Ottenhorst”, Clinic for Companion Animal Medicine and Veterinary Oncology Referral Centre, part of the “Animal Hospital Zeeuws-Vlaanderen”, where he is principal oncologist. He has given presentations on oncology topics on national and international conferences, and published in several peer-reviewed international journals. Former Treasurer and Membership Secretary of the European Society of Veterinary Oncology (ESVONC) 2004-2012, founder and President of the Dutch Animal Cancer Foundation (NKFD); co-founder and Past-President of the Dutch and Belgian Collaborating Veterinary Cancer Centres (SDK); co-founder of the World Veterinary Cancer Congress. Dr. de Vos is Honorary Member of the Brazilian Association of Veterinary Oncology (ABROVET), Co-winner of the Alberto Vittone Award 2007, of the Società Italiana Bovaro del Bernese, and Voorjaarsdagen & Hill’s “Excellence in Veterinary Health Care Award” 2012.

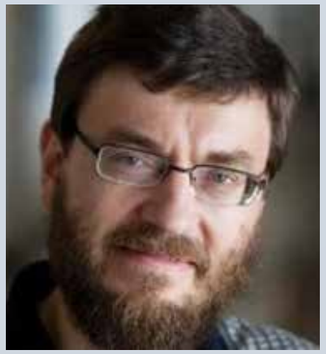
Takuo Ishida, President of Japanese Veterinary Cancer Society (JVCS)
President of Japanese Board of Veterinary Practitioners (JBVP)
President of Japanese Society of Feline Medicine (JSFM)
Medical Director, Akasaka Animal Hospirtal
Graduated from Nippon Veterinary and Zootechnical College (NVZC) with DVM degree in 1976. Obtained Ph.D. in 1981 at University of Tokyo. Postdoctoral research fellow for 3 years at Oncology Section, Department of Surgery, School of Veterinary Medicine, University of California, Davis with Drs. Gordon Theilen and Niels Pedersen as sponsor professors. Associate professor of clinical pathology at NVZC for 13 years as a JCVP board-certified pathologist. Joined one of the largest animal hospitals in downtown Tokyo, Akasaka Animal Hospital, as the Medical Director in 1998 specializing in hematology, virology, cytology, immunology and oncology.

Carlos Roberto Daleck
Graduated in Veterinary Medicine from the Universidade Estadual Paulista Julio de Mesquita Filho (1976), master’s degree in Veterinary Medicine from the Federal University of Santa Maria, RS (1982) and Doctorate in Veterinary Medicine from the Universidade Estadual Paulista Julio de Mesquita Filho (1986). Now retired, he has been Assistant Professor of Universidade Estadual Paulista Julio de Mesquita Filho. Dr. Daleck has experience in the area of Veterinary Medicine, with emphasis on Surgical Animal Clinic, surgery, dogs, chemotherapy and Oncology. Pós doctor at Colorado State University (USA) with an emphasis on oncology of dogs and cats. Author of the book: Oncology of Dogs and Cats, Roca, Brazil. Dr. Daleck is one of the pioneers in practicing and teaching Veterinary Oncology in Brazil.

Maria L. Z. Dagli, President of ABROVET
Dr. Dagli graduated in Veterinary Medicine at the University of São Paulo, Brazil. She obtained a Masters and PhD in Experimental and Comparative Pathology at the same University, and a post doc at the International Agency for Research on Cancer, IARC, Lyon, France. Since 1987, she is a Professor of Veterinary Pathology at the School of Veterinary Medicine and Animal Science of the USP, and is the head of the Laboratory of Experimental and Comparative Oncology at the same Vet School. In 2004, Dr. Dagli, with the help and support of the undergraduate students Carolina Scarpa Carneiro, Katia Cristina Pinello and Patricia Bonifacio Flor, and of colleagues, like Prof Drs. Julia Maria Matera and Silvia R. Ricci Lucas, founded the Brazilian Association of Veterinary Oncology, ABROVET.



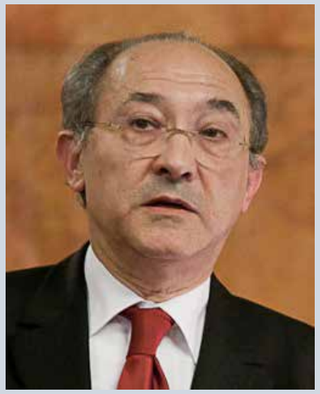
Chand Khanna
Dr. Khanna is a graduate of the Western College of Veterinary Medicine in Saskatoon, Saskatchewan. His specialty training included a small animal medicine and surgery internship at the Ontario Veterinary College in Guelph, Ontario, followed by a residency in internal medicine and oncology at the University of Minnesota. He is board certified with the American College of Veterinary Internal Medicine (Oncology). Dr. Khanna’s training in cancer research includes a PhD from the University of Minnesota, where he studied immunotherapy of metastatic cancers, and a postdoctoral fellowship at the National Institutes of Health, where he was a Senior Staff Fellow at the National Cancer Institute. Since moving to the Washington, D.C. area, he has been able to couple his research interests with his love for clinical veterinary oncology through his work with The Oncology Service, LLC, and his development of the Animal Clinical Investigations, LLC. Dr. Khanna has authored several manuscripts and textbook chapters in the field of veterinary oncology and cancer biology.



Andrei Seluanov
Dr. Seluanov is Associate Professor at the University of Rochester, Department of Biology. His research focus is on understanding the mechanisms of longevity and cancer resistance. Aging is the major cause of death in developed countries. By finding ways to delay aging it will be possible to delay the onset of multiple age-related diseases. Cancer is another major killer in developed world, where 25% of human mortality is caused by cancer. Cancer incidence increases exponentially with age and to achieve long-life species must evolve efficient tumor suppressor mechanisms. Our goal is to understand such mechanisms in mammalian species that are naturally cancer-resistant.

Elizabeth Murchison

Dr. Murchison is a professor at the Department of Veterinaty Medicine of the University of Cambridge, United Kingdom, and the head of the Transmissible Cancer Group. The goal of her lab is to understand how cancers can become transmissible. Cancers arise when cells in the body acquire mutations that cause them to grow and divide uncontrollably. As cancer progresses, some cancer cells can leave the primary site and move around the body in a process of metastasis. However, cancer cells do not normally survive beyond the body of the host that first spawned them. Her research is on two unusual cancers, known as the canine transmissible venereal tumour (CTVT) and the Tasmanian devil facial tumour disease (DFTD). These are cancers that can be transmitted through the population by the transmission of living cancer cells between individuals



Lluis Maria Mir

Dr. Mir is the leader of the Laboratory of Vectorology in anticancer therapy at the Institut Gustave Roussy, France. The role of this unit is to develop viral and non-viral vectors for anti-tumour gene therapy. Dr. Mir was a pioneer and founder of Electrochemotherapy. The technique was first developed in the early 80s and, despite the existence of other researchers at the same time, Dr. Mir was responsible for introducing the actual procedure in the medical world. Nowadays, the technique is widely used in Veterinary Oncology.

Clemens Lowik

Dr. Clemens Lowik is the Head of Experimental Molecular Imaging at the Leiden University Medical Centre, Department of Radiology Netherlands, and the President of the European Society for Molecular Imaging. He is the leader of the MUSIS project. This project represents a highly multidisciplinary research effort that will bring near infrared optical imaging into the clinic for image guided cancer surgery. It is a unique collaboration in the Netherlands between leading scientists, technical universities, companies and surgeons, and has the potential to revolutionize surgical oncology by providing surgeons with a real-time fluorescence based tumor imaging technique to guide surgery for radical resection of tumor tissue and identification of sentinel nodes. By allowing highly tailored surgical treatment, it could significantly improve cancer survival rates.

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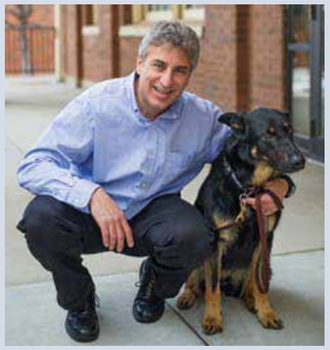
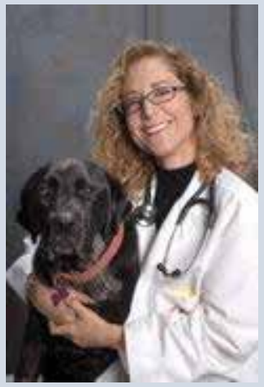


Jaime Modiano

Dr. Jaime Modiano joined the College of Veterinary Medicine and the Comprehensive Cancer Center at the University of Minnesota, USA, in July of 2007, where he continues his research program as Professor of Comparative Oncology holding the Al and June Perlman Endowed Chair. His research program has had uninterrupted support from federal and private sources for 18 years, leading to co-authorship of more than 80 peer-reviewed scientific manuscripts, and ~200 abstracts, presentations, and book chapters focused on various aspects of immunology, cancer cell biology, the genetic basis of cancer and applications of gene therapy.



Cheryl London

Dr. London is a Professor at The Thekla R. and Donald B. Shackelford Professorship in Canine Medicine, Department of Veterinary Biosciences, of The Ohio State University, USA. Her research interests are: the use of spontaneous tumors in dogs and cats as models for human cancer, Investigation of kinase dysfunction in animal tumors, Mouse models of Kit dysregulation, Cellular Biology, Molecular Oncology, Mast Cell Disorders.

WORLD VETERINARY CANCER CONGRESS
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ABSTRACTS

How to Write a Scientific Abstract: Preparing for Congress Submission and Review

By Iain A. Grant BVSc MRCVS DipACVIM (oncology) RCVS
Registered Specialist in Veterinary Oncology

Writing a good scientific abstract requires guidance and practice and in this review article, I will present some ideas that I hope will make your next scientific abstract outstanding. If you are a young clinician or scholar in training, I also hope this will inspire you to seek outlets to present your scientific work. This is the best way to contribute to the advancement of our profession and disseminate medical knowledge worldwide. If you are a seasoned presenter then perhaps this will be useful revision, or it may motivate you to mentor a less experienced colleague in the process of scientific writing.

Purpose

Scientific abstracts are summaries of research work. Although they may serve a number of different functions, in this article we shall focus on their role in summarising an oral presentation or poster to be considered for acceptance at a veterinary or medical conference. Conference organising committees seek to draw up a program of the highest scientific interest and quality. All abstracts are therefore scrutinised by a panel of reviewers for the research they present but also for their written content. Poor quality abstracts that lack preparation or that are not complete, concise and clear are obvious to reviewers and will generally be rejected. Conference organisers generally post guidelines on their website summarising the preferred structure for abstract submissions. Please read these guidelines carefully. They provide vital 'go-to' information, including the criteria by which abstracts will be evaluated and the word limit when writing the abstract, which must be adhered to. A scientific abstract provides a vital first impression of you as a scientist, author and presenter. It is not the time to express your personal writing style; as you will see below, scientific writing is highly stylised and structured. Almost certainly a good abstract, if accepted, will encourage attendance at your forthcoming conference presentation. A poorly written abstract will represent a lost opportunity for you to share the results of the research work that you spent so much time and energy carrying out.

Structure

Most conferences request a structured abstract. This follows the IMRAD format (Introduction, Materials and Methods, Results and Discussion). Research has shown that the structured abstract is easier to read, recall and peer review and is preferred by readers. Broadly speaking, each section poses a question.

1. Introduction : Why was this research carried out? The Introduction puts you research into context and explains how it contributes to the current body of knowledge. You should demonstrate your understanding of the current literature relevant to your study. Crucially, the introduction section ends by stating the aim or aims of the research. This is frequently omitted.

2. Materials and Methods : How was this research carried out? In this section you may want to state the sample size, the population from which the study group were taken, the nature of the intervention and how data was gathered and analysed. An in depth discussion of technical methodology is not appropriate in the abstract and can be discussed at the forthcoming oral or poster presentation.

3. Results : What was discovered? Although no one section of the abstract should carry more importance than another, a reader or conference delegate is potentially most interested in the results of your study. Present clear and concise data; include numerical values such as absolute numbers, percentages or p-values. Negative outcomes should be stated. It is not good scientific practice to present additional or new data later at the Congress which differs from the abstract, unless absolutely unavoidable. Try to include your final data set in the abstract submission.

4. Discussion : What does the data mean? This may include the implications for clinical practice or how your

study has altered the understanding of the pathophysiology, diagnosis or treatment of disease. You may want to state how your study could guide future research work. The discussion section should explain the importance of the study's findings and provide some interpretation.

5. Conclusions should be stated based on the data presented in the results section. No new data should be introduced in the discussion.

After completion of the abstract it is important to review, edit and correct it a number of times. If you are not a native English speaker, then ensure it is read by someone who can correct for grammatical and spelling errors or errors in English constructions. Remove unnecessary words and ensure that sentences are short, simple and purposeful.

Peer review by a colleague, especially someone experienced in scientific writing or abstract preparation can improve your work before final submission. For this reason, make sure you prepare well in advance of the submission deadline.

Choosing a title

The choice of title is very important. It not only conveys the content of the abstract, it is the first opportunity to attract the reader's attention to the importance or findings of your work. It should be 10-12 words in length, and may be informative (outlining the nature of the study) or may be descriptive (giving an insight into the results of the study). Do not overlook the value of a good choice of title.

The abstract review process – in summary

In brief, abstracts will be assigned to a number of different sub-categories for the purpose of review: pure clinical, basic science, radiation oncology/imaging, surgery and pathology.

A panel of 3 reviewers will be assigned to each category, and the abstracts will be scored using an online scoring system. The process of review will be equally rigorous for both oral and poster presentation formats and failure to be accepted for an oral presentation does not automatically mean acceptance for the scientific program in poster format. The following criteria will be evaluated, although not all assessment criteria carry the same importance.

Points will be assigned for each criterion.

1. Is the study relevant to the congress attendees?
2. Does the study present new information?
3. Is the study design clear and appropriate?
4. Is the objective/hypothesis of the study clearly stated?
5. Is the abstract clearly and concisely written?
6. Are the study conclusions supported by the abstract content?
7. Does the abstract title reflect the content of the study?

Based on the score, a rank order will be drawn up with the highest scored abstracts at the top. Individual reviewer's scores will be compared amongst the 3 reviewers in each of the panels and the final decision on whether the abstract is accepted or rejected will be made based on a consensus amongst the group.

The reviewers' decision will be final and no correspondence in any format will be able to be entered into between the researcher and the review panel.

In addition to deficiencies in the writing of an abstract, further reasons for rejection may include inappropriate study design or lack of originality. If information has been presented previously or already published or the outcomes of the study present no new data that contributes materially to the current body of veterinary oncology knowledge, an abstract is likely to be rejected. In a very small number of cases, rejection may be based on ethical considerations. This may include such concerns as inadequate short or long term monitoring

of side effects of a newly proposed therapy. Single case reports in either oral or poster format will not be accepted.

In some cases, when a submission is unsuccessful at being accepted for an oral presentation, a poster format may be offered. Reasons for this may include small case numbers presented, the impression that the research may have been of limited relevance to all conference delegates but would still be valuable to a minority audience and also if English language content is poorer but the science is felt to be valuable. It is thought that this may be more effectively communicated in the less time pressure setting of a poster presentation. On the contrary, although less likely, authors of poster presentation abstracts may be offered an oral presentation opportunity. This is likely to be a reflection of the exceptional quality of the science or when the content of the research work is considered to be highly relevant to the congress audience.

Like any creative endeavor, writing a scientific abstract requires practice and some individuals may be better at this process than others; however reading around the subject and following some of the recommendations made in this review will hopefully assist you the next time you consider a scientific meeting for presentation of your research work. We sincerely hope that this will include forthcoming ESVONC, ABROVET, JVCS and VCS Congresses.

'*Writing for Publication in Veterinary Medicine' is a free online resource provided by Wiley Blackwell. I thoroughly recommend this as a valuable addition to your scientific library.*

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ABSTRACTS FOR ORAL PRESENTATIONS

ORAL ABSTRACTS

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Accuracy evaluation of frozen sections compared to paraffin histological sections - retrospective study of 142 cases.

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Introduction

Material and Methods

We conducted a review of FS and paraffin diagnoses during the year of 2015. It was considered: definitive diagnosis (DD) when the findings were exactly alike; identification of the pathologic process (IPP), when the type of process has been correctly identified, including, if neoplastic, the origin and behavior; incorrect diagnosis (ID) when there was a difference between FS and paraffin and delayed (D) when it was necessary to wait for paraffin results. Was used American Optical portable freezing microtome, toluidine blue staining at 1%, with five to ten minutes to complete the analysis of each sample.

Results

142 cases were reviewed, 29 cats and 114 dogs and the total of 233 samples analyzed by FS. Of these, 68 (29.18%) obtained DD, 136 (58.36%) had IPP, 21 (9.01%) showed ID and 8 (3.43%) were D. The accuracy of the procedure was 87, 67%, being similar to the data from the medical literature and veterinary.

Conclusion

Although DD have been considerably smaller, must be consider the methodology difference in the other studies that used, cryostat and HE staining, providing another sample quality. These results show that the FS is a reliable method for intraoperative diagnosis with portable freezing microtome and stained with toluidine blue.

Both membranous and cytoplasmic cyclooxygenase-2 (COX-2) expression modulates the prognosis of feline invasive mammary carcinoma

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Introduction

Cytoplasmic cyclooxygenase-2 (COX-2) overexpression has been associated with poor prognosis in feline invasive mammary carcinomas (FMCs) and human invasive breast cancers (BC). Membrane COX-2 expression is reported in invasive BC, but is more common in atypical hyperplasia and ductal carcinomas in situ. The aim of this study was to determine if both subcellular locations of COX-2 expression are significant in FMCs.

Material and Methods

180 surgically-treated FMCs, diagnosed in female cats whose outcome was known 2 years' post-mastectomy, were retrospectively included. ER (clone C311), PR (clone 10A9), Ki-67 (clone MIB1), HER2 (clone 4B5) and COX-2 (clone SP21) expressions were determined by automated immunohistochemistry. COX-2 membrane expression was quantified as an Allred score (sum of 0-3 points for staining intensity and 0-5 points for the percentage of positive cells).

Results

Three aspects of COX-2 expression impacted the outcome of cats with FMC. (1) The pattern of membrane COX-2 expression was associated with cancer progression (local recurrence, nodal and/or distant metastasis) with Hazard Ratio HR=1.58 for basolateral or complete COX-2 staining (127/180 FMCs) compared to apical (53/180 FMCs) staining (p=0.03; log-rank test). (2) Cytoplasmic COX-2 expression in at least 30% of the neoplastic cells (20/180 FMCs) was associated with shorter overall survival (HR=1.84; p=0.0092). (3) Membrane COX-2 overexpression (scores 7-8 points, 74 cases) was associated with increased risk of cancer-related death (HR=1.63; p=0.0052).

Conclusion

Membrane COX-2 expression is more common in FMCs than in human BC. In cats, COX-2 overexpression either in the cytoplasm or at the plasma membrane carries a poor prognosis.

Impact of luminal phenotypes on outcomes of cats with invasive mammary carcinoma

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Introduction

In humans, “luminal” invasive breast cancers (BC), positive to Estrogen Receptor (ER) and/or Progesterone Receptor (PR), carry a good prognosis, especially when they coexpress FOXA1 (forkhead box protein A1). “Luminal-AR” BC, defined by Androgen Receptor (AR) positivity, is the least aggressive subtype of triple negative BC (negative to ER, PR and HER2, Human Epidermal Growth Factor Receptor-2). The aim of this study was to investigate the “luminal” phenotypes of feline invasive mammary carcinomas (FMCs).

Material and Methods

Retrospective study of 180 FMCs from 180 female cats with 2-year follow-up post-mastectomy. ER (clone C311), PR (clone 10A9), Ki-67 (clone MIB1), HER2 (clone 4B5), FOXA1 (clone SP88) and AR (clone SP107) expressions were determined by automated immunohistochemistry. Thresholds for positivity were 10% for ER and PR, 2% for FOXA1. AR expression was quantified as an Allred score (0 to 8 points according to staining intensity and percentage of positive cells).

Results

Among the 57 luminal FMCs, FOXA1 positivity (25/57, 44%) was associated with lower risk of local recurrence (HR=0.31; p=0.02), lower risk of distant metastasis (HR=0.31; p=0.03), and lower risk of cancer-specific death (HR=0.39; p=0.0038; log-rank tests). Among the 123 triple negative FMCs, AR overexpression (scores 7-8 points, 21/123 FMCs, 17%) was associated with decreased risk of cancer progression (recurrence and/or metastasis, HR=0.48; p=0.03) and improved specific survival (HR=0.53; p=0.03).

Conclusion

As in human breast cancer, luminal FMCs differ in prognosis according to FOXA1 expression, and there exists a good-prognosis “luminal-AR” subtype of triple negative FMCs.

The use of biomarkers and molecular classification as prognostic indicators in patients with canine mammary tumors in Mexico

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Introduction

The classification of canine mammary tumors (CMTs) based on their molecular expression aims to define molecular subtypes in order to establish appropriate prognostic and therapeutic criteria. Our objectives were to establish molecular classification of CMTs in relation to their clinical and pathological features.

Material and Methods

Immunohistochemical expressions of estrogen receptor alpha (ERα), ErbB2, Ki67, E-cadherin and CD31 were evaluated in 135 CMTs.

Results

Most benign CMTs were ERα positive, with low Ki67 expression, associated with longer survival time (ST). Malignant tumors were generally ERα negative, with high Ki67 expression, associated with shorter ST. ErbB2 was upregulated in small-sized tumors and downregulated in large-sized tumors. The most common molecular subtypes were ErbB2 and Luminal ErbB2. Carcinosarcomas and solid carcinomas were sub-classified as Basal and Luminal B types, respectively; and simple and complex carcinomas were Luminal B-ErbB2.

Conclusion

CMTs are heterogeneous, thus, each patient requires molecular classification. Ki67 and ERα are excellent prognostic markers for CMTs. Ki67 is very useful for molecular subtype classification, discriminating between Luminal Subtypes A and B, and along with ERα, it allows consideration of the Luminal ErbB2 subtype, which is associated with a better life expectancy compared with the ErbB2 subtype. The identification of molecular subtypes of CMTs may help develop individualized treatments in these patients. Biomarkers for CMTs in developing countries like Mexico have not been routinely utilized, perhaps due to socioeconomic factors and the lack of prospective studies in our canine populations. Our findings may provide a starting point for comparative studies with breast cancer in women.

Global changes in microRNA levels during epithelial to mesenchymal transition in canine mammary carcinoma

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Introduction

Epithelial to Mesenchymal Transition (EMT) is a process involved in embryogenesis, carcinogenesis and metastasis. EMT has been implicated as a driver of metastasis as it allows cells to migrate to different organs, and has been shown to promote conversion of cancer cells to ones with stem-cell characteristics. Previously, we found that canine mammary carcinoma cells acquired aggressive characteristics after a TGF-β-induced EMT, confirmed by morphologic, molecular and migratory analyses. MicroRNAs (miRNAs) are small RNA molecules involved in a variety of cellular processes, and can promote or regulate cancer-related genes. We aimed to determine global changes in miRNA expression through screening of canine mammary carcinoma cells during TGF-β-induced EMT. Our hypothesis was that EMT-induced cells would differently express miRNAs compared to control cells not undergoing EMT.

Material and Methods

We sent RNA from these cells, at different time points (0 - 23 days), to Edinburgh Genomics-Roslin Institute for miRNA sequencing. The obtained reads were mapped to known canine miRNAs looking for changes in miRNA expression during TGF-β-induced EMT in canine mammary carcinoma cells.

Results

Four different miRNAs (cfa-miR-380, cfa-miR-381, cfa-miR-410, cfa-miR-411) were significantly upregulated in TGF-β-stimulated canine mammary carcinoma cells compared to unstimulated cells. These findings were then validated by qRT-PCR.

Conclusion

Previous studies in humans have determined that these 4 miRNAs have roles in carcinogenesis. The discovery of new miRNAs and understanding of action of known miRNAs during EMT in canine cancer cells could be very useful for cancer research in companion animals. Inhibition of miRNAs might become a therapeutic option in future.

Melatonin as pro-apoptotic and anti-angiogenic therapy in canine mammary tumor cells

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Introduction

Melatonin is an anti-angiogenic, anti-inflammatory and pro-apoptotic agent studied in many tumor types. There are few studies evaluating the melatonin effects on tumorigenesis of dogs, so, we purpose to determine the action of melatonin in canine mammary tumor cells, by the modulation of apoptosis and angiogenesis.

Material and Methods

Metastatic (CF-41) and non-metastatic (CMT-U229) canine mammary tumor cells were cultured in monolayer and tridimensional spheroids and treated with melatonin in different concentrations by 48 hours. Cell viability was measured by MTT assay, gene and protein expression of cleaved caspase-3 and VEGF-A was performed by qRT-PCR and immunofluorescence, respectively, and, an apoptosis antibody array was performed in metastatic cells after melatonin treatment.

Results

Treatment with pharmacological concentration of melatonin (1 mM) was able to reduce viability of both tumor cells, increased cleaved caspase-3 and reduced VEGF-A protein expression ($p<0.05$). In addition, melatonin was able to modulate another apoptotic protein involved in intrinsic and extrinsic apoptosis pathway ($p<0.05$). For 3D culture, that mimics tumor microenvironment, it was observed enhance of apoptotic spheroids after melatonin treatment.

Conclusion

Antiproliferative activity of melatonin involves different mechanisms and it was confirmed in canine tumor cells, being effective in control of tumor growth. Besides that, the modulation of apoptosis and angiogenesis together represent an effective approach in control of tumorigenesis. Conclusion: Our study is the first one in canine specie evaluating the potential of melatonin as oncostatic, pro-apoptotic and anti-angiogenic agent and could represent a new therapeutic alternative to control growth in canine mammary tumors.

IL-25/siIL-17B modulates apoptosis, angiogenesis and NF-kB signaling in the control of canine mammary tumor cells

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Introduction

IL-25 is an active cytokine related to cancer development. The interaction with its receptor IL-25R induces apoptosis, but there is a competition for the site of action with IL-17B in neoplastic cells, contributing to tumor progression, in part, by activation of nuclear factor (NF)-kB, that leads the cancer progress. The aim of this study was to evaluate the potential of IL-25 and IL-17B silenced (si) in modulation of apoptosis, angiogenesis and NF-κB expression in canine mammary tumor cells.

Material and Methods

Metastatic (CF-41) and non-metastatic (CMT-U229) canine mammary tumor cells were cultured in monolayer and tridimensional model and treated with IL-25 purified and siIL-17B. Gene and protein expression of cleaved caspase-3 and VEGF-A were evaluated by qRT-PCR and immunofluorescence, and, NF-κB expression by Western Blotting.

Results

IL-25 and siIL-17B treatments increased cleaved caspase-3 in monolayer cells and IL-25 enhanced apoptotic spheroids ($p<0.05$). For angiogenic marker, both treatments reduced VEGFA in both tumor cells ($p<0.05$). Furthermore, NF-κB was reduced after both treatments in CMT-U229 cells ($p<0.05$) and, for CF-41 cells, only siIL-17B reduced the nuclear factor but it was not significant.

Conclusion

The siIL-17B strategy allows IL-25/IL-25R interaction, sending a death signal to breast cancer cells, stimulating FAS receptor and TNF-1 that block NF-kB pathway. Besides that, both therapy strategies were efficient in modulating apoptosis and angiogenesis and, until the moment, there are no studies evaluating these proteins in canine mammary tumor cells. Conclusion: IL-25/siIL-17B signaling is a potential agent in controlling tumorigenesis of canine mammary tumor cells and could be a promise in clinical practice.

FAK and mTOR inhibitors, promising therapeutic agents, modulate cancer cell stemness in both canine and human mammary cancer

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Introduction

Focal Adhesion Kinase (FAK) overexpression is associated with poor overall survival in human breast cancer. In dogs, metastases have higher FAK mRNA levels than primary mammary tumors. FAK activates AKT/mTOR pathway that is deregulated in tumors of both species. FAK, AKT and mTOR interact with nucleostemin, a regulator of cancer stem cells (CSC), and modulate its expression. FAK and mTOR inhibitors are in development, and it is essential to know which tumor types are responsive to these inhibitors in human and veterinary medicine.

Material and Methods

Here, we investigate the effects of FAK and mTOR inhibitors on CSC using a panel of human (MCF7, MDA-MB-468, MDA-MB-231), mouse (4T1) and canine (CMT-U-27 and CMT-U-309) mammary cancer cell lines. Cell lines considered sensitive have an impairing of cell proliferation, a decreased number of ALDH (CSC marker) positive cells by FACS and a reduction in nucleostemin expression by Western Blotting.

Results

Only cells (MCF7, MDA-MB-231, 4T1), with lower levels of AKT phosphorylation, were sensitive to FAK inhibitor (PF-271). There is no correlation between FAK phosphorylation levels and PF-271sensitivity. Cells with higher levels of AKT phosphorylation, MDA-MB-468, CMT-U-27 and CMT-U-309, are resistant to PF-271 and sensitive to mTOR inhibitor (Rapamycin). Rapamycin did not affect the number of CSC in PF-271-sensitive cells (4T1 and MDA-MB-231), indicating that FAK and AKT/mTOR pathways can regulate nucleostemin and stemness independently.

Conclusion

These results suggest that tumors with low levels of AKT phosphorylation might respond better to FAK inhibitors, while mTOR inhibitors might benefit patients with tumors that have high AKT phosphorylation.

Inflammatory response following thalidomide treatment in the 4T1 murine mammary carcinoma

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Introduction

The potential immunomodulatory effect of thalidomide has been described as an important strategy in the treatment of multiple myeloma, melanoma, and liver carcinoma. The aim of the present study was to evaluate the effect of different doses of thalidomide in mice with murine mammary carcinoma 4T1, identifying cell proliferation rates and the angiogenic and inflammatory behavior of the primary tumor and lung metastasis.

Material and Methods

Balb/c mice were inoculated with 2.5x106 4T1 tumor cells and divided into three groups according to different daily doses of thalidomide (50, 100, and 150 mg/kg). The animals were treated from the fifth to the twenty-eighth day of tumor development, with subsequent euthanasia and collection of the primary tumor and lungs.

Results

Treatment with 150mg/kg of thalidomide significantly reduced the 4T1 tumor growth. Histologic, morphometric, immunohistochemical, and immunofluorescence analyses of the primary tumor demonstrated an increase in inflammation and a reduction in neoplastic size, neoplastic proliferative rates, and tissue macrophages in response to increased concentrations of thalidomide. A reduction in the neoplastic proliferative rate and in the number of vessels was observed in lung metastases, with an increase in NAG, CCL2, TNF-α levels and the number of macrophages in response to increased concentrations of thalidomide.

Conclusion

These results open perspectives for the immunomodulatory and antitumor effects of thalidomide according to the tumor site and doses studied, particularly regarding the immunomodulatory role of thalidomide in the recruitment and activation of tumor-associated macrophages.

Claudin Gene Expression Analysis of Canine Mammary Non-Neoplastic and Neoplastic Tissue Derived Cell Cultures

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Introduction

Cells derived from non-neoplastic or neoplastic tissues are commonly used in medical research or evaluation of new application logs. Cell connectin claudin proteins are deregulated in canine epithelial tumors. Claudin-expressing tumour cells were killed by application of claudin targeting toxins. Claudin gene expression of cultivated cells was found to be altered compared to the original tissue. This study aimed at the identification and characterization of claudin expressing cell cultures. Claudin expression analysis was performed on 17 canine mammary non-neoplastic and neoplastic tissue derived cultures during cultivation until passage 30 and corresponding tissue samples.

Material and Methods

17 canine mammary tissue samples (five non-neoplastic tissue samples, two lobular hyperplasias, one simple adenoma, one complex adenoma, two simple carcinomas, three complex carcinomas, one carcinoma in a benign mixed tumor, two benign mixed tumors), thereof derived cultures. Cell culture until passage 30, RNA-isolation, cDNA-synthesis, PCR, quantitative real-time PCR, luminex bead conjugated technology.

Results

Claudin-1, -3, -4 and -7 gene expression persisted in cell cultures derived from a lobular hyperplasia, a simple adenoma and a complex carcinoma during cultivation until passage 30.

Conclusion

Claudin-1, -3, -4 and -7 gene expression of canine mammary neoplastic and non-neoplastic tissue derived cell cultures was analysed using PCR, qPCR and luminex bead conjugated technology. Cell cultures derived from a lobular hyperplasia, a simple adenoma and a complex carcinoma were identified to positively express the claudins-1, -3, -4 and -7. They classify as candidates for further research regarding claudin-targeting tumor therapeutics killing claudin expressing tumour cells using claudin targeting molecules.

Surgical approach of mammary tumors in small animals: a survey involving Brazillian veterinary physicians

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Introduction

Mammary tumors are the most common neoplasms in bitches and the third most common in cats. Even so, there is great variability between physicians regarding surgical treatment. The aim of this study was to assess the surgical data of these tumors in different Brazillian regions.

Material and Methods

Brazillian veterinary physicians were asked a series of questions including professional degree and aspects concerning surgical approach of mammary tumors. Questions were applied via e-mail.

Results

Of a total of 300 physicians, 59% were graduated 5 years before the study and 52% did not exclusively work with oncology or surgery. Approximately 65% of the professionals do not use cytology, however 90.7% perform the histopathological exam after mastectomy. The TNM staging system is performed by 52.3% of the physicians and 14% are not familiarized with it. Most professionals neuter the patients (94%) and 70.5% do so during mastectomy. Approximately 75% of the physicians do not detect sentinel lymph nodes, but most excise the nodes for histopathology. Surgical treatment is more radical for cats. In dogs, 80% of the surgeons perform bilateral mastectomy in two times. Reconstructive surgery is not usually performed during closure of the surgical wound. Regarding metastasis, 87% of the physicians adapt treatment to improve life quality.

Conclusion

Cytology, tumor staging and sentinel lymph node detection are not commonly used when approaching mammary tumors. There is still great divergence regarding whether to neuter these patients and there is no standard technique for each situation. There appears to be greater consensus about using aggressive techniques in cats.

Enhanced importance of mammary tumour lymph nodes in radical treatment and prognosis in companion animals

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Introduction

The treatment of mammary tumours is the surgical excision, but the success of the treatment is dependent on residuum free excision. Finding tumour-infiltrated lymph nodes during the operation is a challenging process. Aim of our study was to introduce an intraoperative method for the visualisation of the tumour-infiltrated lymph node and demonstrate the positive effect of radical mammectomy on survival and the prognosis of local recurrence.

Material and Methods

Bitches (170) and queens (24) were diagnosed with multiple, malignant mammary tumours. Tumours of 110 bitches and 16 queens were peri-injected 5-10 minutes preoperatively with 4x0.1 ml nano size particle Patent blue 1% solution (Sigma-Aldrich Co.) (PB). Sixty dogs and 8 cats as controls (C) were operated without infiltration. Histopathology was based on HE stain and as prognostic factors Ki67, ER, PgP, VEGF and EGFR immunhistochemie occurred.

Results

The number of affected islets differed from 3 to 10. Incidence of malignant tumours was 78.5 % and metaplasia were present in 30 % of the cases. Lymphoid metastases were detected in 22 PB (17.4 %) and 7 C (10.2 %) lymph nodes. Affected lymph nodes had similar immunhistochemical characteristics as the primer tumours. Survival of patients was 4.5 year in average and tumour recurrence was 13.5 % (17) in group PB and 19.1 % (13) in group C.

Conclusion

The visualisation of tumour infiltrated lymph nodes with PB increased the chance of reaching a tumour free condition. Immunhistochemical examination of tumours and lymph nodes helps to state the prognosis.

Use of adjuvant chemotherapy in canine mammary gland carcinosarcomas

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Introduction

Carcinosarcoma (CSS) is an infrequent histological type of the canine mammary gland and is related to a guarded prognosis. The aim of the work was to evaluate the overall survival (OS) of female dogs diagnosed with CCS of the mammary gland according to clinical staging and different treatment protocols, including surgery and adjuvant chemotherapy.

Material and Methods

A retrospective study of female dogs diagnosed with CSS of the mammary gland admitted at the Veterinary Hospital of the Federal University of Minas Gerais (UFMG), between 2000 and 2014, was performed. Neoplasms were diagnosed at the Laboratory of Comparative Pathology (UFMG). Chemotherapy protocol consisted in four cycles of 300mg/m2 of carboplatin and 30 mg/m2 of doxorubicin, every 21 days.

Results

Fifty-five CSS cases were obtained. Mean age at the time of diagnosis was 10,4±2,68 years. Twenty-five (55%) cases were classified as clinical stage I-III and 20/44 (45%) stage IV-V, presenting regional or distant metastasis. Stage IV-V patients presented a median OS of 197 days, while stage I-III patients did not reach the median OS (p=0.0005). Adjuvant chemotherapy was performed in 11/55 (20%) cases. Patients submitted to chemotherapy presented a longer overall survival when compared to patients treated only with surgery, 475 and 128 days, respectively (p=0.0046).

Conclusion

Clinical staging was considered an important prognostic factor and adjuvant chemotherapy increased OS in female dogs diagnosed with CSS of the mammary gland.

Mycobacterium Cell Wall-DNA complex (MCW-DNA) as an aid in the treatment of chemotherapy- induced neutropenia in healthy dogs

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Introduction

Currently, there is no efficacious veterinary product available for the treatment of chemotherapy-induced neutropenia. Immune response modifiers have been used for cancer treatment for many years. Here, we report the results on the use of Mycobacterium Cell Wall Fraction (MCWF) non-specific immunomodulator in restoring chemotherapy-induced neutropenia in healthy dogs.

Material and Methods

The study design included four experimental groups with ten dogs per group. On Day 0, all dogs received 3 mg/m2 of vinblastine intravenously (I.V) and upon diagnosis of neutropenia, each dog was randomly allocated to one of four experimental groups. MCWF was I.V administered to all dogs within 24 hours following onset of neutropenia. MCWF dosing was 100, 200 or 500 µg/kg, or no treatment. The end of the study was defined as a neutrophil count ≥ 2000/µL for two consecutive days or Day 10, whichever came first.

Results

There was a clear dose-response observed in the time to recovery from neutropenia. The mean duration of neutropenia was 4.2, 2.5, 1.8, and 1.2 days in the control, 100, 200 and 500 µg/kg MCWF-treated groups respectively. There were statistically significant differences ($p<0.01$ and $p<0.0001$) in the mean duration of neutropenia between the control group and all MCWF-treated animals.

Conclusion

Administration of MCWF demonstrated efficacy in restoring chemotherapy-induced neutropenia in healthy dogs. Additional studies are underway to demonstrate the efficacy of MCWF in preventing chemotherapy-induced neutropenia following concurrent administration.

Serum Cytokine Induction After a Single Mycobacterium Cell Wall Fraction (MCWF) treatment in Tumor Bearing Dogs

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Introduction

Mycobacterium cell wall fraction (MCWF) is a non-specific immunomodulator with antitumor activity that is regulator-approved for intratumoral injection. MCWF can activate macrophages and monocytes to directly destroy cancer cells, or indirectly control tumor growth by cytokine production. The aim of this study was to evaluate the ability of MCWF to induce serum cytokine synthesis after single intravenous treatment in dogs with different solid tumors.

Material and Methods

Ten naïve dogs with solid tumors were treated with 50ug/Kg of MCWF to a maximum dose of 1.5mg diluted in 100mL of 0.9% NaCl administered as slow drip infusion over 45 minutes. Canine-specific IL-2, IL-6, IL-10, IL-12, IFN-γ, GM-CSF and TNF-α were measured using a multiplex ELISA kit at 0, 2, 4, 6, 12, 24, 48 and 168 hours' post-treatment. Clinical signs, CBC and biochemistry parameters were monitored following treatment and no clinically significant abnormalities were observed during the course of the study.

Results

Two hours following treatment, transient decrease of IL-2, IL-6, and TNF-α was observed in all dogs. In parallel, levels of IL-10 were continuously increasing and were statistically significant compared to pre-treatment (p=0.0306).

Conclusion

These observations are expected as it is known that IL-10 could suppress the expression of IL-2, IL-6 IFN-γ and IL-12. In addition, there were no statistically significant differences in GM-CSF, INF-γ and IL-12 levels at all time points during the course of the study. IL-10 is a multifunctional cytokine with the ability to inhibit T-helper (Th1) activity, stimulate natural killer cells, inhibit tumor-induced angiogenesis and enhance the production of nitric oxide.

Genomic copy number variation in canine cutaneous mast cell tumors

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Introduction

Mast cell tumors are the most common cutaneous malignant tumors in dogs. Although there are several prognostic factors, the clinical behavior of this tumor is highly variable, making difficult to select the most appropriate treatment. Molecular techniques are used to evaluate a large number of genes involved in the neoplastic process, which helps the selection of candidate genes related to prognostic and predictor factors. The CGHarray technique (comparative genomic hybridization) allows the investigation of numerical and structural changes in DNA isolated from tumor cells.

Material and Methods

This study was designed in order to compare the copy number variations (CNVs) in canine cutaneous mast cell tumors from animals that survived less than six months (SV<6) and animals that survived more than twelve months (SV>12).Material and methods: Ten animals were selected, four from SV>12 group and six animals from SV<6 group. Genomic DNA was extracted and array-CGH was done using Agilent Canine Genome CGH Microarray 4x180. Data analysis was performed using Nexus program version 5.0 (Biodiscovery).

Results

We detected an overall mean of 55.5±25.3 CNVs in the 10 animals. The group SV>12 presented 11±3.3 CNVs while the SV<6 group presented 85±38.5 CNVs.

Conclusion

Regions of loss in PTEN and FAS and regions of gains in MAPK3, WNT5B, FGF, FOXM1 and RAD51 were detected in mast cell tumors with shorter survival time and thus, worst prognosis, allowing the identification of potential candidate genes to more detailed studies.

Association between frequency and intensity of gastroduodenal lesions and plasma histamine levels in dogs with cutaneous mast cell tumour

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Introduction

Gastroduodenal ulceration secondary to neoplastic mast cell degranulation is cited as the main complication associated with canine cutaneous mast cell tumor. This study evaluated the occurrence and severity of gastroduodenal lesions in dogs having cutaneous mastocytoma along with their plasmatic histamine concentrations at the time of diagnosis.

Material and Methods

Forty patients, from the Veterinary Hospital of the Federal University of Rio Grande do Sul, underwent gastroduodenal endoscopic examination after surgical removal of a mast cell tumor. Histological samples from gastrointestinal tract were evaluated according to the criteria proposed by Day et al. (2008). Quantitative plasmatic histamine was determined by a competition ELISA test, detecting a minimum of 0,02ng/mL. Plasmatic histamine was assessed in all 40 dogs having mast cell tumors and in other 18 healthy ones (control group).

Results

In the control group, the median and maximum values detected were 0.0975 and 1.7293 ng/mL, respectively, while in dogs with mast cell tumor, they were 0.0160ng/mL and 2.5675 ng/mL, respectively. No statistical difference was observed in the histamine plasmatic concentration between groups. Gastroduodenal lesions were present in 17 dogs (41.5%) with mast cell tumor, being one (5.9%) graded as moderate and the other 16 (94.1%) as mild. Gastroduodenal ulceration was not observed in any of the dogs.

Conclusion

In the population studied, gastroduodenal lesions were not associated with plasmatic histamine concentration, suggesting that other factors may influence the occurrence of gastroduodenal lesions in dogs with mast cell tumor.

Retrospective evaluation of masitinib and prednisolone compared to masitinib alone in canine mast cell tumours

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Introduction

Masitinib mesylate is a TKI approved for treatment of non-resectable grade II / III mast cell tumours (MCTs). Prednisolone can be beneficial in management of MCT and may be combined with masitinib but the advantage or disadvantage of this combination has not been investigated. The aim of this study was to compare tumour response and survival of patients treated with masitinib + prednisolone versus masitinib alone.

Material and Methods

Records of dogs with MCTs treated with masitinib +/- prednisolone at the Queen's Veterinary School Hospital, between August 2009 and May 2014 with follow up were reviewed. Response rate, progression free interval (PFS) and overall survival (OS) were analysed and Kaplan Meier survival curves were generated for PFS and OS to compare the two groups.

Results

Forty-five dogs were included in the study, 16 received masitinib alone and 29, masitinib + prednisolone. 49% of cases with measureable disease (35 patients) responded to treatment (20% CR 28.6% PR). Response to masitinib was associated with a significant increase in MST (P=0.0008) (284 versus 89 days) and progression free survival, (243 versus 48 days) compared to no response. Grade and stage were not associated with significant differences in MST and PFS. Patients treated with masitinib + prednisolone showed lower overall response and survival compared to those treated with masitinib alone (34.8% versus 66.7%, OS from 471 to 160 days and PFS 177 to 103 days respectively).

Conclusion

In this study the addition of prednisolone to masitinib reduced overall response rate, OS and PFS in dogs with MCT.

New insights in dose-escalation of vinblastine in dogs with biologically aggressive mast cell tumours

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Introduction

Established vinblastine dose in dogs is 2.0-3.5 mg/m² (humans 3.7-18.5). The objective of this retrospective study in mast cell tumour (MCT) bearing dogs was to evaluate vinblastine dose-escalation >3.5 mg/m², check for the only two missense variants (rs24334216-rs24334217) with a Sorting-Intolerant-From-Tolerant (SIFT)-score<0.05 in CYP3A12, possibly influencing vinblastine pharmacokinetics, and assess Progression Free Survival (PFS).

Material and Methods

Selected were chemo-naïve dogs with non-resectable, or (in)completely excised subcutaneous or grade-2/3 cutaneous MCT, with the intention-to-treat weekly for 12-weeks with a dose-escalating vinblastine protocol (+/-prednisolone). Initial dose (2.0-3.0 mg/m²) was increased weekly by 0.5 mg/m². Adverse events (AEs) were assessed according to VCOC-CTCAE v1.1, response to therapy following RECIST v1.1. Neutrophil count of 1.5-2.0x10^9/l and/or other grade-2 AEs required 0.5 mg/m² dose-reduction. Dogs still alive (n=3), had Sanger sequencing performed in a 287-bp amplicon of CYP3A12, containing rs24334216-rs24334217, on a 3730XL sequencer.

Results

Seven of thirteen dogs included were dose-escalated between 4.0-5.5 mg/m². Five completed the protocol in the high-dose group (HDG: 4.0-5.5 mg/m²), three in the low-dose group (LDG: 2.0-3.5 mg/m²). Five (three LDG, two HDG) prematurely discontinued vinblastine, tumour/therapy unrelated. Dose-limiting AEs were only detected in LDG: 4x neutropenia, one grade-2 anorexia, one grade-3 anaemia. Median PFS for LDG (4CR/2PR) was 324 days (230-418), for HDG (6CR/1PR) 329 days (238-843). No rs24334216-rs24334217 mutations were found in one HDG-dog, while two LDG-dogs were heterozygous for both missense variants.

Conclusion

Vinblastine dose-escalation >3.5 mg/m² was possible in a cohort of dogs, without AEs. Survival benefit and the role of missense variants in CYP3A12 require further investigation.

Heterogeneity in c-kit profile and expression in canine mast cell tumour and its metastasis

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Introduction

Mutations in c-kit oncogene occur in 15-40% of canine mast cell tumours, and internal tandem duplications in exon 11 were associated with a worse biological behavior and outcome. In fact, those are the main abnormalities found in this context and are predictive for treatment with tyrosine kinase inhibitors. This study had the objective to describe abnormalities in the KIT receptor expression and mutational status of exon 11 of the c-kit gene in canine mast cell tumours presenting lymph node metastasis and satellite nodules.

Material and Methods

Thirteen dogs, presenting cutaneous mast cell tumours and advanced metastatic lymph node (HN3) underwent surgical treatment and had their lesions individually assessed on the immunohistochemical expression of the KIT receptor and the presence of mutations in exon 11 of the c- kit oncogene, using a PCR technique.

Results

Surgical excision resulted in 13 primary lesions, 15 lymph nodes and a skin satellite nodule Three patients presented aberrant expression of KIT in lymph node while normal expression in primary tumour, and five patients presented mutations in metastases without any abnormalities in primary tumours.

Conclusion

Mutations in c-kit oncogene appears to be more frequently in patients presenting advanced stage and those, may be acquired in the later course of the disease, contributing to its progression. This may explain the favorable response to tyrosine kinase inhibitors, in patients whose mutation was not initially found. Molecular analyzes of both primary tumour and its metastasis may be important to a proper therapeutic decision in canine mast cell tumours.

Prognostic value of Ki67 and mitotic index in dogs with diffuse large B cell lymphoma

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Introduction

Diffuse large B cell lymphoma (DLBCL) is the most common type of lymphoma in dogs and humans. Prognosis is quite variable because of its aggressiveness and heterogeneous behavior and few prognostic factors for DLBCL are described. Ki67 and mitotic index (MI) are proliferative markers that have been correlated with prognosis in different tumors, but have not been studied specifically in DLBCL in dogs. The authors hypothesis is that they might constitute a practical prognostic factor in this disease.

Material and Methods

Thirty dogs diagnosed with DLBCL were selected for MI counting and for immunohistochemical evaluation using MIB-1 for Ki67 expression. A grid reticle was used for counting Ki67 positive cells in a total area of 1 cm^2. MI was counted as described elsewhere. Cutoff values were calculated based on the mean score for both markers. Patients were classified into two groups (low and high) according to the cutoff value (Ki67:107, MI:21).

Results

Survival times for patients in groups Ki67-high (n=21) and MI-high (n=21) were lower when compared to groups Ki67-low (n=9) and MI-low (n=9), however a significant difference was found only in group Ki67 (281 days versus 98 days, $p<0.05$). A positive correlation between Ki67 immunoexpression and MI was also found.

Conclusion

Ki67>107 positive cells, counted using a grid reticle, can predict lower survival times and therefore may constitute a practical prognostic factor in dogs with DLBCL. Further studies about molecular pathways that may be associated with high Ki67 immunoexpression are needed. This study reinforces the relationship between Ki67 immunoexpression and MI in canine DLBCL.

Comparative genomic sequencing of the canine B-cell lymphoma cell lines CLBL-1 and CLBL-1M

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Introduction

Cell lines are key tools in cancer research allowing the generation of neoplasias in animal models. Canine lymphoma is the major hematopoietic malignancy in dogs, accordingly stable in vitro and in vivo model systems are of critical value for the development of therapeutic approaches. Herein we describe the comparative genomic characterisation of two canine B-cell lymphoma cell lines by whole genome sequencing.

Material and Methods

CLBL-1 was injected into Rag2-/-γc-/- mice, allowing to generate xenografts, which were used to derive the CLBL-1M cell line. Genomic DNA of both cell lines was sequenced by NGS technique in order to identify copy number variations and both cell lines were comparatively karyotyped.

Results

CLBL-1 and CLBL-1M are hemizygous for chromosomes CFA14 and CFA35. Large focal deletions (>10Mbp) on CFA12, CFA22, CFA33 and smaller deletions (<10Mbp) on 8 different chromosomes were detected. CFA13 is duplicated in both cell lines and 20 focal amplifications ranging from 30kbp-20Mbp were detected on 14 chromosomes. Karyotyping showed similar cytogenetic aberrations in both cell lines. Only, one focal deletion at CFA32: 5Mbp-7.5Mbp is exclusively present in CLBL-1.

Conclusion

The comparative analyses showed that CLBL-1 maintains its genomic characteristics, when inoculated in vivo and further re-cultured in vitro. This is in line with our previously reported immunophenotype, histopathologyic response to IL-2 and DSP30, PARR and expression analyses, which revealed stability of CLBL-1 in in vitro and in vivo models. These data indicate that CLBL-1 derived in vivo models provide a highly stable tool for B-cell lymphoma research in veterinary medicine.

Immunophenotypic quantification of T regulatory cells in dogs with multicentric lymphoma submitted to chop chemotherapy protocol associated or not to Firocoxib

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Introduction

Regulatory T cells inhibit the effective function against tumors, resulting in disfunction in T cells in humans and dogs with cancer. The increase of cyclooxygenase-2 enzyme (COX-2) expression by tumor and inflammatory cells stimulates Tregs development. Thus, treatment with COX-2 inhibitors, like non steroidal anti-inflammatory is a new strategy to suppress Tregs. This study aimed to evaluate Tregs in a quantitatively way, by flow cytometry, submitted to CHOP chemotherapy protocol, associated or not to a COX-2 inhibitor (Firocoxib).

Material and Methods

Three groups were formed, as following: (G1) 12 healthy dogs; (G2) 12 dogs with multicentric lymphoma treated with regular CHOP protocol (with prednisone) and (G3) 12 dogs with multicentric lymphoma treated with CHOP protocol in which the prednisone was substituted by firocoxib. Tregs were evaluated, by flow cytometry, at the time of diagnosis, at the end of induction phasis (5th week) and in the end of the protocol (20th week).

Results

Results showed that dogs with lymphoma had higher percentages of Tregs (17.08±1.62) than healthy dogs (5.67±1.62) ($p<0.0001$). Additionally, in dogs with lymphoma Tregs decreased at the end of treatment either in G2 (8.648±1.736) and G3 (5.903±1.126) ($p<0.0001$). Between G2 (20.66±2.487) and G3 (12.79±0.7673) groups there were no significant diferences in Tregs, the only difference occurs at the time of diagnosis ($p<0.0114$). There was no statistical difference in survival curves between groups (p=0,4201).

Conclusion

We concluded that dogs with lymphoma had an increase in Tregs and during CHOP chemotherapy protocol, with prednisone or firocoxib, those cells had a significant decrease.

L-asparaginase activity and plasma amino acid profile in healthy cats after a single injection of PEG-L-asparaginase or native E. Coli L-asparaginase: a pilot study with potential therapeutic consequences

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Introduction

In people, polyethylene-Glycol-L-asparaginase (PEG-ASNase) has reduced immunogenicity and longer half-life compared to native E. Coli L-asparaginase (ASNase). In patients with ALL, serum target trough level is >100 IU/L. PEG-ASNase pharmacokinetics in cats are not established. The objective of the study was to determine plasma L-asparaginase activity (PLAA) and relevant amino acid concentrations after a single intramuscular PEG-ASNase or ASNase injection in healthy, L-asparaginase-naïve cats.

Material and Methods

Three cats received 40 IU/kg PEG-ASNase, one cat 400 IU/kg ASNase. At D0 (baseline), and during the 31 days post-injection, plasma amino acids, including asparagine, aspartate, glutamine and glutamate, were determined, using hydrophilic interaction liquid chromatography coupled with tandem mass-spectrometry. PLAA was determined, using a microplate reader-based method described by Lanvers et al. (PMID:12381370).

Results

Following PEG-ASNase, PLAA, with inter-individual level differences, was detectable between D1-27. The mean($\bar{x}$)-maximum activity was 798 (366-1070) IU/L between D4-7, lowest 28 IU/L. The $\bar{x}$-asparagine baseline concentration was 78 µmol/L, and complete depletion occurred as long as PLAA was measurable. Aspartate and glutamate $\bar{x}$-baseline concentrations increased from 10 and 43 µmol/L to $\bar{x}$-maximum of 40 (D14) and 437 (D7) µmol/L, respectively, and normalized when PLAA was non-measurable. Glutamine $\bar{x}$-baseline concentration (737 µmol/L) remained similar post-injection. In contrast, following ASNase, PLAA was maximal (1174 IU/L) after 12 hours, and declined to 20 IU/L at D9. During this period asparagine was not detectable.

Conclusion

PEG-ASNase showed considerably longer PLAA compared to native ASNase. Measurable PLAA, even <100 IU/L, led to complete asparagine depletion. This observation supports further exploratory use of PEG-ASNase in cats with lymphoma.

Canine Histiocytic Sarcoma: toward diagnostic and therapeutic options.

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Introduction

Histiocytic sarcoma (HS) is a rare and aggressive cancer in the dog, but a few breeds are highly affected notably Bernese Mountain Dogs, Rottweilers and Retrievers. Since there is no available efficient treatment, a better understanding of the genetic mechanisms leading to HS is needed to improve treatment of affected dogs. Finding somatic alterations in HS represents a powerful way to develop diagnostic and therapeutic opportunities to fight against this devastating cancer.

Material and Methods

Whole transcriptome sequencing on four canine HS samples was used.

Results

We identified 214 somatic variants. Among those, we confirmed the alteration of the MAPK pathway in 55 of 100 HS cases with the findings of recurrent and exclusive somatic mutations in two genes. One of it is a major oncogene harboring mutations in 50% of HS cases, at the same hotspots than those identified in human hematopoietic cancers. HS cases present specifically somatic mutations of this oncogene, independently of its clinical presentations or the breeds. Moreover, using plasma samples, we also showed that these mutations can be detected in cell-free circulating DNA of affected dogs with thus a diagnostic value. Furthermore, we demonstrated that HS cell lines harboring these mutations, established in our lab, showed (i) an over-activation of the MAPK pathway and (ii) a significant sensitivity to human available drug targeting this pathway.

Conclusion

All together, these genetic analyses establish a landscape of driver mutations in HS and open the field for new diagnosis methods and treatment of HS in dogs and hopefully in the corresponding human cancer.

Combination of etoposide and piroxicam: A potential treatment for canine osteosarcoma

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Introduction

Canine osteosarcoma (cOSA) is a highly malignant bone tumor. Despite of advances in various cancer therapies, its prognosis remains poor with 80% of dogs succumbed to metastases. Etoposide is an effective chemotherapeutic drug for various human cancers; however, its use to treat cOSA is yet to be explored. Non-steroidal anti-inflammatory drugs have been shown to enhance cytotoxicity of chemotherapeutic drugs in various tumors. Thus, the purpose of this study was to investigate the effects of etoposide and combination therapy with piroxicam on cOSA cells, and the mechanisms involved.

Material and Methods

Three cOSA cell lines were exposed to etoposide and/or piroxicam at various concentrations. Effects of treatment on cell viability and colony formation were assessed. Cell cycle analysis and apoptosis assay were performed by flow cytometry, and cell cycle checkpoint and apoptosis elements were evaluated by western blot analysis.

Results

Etoposide alone caused significant inhibition of cell proliferation in all cells, and combination with piroxicam further enhanced the effect in a dose dependent manner. Number of colonies reduced 90–99% when exposed to etoposide. In flow cytometric analysis, etoposide treatment increased the number of G2/M, sub-G1, and early apoptotic cells ($p<0.05$). These effects were more prominent when piroxicam was added. Combination therapy upregulated cyclin B, phosphorylated-cdc2 and cleaved-PARP expression, and downregulated survivin expression.

Conclusion

Piroxicam enhanced the anti-tumor effects of etoposide by promoting G2/M arrest followed by apoptosis. These findings suggest combination of etoposide and piroxicam might be a potential therapy for cOSA patients. Further studies are needed to evaluate its potential for clinical applications.

Investigation of sodium dichloroacetate for the treatment of canine osteosarcoma: in vitro preclinical assays.

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Introduction

This investigation aims to investigate the DCA (sodium dichloroacetate) as a possible new cancer therapy for canine osteosarcoma tumors performing in vitro preclinical assays. The DCA can initiate the remodelling of cell metabolism, opening transient pores and increasing the levels of reactive species of oxygen pro- apoptotic. High levels of reactive species of oxygen can inhibited the tumor growth and result in apoptosis.

Material and Methods

In this research we used an osteosarcoma primary cell line called CL3. We did a growth curve for CL3, by the method of crystal violet, to determine a better concentration of cells that we need to use in the experiment with DCA. Next, we treat this cells whit DCA follow the doses 50mM, 20mM, 10mM, 5mM, 1mM, 0,5mM for 72 hours by the method of crystal violet, we read the optical density by spectrophotometer (570nm) and analyze the results of the cellular viability in 24 h, 48h and 72 h.

Results

In the cell line CL3, we found a statistically significant reduction in cell viability at the concentrations of 20mM and 50mM in 48 hours and 72 hours. The optical density is smaller in cells with treatment by DCA than untreated cells.

Conclusion

As we know the treatment to osteosarcoma is very limited, so this research will be able to help increase the prognosis life of our patients. The next step is establishing a longer treatment in vitro to see if smaller doses of DCA may be effective against these cancerous cells.

The effect of Dichloroacetate in canine cancer cell lines

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Introduction

The Warburg effect describes the ability of cancer cells to produce energy via aerobic glycolysis instead of oxidative phosphorylation of pyruvate. This failure in mitochondrial metabolism inhibits apoptosis allowing proliferation of cancer cells. Dichloroacetate (DCA) was successfully used in human cancer cell lines to reactivate oxidative phosphorylation. Aim of this study is to characterize the effect of DCA on canine cancer cells.

Material and Methods

Eight canine cancer cell lines derived from prostate adenocarcinomas, transitional cell carcinomas and mammary carcinomas were exposed to 10mM DCA for 48 hours. Survivin and PDH protein expression were analyzed using Luminex bead assays. Further culture media supernatant was used for lactate measurement. The metabolic activity was analyzed with a tetrazolium compound. Statistical analysis was performed with SAS.

Results

Dichloroacetate significantly decreased cell number of all cell lines except one prostate adenocarcinoma and had a significant lactate lowering effect in 4/8 cell lines. Three of eight investigated cell lines showed a significantly decreased metabolic activity. Further DCA decreased survivin production in 3/8 cell lines and the inactive form of PDH in all cell lines.

Conclusion

The switch from aerobic glycolysis to oxidative phosphorylation is shown in decreased inactive PDH and lower lactate levels. DCA decreased survivin and results in lower metabolic activities. These results show that these cell lines are well suited for determining the effect of DCA on canine cancer cells. For an evaluation of the anticancer therapeutic potential of DCA more in vitro and especially in vivo experiments are necessary.

Autologous vaccine for dog osteosarcomas

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Introduction

Cancer cells generally synthesize heat shock proteins. These molecules are chaperone proteins and thus are associated with almost all the normal and numerous abnormal peptides synthesized by the cancer cells. They also participate in the cross-priming of CD8 cells against the cancer cells secreting abnormal peptides and proteins. The aim of this study was to check whether a method of vaccination based on the injection of autologous heat shock proteins could have a clinical effect on naturally-occurring osteosarcomas in veterinary medicine.

Material and Methods

Twelve dogs consulting for an osteosarcoma were enrolled in the study. Three dogs were amputated at the request of their owners. The dogs were staged and monitored up to their death. Both OS and PFS were noted and compared to results published in the literature. A test of APC stimulation was developed to check that the loaded powders could stimulate these cells.

Results

A group of short survival (n=3, < 150 days) and a long survival group (n=7, > 150 days) could be differentiated. In this late group, it was shown that the overall survival rate of the dogs, whether amputated or not, was much higher than the median OS of untreated dogs reported in the literature. The OS of the amputated dogs was significantly higher (median 531 days) than non- amputated (median 260 days).

Conclusion

This kind of immunotherapy allows obtaining long survival of dogs suffering from osteosarcomas and this study could be a model for immunotherapy of human osteosarcoma.

CDH1 hypermethylation is responsible to E-cadherin plasticity and down-regulation in metastatic process of canine prostate

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Introduction

Canine prostate cancer (PC) is a very aggressive disease with high metastatic rate and poor prognosis for the patients. Previously, our research group had shown an evidence of epithelial mesenchymal transition (EMT) in canine PC with a very dynamic E-cadherin expression. We demonstrated E-cadherin loss in primary tumors associated with re-expression in their respective metastasis. This finding suggests an epigenetic regulation of CDH1 gene. Due to importance of metastasis in canine PC this research aimed to evaluate the protein and gene expression and the DNA methylation pattern of CDH1 gene in canine PC.

Material and Methods

We selected 37 normal samples (10 to immunohistochemistry, 10 to gene expression, seven to western blot and 10 to pyrosequencing) and 42 PC (10 immunohistochemistry, 11 to gene expression, seven to western blot and 14 to pyrosequencing). We performed the gene expression by qRT-PCR, the protein expression by western blot (WB) and immunohistochemistry (IHC) and the methylation analysis by Pyrosequencing. The statistical analysis was performed using a computational program and we compared normal with PC samples.

Results

We found low E-cadherin protein expression (p=0.005) by WB and low CDH1 transcript levels (p=0.0001) in PC compared to normal prostate. Normal samples showed a specific membranous E-cadherin staining and it was possible to note the loss of protein expression in PC (p=0.003). We found CDH1 hypermethylation in all canine PC (14/14) compared with normal samples (p=0.00001).

Conclusion

Our results suggest a dynamic expression of E-cadherin in PC with CDH1 hypermethylation responsible to E-cadherin silencing and re-expression in EMT process.

Characterization of the mediated effects of two arylindolylmaleimides on canine and human prostate carcinoma cell lines

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Introduction

Presently available treatment options curing canine prostate cancer are restricted and chemotherapeutic approaches showed limited success. Beside humans, dogs are the only mammalians that develop spontaneous prostate cancer. Therefore dogs become a model organism for human research. For humans, existing agents are effective against early stages but survival after development of androgen independency remains very short. Consequently, evaluation of new treatment strategies is essential for both species. Arylindolylmaleimides, synthetic molecules, showed promising results as new chemotherapeutics in lab animals. The aim of the study was to investigate the impact of two new arylindolylmaleimide derivatives on canine and human prostate carcinoma cell lines.

Material and Methods

Proliferation ELISA BrdU-Assay, cell count analyses, analyses of apoptosis and necrosis as well as Life Cell Imaging for verification was performed with increasing incubation periods and various concentrations of two derivatives (A, B) on one canine and two human prostate cancer cell lines.

Results

Both of the arylindolylmaleimides have an effect on the canine or human cell lines. Derivative A had no effect on the proliferation of the canine cells, but a dose independent effect on human cells. The impact of derivative B increased with higher concentrations and incubation periods. Cell count analyses showed that in all tested cell lines the cell count decreased after treatment with this derivative.

Conclusion

For one of the two derivatives, the results showed an increasing amount of non-vital cells for all three tested cell lines with increasing arylindolylmaleimide concentrations. These are promising results leading to further studies regarding the chemotherapeutic potential of derivative B.

Serum levels of urokinase-type plasminogen activator in canine patients with cancer

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Introduction

Urokinase plasminogen activator (uPA) system contributes to the progression of certain cancers and is associated with human patient outcomes, although in veterinary oncology it is scarcely studied. The aim of this study was to describe uPA serum concentrations in healthy and cancerous dogs to investigate its potential value as a tumour biomarker.

Material and Methods

In this prospective case-control study, serum concentrations of uPA were measured using a canine-specific ELISA assay in 21 healthy dogs and in 20 dogs with spontaneous tumours, attended in two veterinary hospitals (FMV-ULHT and UP-VET-ICBAS). The relationship between uPA levels and tumour characteristics –type, lymph node status and distant metastasis, was analysed with the statistical package SPSS 23 (P value was considered significant when less than 0.05).

Results

The mean serum values (ng/ml) of controls (0.29 ± 0.13) were not influenced by gender or age, and were not significantly different from cancer patients (0.33 ± 0.33). There were no significant differences between dogs with benign or malignant neoplasms either ($p=0.58$). However, there was an increase in uPA serum levels in dogs with metastatic tumours ($p<0.05$).

Conclusion

This study described for the first time the serum levels of uPA in healthy dogs and dogs with cancer. These preliminary results do not support uPA as a general tumour biomarker, probably due to the inclusion of tumours with different biological behaviors. Higher uPA levels in dogs with invasive tumours may reflect the role of the enzyme in tumour dissemination, suggesting that it could be a seric biomarker of tumour aggressiveness.

Calculation of body surface area using computed tomography-guided modeling in dogs

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Introduction

The dosage of cancer chemotherapeutic drugs given to dogs is calculated using an estimation of body surface area (BSA) which can correlate with physiological processes better than weight alone. The therapeutic margin between toxicity and efficacy is narrow for chemotherapy and dose must be accurate. Currently, BSA is calculated using K·(W2/3)/10000, where K is the shape constant for the species (10.1 for dogs) and W is weight in grams. Previous studies found increased chemotherapy toxicity in small dogs using BSA-based dosing. Conversely, large dogs may be under-dosed, leading to decreased efficacy. Shape and conformational differences including body composition among dogs are vast, and using one uniform shape constant may not be accurate. Additionally, K may vary with the size of the animal. The current formula was derived many years ago from only a few dogs. Computed tomography (CT) can be used to help accurately determine BSA. While CT is expensive, using it to calculate BSA for various sized dogs can lead to verification or modification of the current K constant used in BSA calculations.

Materials and methods

Full-body CT scans were performed on dogs of varying sizes, and BSA was determined using radiation therapy planning software then compared to the calculated BSA based on weight in kilograms.

Results

Preliminary results show discrepancies of 2-17% between CT-based and calculated BSA, which could result in significant over- or under-dosage of chemotherapy.

Conclusion

These results provide a basis for determining the best predictors of chemotherapy-induced toxicity prospectively.

Frameless stereotactic radiotherapy alone and combined with temozolomide in canine gliomas

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Introduction

The primary aim of this work was to evaluate volume modulated arc radiotherapy (VMAT RT) of canine gliomas. The secondary aim was to assess the efficacy and toxicity of the combination of radiotherapy with temozolomide.

Material and Methods

A prospective study was performed in dogs with imaging based or histologically confirmed gliomas. The cohort was divided into three arms: palliation, RT alone, RT + temozolomide (RT+TMZ). The RT schedule has ranged between 33 Gy/5 fx and 42 Gy/10 fx. Temozolomide was administered at the dose of 65 mg/m2/day during the treatment and then for 5 days monthly for 6 cycles. Serial clinical and MRI examinations were planned 2, 4, 6, 12, 18, 24 months after irradiation. Overall survival was estimated using the Kaplan Meier curves. Multivariate analysis to assess any prognostic factors was performed.

Results

30 dogs were palliated, 22 dogs were treated with RT and 20 with RT+TMZ. Median survival in palliation arm was 94 days. Median survival of RT arm (383 days) and RT+TMZ arm (420 days) were not significantly different (p=0.61). The grade of the tumor (GII-GIII p=0.10; GIII-GIV p=0.68) was not correlated with the survival. The relative volume of the tumor <5% (p=0.013) and the clinical presentation with no alteration of the mental status (p=0.032) were positively correlated with the survival.

Conclusion

VMAT RT is feasible and effective for canine brain gliomas. The combination with TMZ at the used dose don’t elicited any additional improvement in survival rate.

Tolerability of postoperative standardized definitive-intent conformal radiotherapy for dogs with anal sac apocrine gland adenocarcinoma: a pilot study.

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Introduction

Adjuvant definitive-intent radiation therapy (RT) is recommended in dogs following surgery for anal sac apocrine gland adenocarcinoma (ASAC). However, clinically significant late toxicities have been reported in up to 65% of dogs with perianal tumors following RT, particularly following prescribed fractions of 3 Gy or higher. The primary objective of this study was to evaluate tolerability of a standardized RT protocol in a pilot group of dogs.

Material and Methods

Dogs with ASAC were prospectively enrolled if clients elected RT following surgery. The RT protocol consisted of 20 fractions of 2.5 Gy to 50 Gy total dose prescribed to the planning target volume (PTV) over 26 days. Conformal RT was prescribed using 6-10 MV photons with dose constraints placed on the colon/rectum. Acute and late radiation toxicity was graded according to standardized scoring criteria. Routine evaluation was performed following completion of RT for toxicity scoring and restaging.

Results

Eight dogs were included with a median follow up of 454 days. All dogs at risk for toxicity had Grade 2 or 3 acute radiation toxicity and grade 1 late skin toxicity. Acute toxicities included dermatitis/mucositis, colitis and tenesmus, and all resolved within 4 weeks. No clinically significant late toxicities were reported. No dogs developed local recurrence.

Conclusion

Preliminary results indicated that conformal RT may be safely administered to the canine anus and pelvic canal. Further prospective evaluation is necessary to fully evaluate long-term tolerability and efficacy of this conformal definitive-intent RT protocol for dogs with ASAC.

Targeted fluorescence-guided cancer surgery: The use of Prosense 750, a cathepsine targeted probe and the SOLARIS® camera system.

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Introduction

Despite improvements in preoperative imaging techniques, such as MRI and CT, during surgery, the surgeon can only rely on his eyes and hands to determine tumor resection margins. Fluorescence-guided surgery (FGS) aims to improve real-time intraoperative determination of tumor resection margins. FGS relies on a clinically applicable imaging system in combination with a specific tumour-targeting contrast agent. Cathepsine activity is essential for initiation, proliferation, angiogenesis and invasion of tumor cells and is therefore highly expressed in the invasive border of tumors. This study demonstrates the feasibility of intra-operative imaging of tumors in dogs, using a cathepsin-specific targeting agent (Prosense®). Tumor-specific signal at the invasive tumor border was detected using the SOLARIS® intra-operative fluorescence camera system.

Material and Methods

Prosense 750 was administered intravenously to five dogs with mastocytomas or soft tissue sarcomas, 16-24 hours prior to surgery. Intraoperative fluorescence imaging was performed using the SOLARIS® camera system, followed by tumor resection and pathological analysis of the resection margins.

Results

A clear fluorescence signal of Prosense 750 was found in all tumors and could easily be detected by the SOLARIS® camera system, allowing for targeted FGS. Pathological analysis showed tumor border specific fluorescence that co-localized with Cathepsin immunohistochemistry.

Conclusion

This study shows the clinical feasibility of targeted FGS using the tumor-specific targeting agent Prosense in combination with the SOLARIS® camera system.

Multi-wavelength fluorescence-guided cancer surgery: first experiences with the SOLARIS® camera system.

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Introduction

Preoperative imaging techniques, such as MRI and CT, allow for accurate diagnosis, tumor staging and preoperative planning in veterinary patients with cancer. However, during surgery, the surgeon can only rely on his vision (eyes) and palpation (hands) to determine the tumor resection margins. Fluorescence-guided surgery aims to improve real-time intraoperative determination of tumor resection margins. We performed a feasibility study to evaluate the clinical use of the Solaris® camera system in combination with non-specific fluorescent tracers Bremachlorin and Indocyanine Green (ICG). Tumor imaging with these tracers is based on the enhanced permeability and retention (EPR) effect of tumor vasculature and lymphatic system.

Material and Methods

A combination of Bremachlorin (emission 680 nm) and ICG (emission 800 nm) was administered to ten dogs with mastocytomas or soft tissue sarcomas, 16-20 hours prior to surgery. Intraoperative multi-wavelength fluorescence imaging was performed, followed by tumor resection and pathological analysis of the resection margins.

Results

A clear fluorescence signal of both tracers was found in all tumors, demonstrating the feasibility of the technique. However, pathologic analysis showed that some fluorescence signal could also be found in the healthy surrounding tissues, as was expected with non-specific tracers that target the EPR effect.

Conclusion

This feasibility study is the first to demonstrate the use of the Solaris® camera system for multi-wavelength fluorescence-guided veterinary cancer surgery.

Bremachlorin photodynamic therapy in dogs with transitional cell carcinomas of the bladder and/or urethra.

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Introduction

Cancer of the urinary bladder and the urethra accounts for 2% of all canine tumors. No effective treatment is known as these tumors do not respond well to chemotherapy and complete surgical excision is often not possible. Photodynamic therapy (PDT) could prove an effective new treatment modality for tumors in this region. Bremachloin is a promising photosensitizer that is retained in tumor tissue due to the enhanced permeability and retention (EPR) effect. In a previous study we demonstrated the feasibility of this treatment modality and determined the optimal timing of the treatment after Bremachlorin administration and the optimal interval between treatments. With that information a prospective study was designed and the results are shown here.

Materials and methods

Dogs with cytologically or histologically confirmed transitional cell carcinoma of the urinary bladder and/or urethra were treated with PDT using Bremachlorin. The dogs received an intravenous injection of 60 mg/m2 Bremachlorin. After 6-8 hours, a laser fiber delivery system was passed retrograde through the urethra into the urinary bladder or was placed in de urethra. Response to the therapy was assessed with urinary bladder ultrasound. The quality of life was an important parameter in this study and was assessed by weekly questionnaires to be filled in by the owner.

Results

Toxicity was not seen after administering Bremachlorin. In all patients, an improvement in quality of life was seen. Two patients were euthanized before 40 days, three patients are still alive at 63, 112 and 358 days from the first Bremachlorin treatment.

Discussion

PDT using Bremachlorin proved to be a minimal demanding palliative treatment for bladder cancer patients, improving the quality of life.

Electrochemotherapy for the treatment of perianal adenocarcinoma in dogs: 16 cases

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Introduction

Adenocarcinoma representing 3% to 21% of all perianal tumors. Characterized by nodular formation, firm, rapidly growth, ulcerated, adehere to underlying tissues and recur following conservative surgery. The most frequente site of metastasis is the regional lymph nodes. Electrochemotherapy is an antitumor therapy that utilizes locally-delivered, short intense direct current electric pulse to the tumor nodule plus chemotherapy. The aim of the present study was to evaluate the electrochemotherapy treatment of perianal adenocarcinoma.

Material and Methods

Sixteen dogs with adenocarcinoma perianal were treated with electrochemoterapy. All dogs received intratumoral injection of bleomycin (1U/cm3), followed by application of electric pulses. Results and discussion.

Results

Electrochemotherapy resulted in 100% CR 4 weeks after de treatment. In one case two lectrochemotherapy sessions were performed. Complications and adverse effects were not observed. All animals were reavaliated after 90 days. Recurrence and metastasis were not observed in this period.

Conclusion

Surgery is considerated the treatment of choice for adenocarcinoma perianal but the surgical margins for completeness excision may result in increased morbidity. This study shows that electrochemotherapy is an effective and safety treatment of adenocarcinoma perianal in dogs. The advantages of this therapy its simplicity, short duration of treatment sessions, low chemotherapeutic doses and insignificant side-effects and decrease morbidity in compared to surgical excision.

In silico study of electrochemotherapy treatment of tumoral tissue near bone

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Introduction

Electrochemotherapy is effective local tumor treatment combining chemotherapy and electric field applied. The optimal electrochemotherapy effectiveness depends of extracellular drugs presence and local electric field covering all tumoral tissue. Standard operation procedure is for superficial tumors. The planning treatment is required because of anatomy and physiology differences between animals produces an interesting challenging to electrochemotherapy. The computational models enable the preparation of patient-specific treatment plans. The aim of this work is to investigate in silico the influence of bone on electrochemotherapy efficiency.

Material and Methods

The in silico model was based on microtomographic images from metaphyseal bone of rat proximal tibiae with a destructive expanded osteolytic lesion caused by tumor. The in silico electrochemotherapy treatment was performed with plate electrodes, 1000 V/cm, 8 pulses, 100 µs.

Results

Results show that bone affects the electric field distributions. The electric conductivity of bones is lower than other tissues. If the tumor is around the bone, bone attracts the electric field. However, the electric field distributions depend of bone geometry. When the bone integrity was compromised is essential multidirectional application of electric field around the tumor. Intense electric fields are located around the bone protuberances. The tumor inside bone cavity are not affected by electrochemotherapy with plane electrodes.

Conclusion

The in silico analysis in for planning the electrochemotherapy treatment is important to veterinary oncology. The bone produces distortion on electric field distribution. The clinical treatment with electrochemotherapy needs special attention when the tumor is near bones.

A pilot, uncontrolled study of postsurgical treatment with autologous dendritic cell-based immunologic therapy in 10 dogs with splenic hemangiosarcoma

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Introduction

This research shows the result of an autologous dendritic cell-based cancer treatment in 10 dogs suffering from splenic hemangiosarcoma. The production of autologous dendritic cells (DCs) and the ability to present autologous and tumor specific antigens to the immune system yielded promising clinical results.

Material and Methods

The dogs showed hemascus and were undergoing an emergency surgical laparotomy, removal of tumor particles and splenectomy. If the result of the following pathohistological confirmed a hemangiosarcoma, the immunologic treatment was immediately started. A fresh whole blood sample from the patient was processed by gradient centrifugation, followed by adherence steps to derive the patients' monocytes. These monocytes were cultivated with specific cytokines to derive autologous DCs. The culturing lasted for 7 days. The cells were then harvested unprimed, resuspended and injected intradermally. The basic protocol consists of three treatments every 4 weeks with newly cultivated DC's.

Results

10 dogs were treated using this protocol. The the median survival time was 611 days after treatment with DC therapy. The survival rate after 365 days was 71% after 730 days 57%. Patients should receive this therapy immediately post-surgery. Life-long retreatments in a 4 month- interval are recommended.

Conclusion

In this study, surgical excision followed by DC-based therapy yielded promising results in the treatment of canine splenic hemangiosarcoma with no or mild side effects. However, further results from controlled studies are required to investigate and confirm the efficacy of the DC-based vaccine therapy.

Canine oral malignant melanoma: therapeutic anti-cancer pool vaccine and surgical treatment

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Introduction

Oral malignant melanoma (OMM) is the most frequent oral cancer in dogs, with a great metastatic potential and resistance to chemotherapy. Dogs present spontaneous oral melanoma and could be experimental model for cancer immunotherapy. The aim of this study was to evaluate radical surgical treatment plus a pool vaccine in dogs with a diagnosis of oral malignant melanoma and correlate it to recurrence, metastasis, quality of life and survival time.

Material and Methods

Forty-three dogs, several breeds and mongrel, male and female, with ages raging from 5 to 16 years, presenting oral malignant melanoma were used for the study. Tumors were graded according to TNM classification. Dogs were examined, and treated for OMM at different private surgical services in Brazil. All dogs went through radical surgery and received pool vaccine (three doses or more).

Results

By TNM Classification there were four dogs stage II, 24 dogs stage III and 15 stage IV. After the treatment (surgery plus vaccine), five cases presented local recurrence and eight cases presented pulmonary metastasis (these four dogs were euthanized). The remaining 39 dogs have presented an average of survival time of 347 days until present date. In the first year more than 70% of dogs are alive with a good quality of life to the present moment.

Conclusion

In conclusion, this study shows a new vaccine for cancer therapy in dogs, which in the future may be used as a model for the treatment of malignant melanoma in men.

Evaluation of the efficacy of 5-azacytidine and SAHA used alone or in combination with doxorubicin in canine hemangiosarcoma cell lines

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Introduction

Hemangiosarcoma (HSA) is a locally aggressive and highly metastatic neoplasm diagnosed commonly in dogs. Although surgery and systemic therapy are typically recommended, the prognosis remains poor. Therefore, new treatment approaches are desperately needed. In human oncology, epigenetic modifiers are currently in clinical use for various tumor types with encouraging results. The aim of this study is to determine whether exposure of canine HSA cell lines to 5-azacytidine, a hypomethylating drug, or to SAHA, an agent that promotes acetylation, induces cytotoxicity and results in decreased cell viability when used alone or in combination with doxorubicin.

Material and Methods

Four canine HSA cell lines were evaluated: LISS-HSA, DD1, FROG and EMMA. Cells were treated with 5-azacytidine or SAHA used as single agents and in combination with doxorubicin. Dimethyl sulfoxide vehicle was used as control. Cell proliferation and viability were determined by the use of a tetrazolium salt assay.

Results

In all HSA cell lines, doses equal to or higher than 5 uM of 5-Azacytidine and SAHA inhibited cell growth when used as single agents and in association with doxorubicin. The canine HSA cell line FROG was the most sensitive cell line to the anti-neoplastic agents studied, while EMMA was the most resistant one.

Conclusion

Micromolar concentrations of epigenetic agents induced citotoxicity in canine HSA cell lines. These results support futures studies investigating epigenetic aberrations as a potential therapeutic target in canine HSA.

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A second transmissible cancer in Tasmanian devils

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Introduction

Material and Methods

Histology, cytogenetic profiling and genetic analysis of DFT2 were performed and compared with host tissue and DFT1. Genetic analysis involved polymorphic microsatellite loci, structural variants and MHC loci.

Results

DFT2 tumors are histologically distinct from DFT1 tumours. DFT2 bears no detectable cytogenetic similarity to DFT1 and carries a Y chromosome, which contrasts with the female origin of DFT1. DFT2 shows different alleles to both its hosts and DFT1 at microsatellite, structural variant, and major histocompatibility complex (MHC) loci, confirming it as a second cancer that can be transmitted between devils as an allogeneic, MHC-discordant graft.

Conclusion

These findings indicate that Tasmanian devils have spawned at least two distinct transmissible cancer lineages. The discovery of DFT2 raises the possibility that this species is prone to the emergence of transmissible cancers. More generally, our findings highlight the potential for cancer cells to depart from their hosts and become dangerous transmissible pathogens.

Canine osteosarcoma cell line tumor growth on chick embryo chorioallantoic membrane (in vivo studies)

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Introduction

The aim of the research was to create a canine osteosarcoma preclinical in vivo model for drug testing. Latest reports show the ability of selected human osteosarcoma cell lines to form tumors on the CAM. In veterinary medicine only the growth of feline fibrosarcoma cell lines on CAM has been described.

Material and Methods

A commercial canine osteosarcoma cell line D-17 (ATCC, USA) was used. 120 of hatching eggs (Ross 308) were incubated (37°C, 5% CO2, 55% humidity). In the 3rd day of incubation the eggshells were pierced and turned 180°. On the 6th day of incubation the 'windows' were cut out in an eggshells and osteosarcoma cells (5 x 106 cells in 25 µl of medium) were aseptically injected into the silicon rings, previously inserted on the CAM. On the 19th day tumors were collected and histopathologicaly confirmed (hematoxylin and eosin staining).

Results

Tumors were collected from 8 chick embryos. Survival of all chick embryos was 70%. Average tumor diameter was 3mm.

Conclusion

In veterinary medicine canine osteosarcoma tumor growth has not been described yet. We proved that the canine osteosarcoma cell line has the ability to form tumors on the CAM. Achieved results are the first step to create a research model of canine osteosarcoma and may be used to examine the efficiency of anticancer drugs.

Characterization of the TGFß-TAZ Signalling Axis and its role in Chemoresistance in Canine Osteosarcoma Cell Lines

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Introduction

Osteosarcoma (OSA) is the most common bone tumor in dogs, where it commonly metastasizes to the lungs. Metastatic canine OSA is resistant to chemotherapy and responsible for patient mortality. OSA metastasis and chemoresistance mechanisms are generally unknown. Transforming growth factor-β (TGFβ) is a highly conserved cytokine with roles in bone development, bone disorders, cancer metastasis and chemoresistance. TGFβ signalling is carried out through Smad transcription factors, which rely on TAZ to alter gene expression. TAZ is a highly conserved transcriptional coactivator and an integral part of the Hippo pathway controlling organ size, proliferation, differentiation, as well as bone mass and osteoblastogenesis. TAZ was shown to mediate self-renewal capacity and cancer metastasis. We hypothesized that the TGFβ-TAZ signalling axis mediates OSA progression and chemoresistance.

Material and Methods

We first established the functionality of TGFb signalling in a panel of canine OSA cell lines generated in house. Next, we treated the cells for 2, 24 and 48 hours with 0, 0.5 and 5 ng/mL TGFβ1, and determined the pattern of Smad2 activation and TAZ expression using immunoblotting. 24-hour treated cells were also examined for their colony-forming ability after doxorubicin treatment.

Results

All cells expressed TGFb receptors and showed a robust TGFβ1 response, displaying dose-dependent Smad activation that varied with time and cell line. 24-hour TGFβ1 treatment caused a dose-dependent TAZ increase in the majority of cell lines, and impacted their ability to form colonies after doxorubicin treatment.

Conclusion

Further validation of these findings, and of approaches to target this axis, may lead to improved canine OSA therapy.

Chlorambucil inhibits proliferation of canine mammary carcinoma and osteosarcoma cell lines

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Introduction

Chlorambucil has proven effectiveness in the treatment of chronic lymphocytic leukemia in dogs and is currently being used in protocols of metronomic chemotherapy for other neoplastic diseases namely mammary and bone tumours. However, in vitro studies that prove its effectiveness in canine mammary carcinoma and osteossarcoma cell lines are yet to be performed. Our objective is to investigate the anti-proliferative effects of chlorambucil in those canine cancer cell lines, in order to justify its use.

Material and Methods

Two established canine cancer cell lines were used: REM 134 (mammary carcinoma) and KTOSA (osteosarcoma). The cells were treated for 48-72h with chlorambucil at concentrations up to 1000uM and DMSO control correspondent to the highest concentration. Proliferation was measured by applying the resazurin sodium salt reduction method. IC50 values were determined using the IC50 toolkit freely available from www.ic50.tk.

Results

Our results show that chlorambucil reduces cell viability to 50% at a concentration of 396.0uM for REM 134 cells and 115.2 uM for KTOSA cells. For KTOSA cells a plateau is reached at the concentration of 200uM with a residual cell viability of 40%. Cell viability is significantly reduced at concentrations above 25uM for REM134 cells and above 200uM for KTOSA cells.

Conclusion

According to our results chlorambucil has the ability to reduce cell viability in both REM 134 mammary carcinoma cells and KTOSA osteosarcoma cells. Further studies are required to clarify its mechanism of action in the tumour cells and to determine if the chlorambucil anti-tumoural effect could be increased in combination with adjuvant drugs.

Combined effect of masitinib and mitoxantrone on the proliferation of variant cell line of canine mammary tumor

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Introduction

Despite advances in the field of cancer therapeutics, canine mammary cancer (CMC) remains a leading cause of death. Novel therapeutic modalities that improve survival with less toxicity are therefore needed. Overall response may be enhanced by treating a tumor with a combination of agents that have different mechanisms of action and toxicity profiles. Accordingly, we evaluated the interaction between masitinib, a tyrosine kinase inhibitor, and mitoxantrone, a conventional chemotherapeutic drug.

Material and Methods

Canine mammary tumor CMT-U27 and CMT-U309 cell lines were treated with increasing concentrations of single-agent masitinib and single-agent mitoxantrone for 24, 48, and 72 h with cell proliferation evaluated relative to vehicle-treated cells. The combination of masitinib plus mitoxantrone was assessed at the IC50 concentration of each drug and also at 50% and 25% IC50 concentrations, with cell proliferation measured after 72h incubation. Combination index (CI) values were used to characterize the interaction of masitinib with mitoxantrone.

Results

Mitoxantrone inhibited cell growth in a time- and dose-dependent manner. After 24, 48 and 72h incubation the mitoxantrone IC50 for CMT-U27 and CMT-U309 ranged respectively from 3.091-0.219µM and from 2.774-0.751µM. Conversely, masitinib showed weak inhibition of these cell lines, ranging respectively from 9.129-7.498µM and from 15.032-8.545µM. Synergistic effects in growth inhibition (CI from 0.549-0.444) on CMT-U27 and (CI from 0.672-0.830) on CMT-U309 were observed for combinations of 100%, 50%, and 25% IC50.

Conclusion

These results suggest that masitinib potentiates mitoxantrone and may represent a beneficial new therapeutic combination modality for treatment of CMC.

Depression evaluation: tail suspension and anhedonia tests in mice with Ehrlich Tumor

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Introduction

Depression is derived from neurochemical and neuroplastic changes in the brain, which seem arise from factors released in the immune, inflammatory and oncologic context. It was investigated the capacity of anhedonia test (AT) and tail suspension test (TST) to evaluate the depressive behavior in mice with Ehrlich tumor.

Material and Methods

Immobility was evaluated by TST in animals treated daily with fluoxetine 5mg/Kg or saline suspension. Other animals were evaluated to consumption of sucrose solution in AT. Mice were inoculated with Ehrlich's tumor suspension or with buffered saline suspension in dorsal subcutaneous. The test was performed before tumor inoculation and after neoplasia development. Then, mice were euthanized to analyze the neoplastic mass.

Results

In the TST there wasn't a significant difference between groups before the tumor/buffered saline inoculation. After the tumor development the group SALINE+TUMOR (130.2±31.0690s) presented more immobility than the SALINE+BUFFERED (60±51.4319s) and FLUOXETINE+TUMOR (63.6666±24.1453s). In animals subjected to AT, there wasn't differences between groups. There wasn't any significant difference in Ehrlich tumor volume in all groups.

Conclusion

The development of Ehrlich tumor determines depressive like behavior in mice measured in TST as noted previously in Forced Swimming test and was reverted by treatment with Fluoxetine 5mg/kg that significantly reduced the immobility. Maybe the AT test is not suitable to evaluate depressive behavior in mice bearing Ehrlich tumor. These results offer subsidies to understand tumorigenesis and depression relationship.

Down regulated genes on mammary cancer in female dogs in the PI3K-Akt pathway

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Introduction

Mammary cancer cells can exhibit different phenotypes. In this context, the evaluation of gene expression enables uncertain characteristics about molecular biology and the behavior of the tumor. The study's goal was to evaluate the gene expression in mammary cancer in female dogs.

Material and Methods

We selected 12 samples of mammary neoplasia and 4 healthy mammary samples for the assay. Total RNA from all specimens was extracted with TRI Reagent® and purified with Rneasy Mini Kit® according to manufacturer's recommendations. The RNA integrity was assessed by capillary electrophoresis with RNA nano chip kit® and the reading was performed on Agilent 2100 Bioanalyzer. The microarrays were made with the commercial kit Affymetrix® Canine Gene 1.0 ST Array, GeneChip® WT PLUS Reagent Kit, GeneChip® Hybridization Wash and Stain Kit® and scanned by Affymetrix GeneChip 3000 according to manufacturer's recommendations. The p-value was calculated to adjust t-statistic with the method "Benjamini and Hochberg" to control false discovery rate. Genes whose adjusted p-value was ≤0.05 were considered differentially expressed, but only those genes whose Fold Change log was ≤-1 or ≥ 1 were detailed.

Results

We have identified 13 down regulated genes involved in the PI3K-Akt pathway (PPP2R2B, PDPK1, TP53, MET, FZD8, IKBKB, JAK1, PPARG, IL8, IL2RA, LAMA4, CSF3R, PRKAA1) in the cancer group.

Conclusion

Many of these genes are involved in cell cycle regulation, proliferation and apoptosis, important phenomena that may be related to the evolution and progression of the disease. Understanding the pathways in cancer is important because it is possible to devise personalized treatment management.

Dual upa (urokinase) and MMP (metalloproteinase) -activated engineered anthrax toxin for treatment of canine oral melanoma cells (TLM-1, GMGD-2 AND GMGD-5).

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Introduction

Anthrax toxin is a three-part toxin secreted by Bacillus anthracis, consisting of protective antigen (PA, wild type), edema factor (EF) and lethal factor (LF or FP). PA binds to cell surface receptors called tumor endothelial marker 8 or capillary morphogenesis gene 2, where it is cleaved by furin-related proteases to form active heptameric channel complex PA63 to translocate EF and LF into the cytosol to cause cell death. Tumor-selective modified Bacillus anthrax toxins which can only be activated by uPA and MMP, proteinases that are overexpressed in many types of malignant tumors, were constructed to target treatment of cancers by Liu et al. The aim of this study is to evaluate the cytotoxicity of a dual uPA and MMP activated engineered anthrax toxin in canine oral melanoma cells.

Material and Methods

Three canine oral melanoma cell lines were incubated for 48h with toxins associations. The cell viability was measured using an MTT assay.

Results

PA in combination with FP killed all cell lines demonstrating the presence of functional anthrax toxin receptors. All canine oral melanoma cells were sensitive to modified toxin and FP indicating functional uPA and MMP expression. TLM-1 cells were not sensitive to PA or reengineered toxin combined to LF, showing that MEK activity is dispensable for these cells. GMGD-2 was sensitive and GMGD-5 cells was not sensitive to modified toxin associated to LF.

Conclusion

The requirement of both cell surface uPA and MMP activity, associated with MAPK kinase pathway for survival on tumors cells are important to action of this constructed toxin.

Effect of deracoxib and doxorubicin combination on canine mammary epithelial cells under in vitro condition

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Introduction

Material and Methods

The antiproliferative effect of deracoxib and doxorubicin combination was determined by MTT assay. Apoptosis was characterized by flow cytometry. Cell nitrite concentrations were measured via Griess reaction.

Results

We found that deracoxib at 50 and 100 µM decreased the cytotoxic action of doxorubicin at 0.9 µM in normal canine mammary epithelial cells, from 33.63 % to 13.4 and 25.82 %, respectively. Also our results showed that the reverse effect of deracoxib on the antiproliferative activity induced doxorubicin alone in the cells is associated with a marked decrease (3.04-3.57 fold) in apoptosis. In additional studies identifying the mechanism of the observed effect; deracoxib exhibited an activity to prevent doxorubicin-mediated overproduction of nitric oxide in the cells.

Conclusion

Our in vitro study results indicated that deracoxib (50 and 100 µM) can be beneficial to protect normal cells from toxic effect of doxorubicin in conjunction with apoptosis by the modulation of nitric oxide production. We have concluded that the addition of deracoxib to doxorubicin therapy may beneficial to minimize damage to normal tissues.

Effects of curcumin in combination with cyclophosphamide on canine mammary tumour cell lines

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Introduction

The present study was undertaken to evaluate the possibility that the combination of curcumin and cyclophosphamide could show synergistic antiproliferative effects towards CMT-U27 and CMT-U309 canine mammary tumour cells and to clarify its related mechanism.

Material and Methods

The anti-proliferative activitity was determined using the MTT and LDH assays. 50% inhibition of cell viability (IC50) and combination index values were calculated. Apoptosis and cell cycle analyses were performed by flow cytometry. Expressions of apoptosis related proteins Bax and Bcl-2 were determined by immunocytochemical stainings.

Results

Curcumin and cyclophosphamide induced a dose- and a time-dependent decrease in cell viability. The interaction between drugs was synergistic when IC50 and ½ IC50 concentrations of curcumin and cyclophosphamide were added concurrently to the cultures. This synergy was characterized by a significant increase in the percentage of early and late apoptotic cells. However, internucleosomal excision of DNA was not observed by DNA fragmentation assay. Cells treated with curcumin and cyclophosphamide were arrested at the G2/M and S phases of cell cycle, respectively. In combined treatments cells were arrested in both phases of the cell cycle. Curcumin induced apoptosis by the modulation of Bcl-2/Bax protein expression, as the expression of Bcl-2 was decreased and Bax was increased. This effect was more pronounced in combination treatments.

Conclusion

This finding provided a molecular basis for the development of naturally compounds as novel anticancer agents by lowering the dose of cytotoxic agent and lead to a more specific and less toxic therapy for mammary cancer in dogs.

Efficacy of Mycobacterium Cell Wall Fraction (MCWF) in the prevention of chemotherapy-induced neutropenia following concurrent administration

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Introduction

Chemotherapy-induced neutropenia in clinical oncology may have negative implications as it increases the risk of infection, interrupts chemotherapy protocols and requires treatment dose reduction. Currently, there is no veterinary product available for use in the prevention of chemotherapy-induced neutropenia. Here, we demonstrated the potential of Mycobacterium Cell Wall Fraction (MCWF), a non-specific immunomodulator derived from a non-pathogenic mycobacterium, to prevent chemotherapy-induced neutropenia in healthy dogs following concurrent administration.

Material and Methods

The study design included one experimental group with ten dogs. All dogs concurrently received 3 mg/m2 of vinblastine (VBL) and 200 µg/kg of MCWF via intravenous route (IV) on two occasions seven days apart. All animals were closely monitored for occurrence of any AE's, CBC and clinical biochemistry parameters were measured. The efficacy of MCWF in the prevention or reduction of neutropenia was determined by comparing the incidence, duration and severity of neutropenia during the two VBL/ MCWF treatment cycles within same group. In addition, the incidence, duration and severity of neutropenia was compared between the dogs receiving VBL only using historical data from our previous study.

Results

Our results revealed that concurrent administration of MCWF with VBL significantly reduced the incidence (10% vs 90%), duration (days: 1.9±0.52 vs 3.9±0.66) and severity (Grade 4 [1] vs [9]) of neutropenia compared to dogs that received VBL alone.

Conclusions

These findings could have significant importance from a clinical standpoint and could support the use of MCWF in conjunction with standard chemotherapy protocols. Additional studies in tumor-bearing dogs and in combination with chemotherapeutics are underway.

Establishment, characterization and ultrastructural analysis of primary culture of grades 1, 2 and 3 canine cutaneous mast cell tumors.

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Introduction

Mast cell tumor is the most common cutaneous neoplasm in dogs, accounting for about 7-21% of all skin tumors and 11-27% of all malignant tumors of this species. Our objective was to establish primary cultures of different grades of canine cutaneous mast cell tumors and cells from canine bone marrow that differentiate into normal mast cells under stimulus.

Material and Methods

We established primary cultures from fragments of canine mast cell tumors and aspirated bone marrow. Analysis of cell cycle and DNA ploidy was made by flow cytometry and proliferation by Carboxyfluorescein succinimidyl ester (CFSE). Ultrastructural evaluation of mast cells in culture was made by transmission electronic microscopy.

Results

We successfully established and characterized primary cultures of the three mast cell tumor grades. The cell cycle analysis showed that the tumor stem cells are predominantly diploid. The analysis revealed that the CFSE cells reach the proliferation peak in 24 hours. The evaluation of cellular ultrastructure showed mast cells in different stages of maturation.

Conclusion

This study allowed us to learn more about the biological behavior of neoplastic and normal mast cells in vitro and provided a rich material for future studies.

Expression of hedgehog signaling molecules in canine osteosarcoma

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Introduction

Osteosarcoma (OSA), the most common primary canine bone tumor, is associated with a poor outcome. Dysregulation of the evolutionary hedgehog (Hh) signaling pathway is linked with tumorigenesis in human OSA. Small molecule inhibitors targeting key Hh components have shown promise in vivo for OSA, making the pathway an excellent candidate for therapeutic intervention. The purpose of this study is to evaluate the frequency of expression of Hh pathway signaling molecules in canine OSA.

Material and Methods

Nineteen OSA patient tumor samples and 4 normal bone samples were analyzed. RNA was extracted with a Qiagen RNeasy Mini Kit. The NCBI Dog Genome Resource was used to identify the mRNA sequence of Hh pathway genes of interest. qPCR data was normalized to two reference genes, canine ribosomal protein S5 and ribosomal protein S19. All qPCR experiments were done at least in duplicate. Statistical comparisons were made with the Pfaffl method using the Relative Expression Software Tool.

Results

Thirteen genes related to the Hh pathway were assessed. Analysis revealed that patched 2 (PTCH2) and smoothened (SMO) had increased expression as compared to normal bone, 6.5 (p=0.036) and 5.9 fold (p=0.003), respe

Conclusion

Increased expression of PTCH2 and SMO, two critical components of the Hh pathway, in canine OSA samples suggests dysregulation of the Hh pathway may be involved in disease pathogenesis. SMO is the most readily inhibited portion of the pathway by way of small molecule therapeutics. Further investigation of the potential role of SMO inhibitors in treatment of canine OSA is warranted.

Growth inhibition of canine mammary cancer cell lines by the selective tyrosine kinase inhibitor, masitinib

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Introduction

Since the discovery of kinase activity dysregulation due to mutations is found in some tumours of dogs and cats, veterinary oncology research with a focus on targeted therapy using tyrosine kinase inhibitors (TKIs) has dramatically increased. Masitinib is a TKI that potently and selectively targets the c-kit cytokine receptor, the platelet-derived growth factor receptors α and β (PDGFR-α/β), and the Src family kinases, Fyn, Lck, and Lyn. It is particularly efficient in controlling the proliferation, differentiation and degranulation of mast cells. However, its possible anticancer effect in canine mammary tumors (CMTs) is unknown. In the present study, we have evaluated the in vitro biological activity of masitinib against CMT cell lines.

Material and Methods

The antiproliferative effect of masitinib on CMT-U27 and CMT-U309 cell lines was determined by MTT assay. Apoptosis was characterized by flow cytometry with PI and Annexin-V staining.

Results

Masitinib has an inhibitory effect on the proliferation of CMT-U27 and CMT-U309 cell lines in a concentration and time-dependent manner with IC50 values at 72 hr of approximately 7.498 µM for CMT-U27 and 8.545 µM for CMT-U309. Maximal apoptotic activity was observed with 8 µM of masitinib in both cell lines.

Conclusion

In this study, TKI masitinib caused cell death via induction of apoptosis in CMT-U27 and CMT-U309 cells in vitro suggesting a potential as a therapeutic tool in the clinical setting of mammary cancer treatment in dogs.

Higher Incidence of Lung Carcinomas in Wistar Rats Induced by DMBA

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Introduction

In the early stages of cancers studies, preclinical elaboreted models are used in rodents for inducing carcinogenic tumors. This study aimed to verify the influence of the carcinogenic 7,12-dimetilbenzetraceno compound model (DMBA) on cancer incidence in different organs in Wistar rats, determining the minimum concentration of the compound which is able to induce tumors.

Material and Methods

DMBA was administred, by gavage, to 45 female Wistar rats, 8 weeks of life, using hebdomadary doses of 3 (G1), 6 (G2) or 9 mg (G3) per animal and others 15 animals were used as control group (GC). Animals were weighed and monitored weekly. All rats were euthanized at the end of 34 weeks. At necropsy, representative fragments of tumors were processed and classified (IARC). Immunohistochemistry were performed (ER, PR, C-erb-B2, p53 and Ki-67 biomarkers). Group data difference was evaluated by a modified t-test (p=5%). ANOVA and F-test were further employed to determine the significance of variations within and between groups.

Results

None statistical diferences occured for weight gain and cancer survival time (p=0.935) between groups. In the groups that received DMBA, 41% of the rats developed some neoplasia. G2 presented significantly higher suceptibility to lung cancer (p=0.003), most of them Carcinoma in situ. In addition, incidence of mammary and tracheal neoplasm were observed in all groups, although not statistically significant. GC had none physical alteration. The immunohistochemistry does not revealed statistical difference between groups.

Conclusion

The Wistar rats showed predisposition to lung neoplasms in those conditions, which strengthens the evidence that DMBA was not a specific carcinogen.

Immunocytochemical study of 5-methylcytosine of peripheral leukocyte DNA and canine lymphoma: correlation with proliferation activity

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Introduction

Lymphomas are very prevalent in dogs and reportedly may be triggered by environmental factors. Decrease global DNA methylation level is generally observed in human cancers. Alterations in global DNA methylation may be induced by environmental factors, contributes to genome instability and can be analyzed by immunocytochemistry. The aim of this work was to examine the peripheral leukocyte global DNA methylation of dogs with lymphomas and to correlate them with lymphoma cells proliferation activity.

Material and Methods

Peripheral venous blood samples from nine dogs bearing multicentric lymphomas (2 small-cells, 2 intermediate-cells and 5 large-cells lymphomas) were centrifuged. The buffy coat was separated for cell block production. After immunocytochemistry with anti-5-methylcytosine, leukocytes were counted and classified by their immunopositivity (3=strong, 2=moderate, 1=discrete). Lymph node samples from the same dogs were collected by fine needle aspiration followed by anti-Ki67 immunocytochemistry for proliferation activity assessment. Proliferation index (PI) was achieved by counting positive nuclei.

Results

A high proliferation activity (50-70% positive cells) was found in 3 cases, moderate (20-50% positive cells) in 4 cases and low (<20% positive cells) in 2 cases. 5-methylcytosine positivity in peripheral leukocytes had an average of 210,9 + 12,6 and Ki-67 in lymph nodes had an average of 38 + 22,3. Both results had normal distribution with a correlation coefficient of -0,69 (p=0,0394).

Conclusion

Since peripheral leukocytes methylation and lymphoma PI showed negative correlation, we propose that peripheral leukocytes hypomethylation may indicate more aggressive lymphomas. Further survival analysis may point leukocyte global methylation status as prognosis marker in canine lymphoma.

Immunohistochemical expression of markers of cancer stem cells, pluripotency and cell proliferation in canine mammary tumors

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Introduction

Cancer stem cells (CSCs) are considered the cell subpopulation responsible for mammary cancer initiation, growth, and relapse and are related to malignancy and resistance to chemotherapy. The aim of this study was to detect the presence and distribution of CSC and cell proliferation markers in canine mammary tumors.

Material and Methods

In this retrospective study we evaluated by immunohistochemistry, expression and tissue distribution of proteins: ALDH-1, Oct4, Ki-67, Factor VIII (FVIII) and TK-1 in 34 post-surgical breast tumor samples without discriminating canine histological diagnosis. Tissue immunoreactivity were graduated from negative to (+++) and using the 40X objective 800 cells were counted, expressing as a percentage of positive immunoreactivity.

Results

This study showed varying percentage and distribution of cell immunostaining pattern: ALDH1 (0-14%), Oct4 (1-10%), FVIII (0-40%), Ki-67 (5-23%) TK-1 (5-12%); ALDH1+ cells were located in the alveolar epithelium of the parenchyma (+++) and in the light of the stromal vessels (+), the Oct4+ cells in the alveolar parenchyma (+++), vessels and stromal tissue (++); the FVIII+ cells in the tumor stroma (+++) vessel lumen (+++) and endothelium (+++), the Ki-67+ in the parenchyma (+++) and tumor stroma (+), the TK-1+ cells in the tumor parenchyma (+++).

Conclusion

The presence of cancer stem cells ALDH1+, Oct4+, Ki-67+, FVIII and TK-1+ in the tumor provides information on its potential aggressiveness. Our results show that some canine mammary tumors have cells with immunophenotype CSC and high cell proliferation with different tissue distribution within the tumor, it is important to study the tumor niche.

In-vitro characterization of turmeric and rosemary combination treatment on canine cancer cells

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Introduction

Adjunctive use of nutraceuticals in canine neoplasia is rarely studied. We have previously identified two natural ingredients, turmeric extract (TE) and rosemary extract rich in carnosic acid (RE), which work synergistically to reduce neoplastic cell growth. The purpose of this in-vitro study was to examine mechanisms of action of this cocktail.

Material and Methods

Three canine neoplastic cell lines representing a variety of tumors were used: C2 mastocytoma, CMT-12 mammary gland carcinoma, and D17 osteosarcoma. Cells were treated with 6.3 µg/mL of extract individually, a combination (3.1 µg/mL of each extract), or vehicle control. Apoptosis was investigated via flow cytometry using Annexin V and by a commercially available caspase-3/7 cleavage assay. Cell cycle changes using propidium iodide staining, generation of reactive oxygen species using Dihydrorhodamine123, and cellular accumulation of curcumin were analyzed by flow cytometry. One-way ANOVA followed by Dunnett's post-hoc analyses were performed to assess treatment effect.

Results

The combination treatment induced caspase-3/7 cleavage and apoptosis in all cell lines, beyond the effects of TE alone, after 48 hours' incubation. Both extracts had a significant antioxidant effect after 12 hours ($p < 0.05$). CMT-12 cells were the most susceptible to treatment (40% Annexin V positive). The presence of RE significantly increased the cellular accumulation of TE as indicated by an increase in fluorescence.

Conclusion

TE and RE interact synergistically to induce apoptosis in-vitro. This could be due to RE increasing cellular accumulation of TE. In-vivo studies are warranted to determine the pharmacokinetics and efficacy of this dual treatment.

Melanoma displays evolutionarily conserved resistance to modulation of phagocytic signals

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Introduction

Melanoma is notoriously resistant to apoptosis and to other cytolytic mechanisms used by immune cells; however, the effects of modulating pro- and anti-phagocytic pathways in this disease are unknown. We hypothesized that melanoma cells would display unique resistance to phagocytosis. Furthermore, we hypothesized that this resistance would be mitigated by blockade of the "don't eat me" signal CD47, which in turn would promote anti-tumor T cell responses.

Material and Methods

To test these hypotheses, we examined the phagocytosis of human, mouse, and dog melanoma cells in vitro.

Results

We observed that the susceptibility of lymphoma and melanoma cells to phagocytosis was dramatically different: whereas lymphoma cells were readily phagocytosed by macrophages, melanoma cells were resistant to phagocytosis. This pattern of resistance was conserved among all three species tested. Significant increases in lymphoma cell phagocytosis were observed upon CD47 blockade while we noted only small increases in melanoma cell phagocytosis. Next, we tested whether we could overcome melanoma cell resistance to phagocytosis by increasing expression of "eat me" signals. We showed that treatment with doxorubicin increased phosphatidylserine and calreticulin expression on melanoma cells, and we noted a small increase phagocytosis when we combined chemotherapy and CD47 blockade. However, this did not consistently promote enhanced activation of antigen-specific T cells in vitro or in vivo and did not reduce tumor burden.

Conclusion

Overall, we conclude that melanoma cells display an evolutionarily conserved resistance to phagocytosis, which cannot be fully mitigated by CD47 blockade or by chemotherapeutic upregulation of "eat me" signals.

NKX3.1 is a new tumor suppressor gene candidate to canine prostate cancer progression and development

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Introduction

Canine prostate cancer (PC) represents 13% of all prostatic disorders and has an aggressive behavior. Canine PC characterization is the first step to demonstrate dogs as a model to human PC study and assess their role as useful model for the design of therapeutic targets. NKX3.1 is a tumor suppressor gene in human PC and associated with high-grade tumor. In veterinary medicine, there is one study evaluating NKX3.1 expression. This research aimed to evaluate the protein, gene expression and the DNA methylation pattern of NKX3.1 gene in canine PC.

Material and Methods

We selected 37 normal samples (10 to immunohistochemistry, 10 to gene expression, seven to western blot analysis, 10 to pyrosequencing) and 42 PC samples (10 immunohistochemistry, 11 to gene expression, seven to western blot and 14 to pyrosequencing). We performed gene expression by qRT-PCR, protein expression by western blot (WB) and immunohistochemistry (IHC) and methylation analysis by Pyrosequencing. The statistical analysis was performed using a computational program.

Results

We found loss of NKX3.1 protein ($p=0.005$) by WB and low transcript levels ($p=0.0001$) in PC samples. Normal samples showed cytoplasmic staining and it was possible to note the loss of protein expression in PC ($p=0.003$) by IHQ. We found NKX3.1 hypermethylation in seven canine PC (7/14) and, when compared with normal samples, there were higher CpG island hipermethylation ($p=0.001$).

Conclusion

Our results suggest loss of NKX3.1 protein and gene expression in canine prostate cancer and the methylation results suggests a epigenetic silencing of this gene in canine prostate cancer by methylation.

Owner´s expectations of adverse effects of chemotherapy in small animals

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Introduction

Although chemotherapy increases survival in small animals, we believe that the preconceptions about adverse effects influences the owner's decision making process and their adherence to chemotherapy program. The aim of this study was to survey the knowledge of small animal's owners about adverse effects of chemotherapy.

Material and Methods

The questionnaires were presented to 320 owners of small animals referred to a veterinary hospital.

Results

Analysis of these data suggests that most people of any sex and age knows the fact that adverse effects of chemotherapy are treatable. However, this information are unknown mainly by the domestic workers as compared to other occupations ($p<0.01$). The most adverse effects mentioned by owners were emesis (71%), alopecia (54%), hyporexia (50%), weakness (45%), diarrhea (44%), pain (35%), fadigue (31%), anemia (25%) and death (21%). On the other hand, few people know important effects such as risk of infections (14%), nephrotoxicity (7%), cadiotoxicity (3%) and anaphylaxis (2%). When asked about the rate of adverse effects in small animals, 37% owners thought that most animals present side effects whereas 30% believe that all animals experience them. Surprisingly, 23% people surveyed said they would not allow chemotherapy to their pets because it does more harm than benefit. We conclude that the owners of small animals mainly domestic workers, are unaware of important aspects about adverse effects caused by chemotherapy.

Conclusion

Thus, the guidance of the veterinary is essential to achieve the adherence to chemotherapy. We encourage new surveys involving owners and veterinarians concurrently.

Prognostic significance of c-CBL, phosphorylated VEGFR2, Neuropilin-1 and Beclin-1 immunoexpression in canine Subcutaneous Mast Cell Tumors

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Introduction

Subcutaneous mast cell tumor (MCT) is less aggressive than cutaneous MCT but there are few studies investigating prognostic factors for these tumors. This research therefore aims to evaluate the prognostic significance of markers associated with signaling and autophagy in canine subcutaneous MCT.

Material and Methods

Twenty subcutaneous MCT and the respective patient outcome were available for analysis. Western blotting was performed using canine MCT cell line lysates to validate the specificity and cross reactivity of the primary antibodies. Immunohistochemical (IHC) reactions were performed for Beclin-1, c-Cbl, Neuropilin-1 and pVEGFR2 protein markers and analyzed by ImageJ. Log-rank test was used to analyze overall survival times.

Results

c-Cbl IHC showed focal cytoplasmic expression in MCT from dogs with higher survival time and diffuse cytoplasmic staining in dogs with MCT showing lower survival time. Patients with overall low c-Cbl immunoexpression showed higher disease free interval (p=0.0067) and overall survival time (p=0.0077) when compared with patients showing higher expression. Beclin-1 showed diffuse cytoplasmic expression in neoplastic mast cells, with higher expression of Belcin-1 associated with poor survival. Beclin-1 expression was significantly associated with disease free interval (p=0.0244) and overall survival. Neuropilin-1 showed membranous and cytoplasmic immunoexpression in neoplastic cells and some samples also showed nuclear neuropilin-1 immunolocalization. pVEGFR2 IHC showed diffuse cytoplasmic expression in neoplastic cells. There were no statistically significant differences in survival associated with neuropilin-1 or pVEGFR2, however, patients with higher pVEGFR2 expression showed 5-fold lower overall survival time.

Conclusion

Neuropilin-1, pVEGFR2, Beclin-1 and c-Cbl seem to be promising prognostic markers for subcutaneous MCT

Prognostic value of activated Smad2 and TAZ levels in appendicular canine osteosarcoma: a pilot immunohistochemistry study in a tissue microarray

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Introduction

Canine osteosarcoma (OSA) is a commonly diagnosed and aggressive bone tumour that lacks reliable molecular prognostic markers. Transforming growth factor beta (TGFβ) and transcriptional co-activator with a PDZ-binding motif (TAZ) are cooperative mediators of bone development and cancer progression. Interestingly, their prognostic value in canine OSA is rather unexplored.

Material and Methods

This pilot study employed a tissue microarray comprised of 31 appendicular primary canine OSA tumour samples, to examine the levels of phosphorylated Smad2 (pSmad2), a key effector of classical TGFβ signalling, and TAZ by immunohistochemistry. Markers were scored using the Allred method. Fisher's exact test was used to seek associations between pSmad2 or TAZ levels, and tumour grade or alkaline phosphatase (ALP) status. Associations between marker levels and metastasis or overall survival (OS) were evaluated using Kaplan-Meier plots and the log-rank test.

Results

Neither pSmad2 nor TAZ levels were significantly associated with tumour grade, ALP status, and time to metastasis or OS. However, patients with high pSmad2 or TAZ levels had a shorter median time to metastasis (2.65 times shorter, HR=1.562 for pSmad2 and 2.95 times shorter, HR=2.157 for TAZ, respectively). Patients with high levels of both markers had a 6.8 times shorter median time to metastasis, and 1.7 times shorter OS.

Conclusion

Immunohistochemical determination of TAZ and pSmad2 level has potential prognostic value in canine OSA. Further analyses in larger patient cohorts are warranted.

Regulation of angiogenesis process by metformin and LY294002 treatment in canine mammary tumors

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Introduction

The angiogenesis process is regulated by numerous factors, specially the hypoxia-inducible factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF), which can be inhibited by metformin and LY294002, a PI3K signaling pathway inhibitor. The aim of this study was to evaluate the effectiveness of treatment with metformin and LY294002 in angiogenesis process.

Material and Methods

Cell viability of CF41 cell line was measured by MTT assay. After treatment with metformin (5mM) and LY294002 (5μM), the protein and gene expression of HIF-1α and VEGF were detected by immunocytochemistry and real time PCR, respectively. Cell cycle distribution was assessed by DNA analysis using flow cytometry. For an in vivo study, CF41 were injected in nude athymic female mice and treated with metformin (200 mg/kg i.p.) for 4 weeks and LY294002 (7.5 mg/Kg intratumorally) every 3 days for three times. At the end, mice were euthanized and the tumors were collected to determine the microvessel density by immunohistochemistry for CD31.

Results

There was a significantly decrease of cell viability after treatment with different concentrations of metformin and LY294002 in 24 hours. Both HIF-1α and VEGFA protein expression significantly decreased after treatment with metformin and LY294002 or in combination. All treatments significantly decreased VEGFA gene expression and arrested the cell cycle at G0 phase. Furthermore, in animals, the tumor size and microvessel density decreased after treatments.

Conclusion

Our results suggest the potential effectiveness of metformin and LY294002 acting in angiogenesis process of mammary tumors.

Resveratrol reduces the growth and the expression of VEGFA in Ehrlich tumor

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Introduction

The resveratrol is a compound derived from the grape, related to increased survival in different organisms, suggesting antineoplastic activity as well. In this study, we seek to evaluate the effect of resveratrol on the development of Ehrlich´s tumor in its solid form.

Material and Methods

15 mice BALB-C, female, adult received subcutaneous inoculum containing 5.0x106 cells of Ehrlich´s tumor. 30 days later, the tumors were measured, and the animals randomly divided into control group without manipulation (five animals) and experimental group (ten animals), which received single intraperitoneal inoculum of 0.1ml of resveratrol, being euthanized 3 to 7 days later. Then, the measurement of tumors, immersion in 10% formaldehyde, and histological and immunohistochemical processing were performed, evaluating the proliferation by mitotic count and the expression of VEGFA, considering hot spots.

Results

There was an average of lower growth in animals belonging to the experimental group ($p<0.01$, ANOVA/Turkey-Kramer) without histological and proliferative activity difference, however, with reduction of VEGFA expression in animals subjected to treatment with resveratrol.

Conclusion

It is speculated that the antitumor activity of resveratrol is related to the change in intracellular signaling pathways, leading to proliferative blocking and modulation of local microcirculation, due to activation of sirtuin 1, that is important in HIF-1 deacetylation. In this study, there was no evidence of antiproliferative action, however, the reduction in the expression of VEGFA strengthens the last hypothesis. The use of resveratrol intraperitoneally in a single dose was associated with a reduction in tumor progression, suggesting its potential utility in adjuvant therapy of neoplasia.

Simvastatin induces autophagy on canine mammary carcinoma cells

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Introduction

Mammary cancer is the most prevalent type of tumor in the female dogs. Recent data shows that simvastatin exhibit an antitumor effect and it is able to induce autophagy in some cancer cell lines. However, there is still no evidence in canine mammary cancer cells. Autophagy is a mayor catabolic process that allows the cells to survive in different stress environments, but also may lead to death in apoptosis-resistant cells. Thus, autophagy may play a role in tumor cell death in response to several antitumor agents. The aim of this study was analyze the effects of simvastatin on autophagy and cell viability in canine mammary carcinoma cells.

Material and Methods

CF41.Mg cells were treated with simvastatin for 24 hours and analyzed by flow cytometry using acridine orange dye. The expression of LC3 and beclin-1 in cells treated with simvastatin/3-methyladenine (autophagy inhibitor) by immunocytochemistry was analyzed. Cell proliferation assays with trypan blue dye in presence of simvastatin, Q-VD-OPh hydrate (pancaspase inhibitor) and 3-methyladenine were performed.

Results

Both autophagy activity and LC3/beclin-1 expression was induced in a dose dependent manner by simvastatin ($p<0.05$). These effects were reversed by 3-methyladenine. This statin inhibited the cell viability, even in the presence of 3-methyladenine ($p<0.05$). However, Q-VD-OPh hydrate blocked the anti-proliferative effect of simvastatin.

Conclusion

Our data indicates that simvastatin induces autophagy, modulating LC3 and beclin-1 proteins. This pro-autophagy effect does not enhance the cytotoxic role of simvastatin, since the functional inhibition of caspases fully blocked the cytotoxic effect of the drug.

Transcriptional factor SLUG overexpression induces malignant progression and n-cadherin expression in canine mammary cancer cells

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Introduction

Epithelial-to-mesenchymal transition (EMT) is an important process, which epithelial cancer cells undergo modifications resulting in a fibroblast-like morphology, associated with reduced cellular adhesion and increased motility being responsible for invasion and metastasis. It is controlled by specific transcriptional factors as SNAIL, SLUG, ZEB1, ZEB2, and TWIST that modulate gene expression controlling the EMT. Previously, we determined the SLUG, ZEB1, ZEB2 and STAT3 gene expression in 4 established canine mammary cancer cell lines and verify a high correlation of TFs gene expression and mesenchymal-like phenotype. Here, we evaluated the importance of these TFs on canine mammary cancers by overexpressing SLUG in two epithelial-like canine mammary cancer cell lines and characterizing its effects in vitro.

Materials and Methods

Two epithelial-like canine mammary cancer cell lines previously established in our lab were genetically modified to stably overexpress SLUG. Thus, these cells were compared with null-controls for sphere formation, resistance to doxorubicin and mRNA expression of several genes. Mann-Whitney or unpaired T-test were used for statistical analysis.

Results

Epithelial-like cells expressed very low levels of SLUG in comparison with the mesenchymal-like cell lines. SLUG overexpression in these epithelial-like cell lines increased significantly sphere formation ($p<0.0001$) and size ($p<0.05$) and expression of n-cadherin ($p<0.05$), a marker of EMT. However, no difference was found on resistance to doxorubicin and expression of VDR, HDAC-1 and ABCB1.

Conclusion

Increased sphere formation and size is a key *in vitro* feature of malignant progression, where SLUG overexpression could play a key role for canine mammary carcinomas as demonstrated here.

Why veterinarians are looking for postgraduate courses in Brazil?

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Introduction

Small animal oncology is a relatively new area of veterinary medicine in Brazil whose rapid evolution has driven veterinarians to seek additional training. Therefore, different postgraduate programs are currently offered to veterinarians. The Brazilian Association of Veterinary Oncology (ABROVET) will start the certification in veterinary oncology this year. This study aimed to survey the profile of veterinarians who looked for a postgraduate program in veterinary oncology.

Material and Methods

An email survey was conducted with alumni that has concluded a 2-year postgraduate veterinary oncology course for the past six years (2009-2015). All former students were from a Veterinary Oncology course certified by Brazilian Secretary of Education (Ministério da Educação – MEC).

Results

Sixty-six veterinarians answered a questionnaire comprised of five multiple choice questions. When asked about the main reason for choosing a veterinary oncology postgraduate course, 45 (68%) veterinarians answered that oncology is an area of special interest. The second most frequent reason is the increasing number of cancer cases in small animals practice, mentioned by 17 (26%) veterinarians. The veterinarians were most interested in clinical oncology (33/50%) than surgical oncology (28/42%). After the postgraduate course, 61 (92%) veterinarians are working in small animal internal medicine and only five veterinarians (8%) are working exclusively in veterinary oncology. These professionals believe that the interest in an oncology specialized service increased due to Clinician's (46/70%) or a pet owner's (18/27%) indication.

Conclusion

In conclusion, although postgraduate veterinarians have special interest for oncology, few of them are practicing only this specialty in Brazil.

3rd WVCC 2016

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A retrospective study of hemangiosarcomas in 37 dogs

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Introduction

Material and Methods

Clinical records of dogs with HAS confirmed by histology between 2012 and 2015 were reviewed. Animals with incomplete record or lack of survival data were excluded. Age, breed, sex, and type of HSA were assessed. To access the clinical outcome of the HSA, owners were contacted by e-mail. Surgical treatment, adjuvant chemotherapy, and their association with survival were analyzed.

Results

Thirty-seven dogs (22 males and 15 females) were eligible for this study. The most commonly affected breeds were mixed breeds (13%), Pit bull terrier (13%), Labrador retriever (11%), and Golden retriever (11%) with mean age of 11 years (range 6 – 15 years). The most prevalent form was splenic (53%), followed by cutaneous (18%) and hepatic (8%). Surgical treatment was performed in 37 dogs and chemotherapy in 27. Twenty-seven dogs died (15 with splenic HSA), with mean survival of 246±431 days. In splenic form, survival in dogs with adjuvant chemotherapy (144±153 days) was greater than without chemotherapy (38±30 days) (Log-rank test $p<0.05$).

Conclusion

Splenic is the predominant form of HAS, and the association of chemotherapy with surgical treatment may improve outcome and survival. The cutaneous form is less aggressive, often successfully treated solely by surgery.

Adjuvant chemotherapy and immunotherapy in oral cavity melanoma

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Introduction

Melanomas are the most frequent neoplasm of the oral cavity of the dog, frequently presenting an aggressive biological behavior. The aim of the present work was to evaluate the overall survival (OS) of dogs diagnosed with oral melanomas, regarding clinical staging and different therapy protocols, including surgery, chemotherapy and immunotherapy.

Material and Methods

A retrospective study of canine melanotic and amelanotic melanomas of the oral cavity was performed. Animals were admitted at the Veterinary Hospital of Federal University of Minas Gerais, Brazil. Patient overall survival was evaluated according to clinical stage and divided into two groups: cases treated only with surgery (G1) and cases treated with surgery, four cycles of chemotherapy with carboplatin and six cycles of immunotherapy with interferon-α (G2).

Results

Fifteen cases were evaluated, 3/15 (20%) amelanotic and 12/15 (80%) melanotic. Regarding clinical stage,6/15 (40%) were stage II and 11/15 (60%) stage IIIIV, presenting an OS of 446 and 321 days, respectively ($p=0.64$). Regarding therapy, G1 consisted of 4/15 (27%) case sand G2 of 11/15 (73%) cases. G2 presented a longer OS, with a median OS of 446 days, when compared to G1, with a median of 86.5 days ($p=0.05$).

Conclusion

Literature data demonstrate an OS of 150 and 90 days for stage II and III oral melanomas, respectively, treated only with surgery. Animals treated with surgery and chemotherapy with cisplatin presented an OS of 119 days. The combination of surgery, chemotherapy and immunotherapy was considered beneficial for the treatment of canine oral melanomas.

Comparison of cryosurgery and photodynamic therapy (PDT) for squamous cell carcinoma (SCC) in two differents populations of cats

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Introduction

SCC is a malignant cutaneous tumour in cats usually arising on the head and is associated with exposure to ultraviolet light. Many treatments are effective, however, challenges related to cost, availability and differences in recurrence rates motivates the development of more effective therapies for this tumour.

Material and Methods

This study compared the response of cats with facial SCC, stage T1N0M0, treated with cryosurgery or PDT. Group 1 (lesions on nose-9, eyelid-5, facial-6 and lip-2) were treated with cryosurgery in Brazil and group 2 (nose-19, eyelid-1) were treated with PDT in UK. Lesions in group 1 were frozen by liquid nitrogen spray in 3 freeze–thaw cycles. 5-ALA cream was applied to the lesion in group 2, iluminated with a LED, peak wavelength of 635 nm.

Results

Thirteen cats had cryosurgical treatment. Complete response occurred in all of them (100%). Eighteen of 20 cats (90%) responded to PDT, and a CR occurred in 15 cats (75%). Three cats (15%) demonstrated a partial response and two cats had no response to therapy. Of those that demonstrated a CR, tumors retuned in 4 cats in group1 and 8 in group 2. There was significant difference in DFS between PDT and cryosurgery (46.7% and 81.8%, respectively, P 0.034), but no significant difference in OS between PDT and cryosurgery (60.0% and 92.3%, respectively, P 0.284).

Conclusion

The likelihood of tumor recurrence was significantly reduced with cryosurgery compared with PDT, however these findings are limited by different populations of cats and tumours in different sites.

Cytological classification and chemotherapy response in Transmissible Venereal Tumor diagnosed in Parana State – Brazil.

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Introduction

The transmissible venereal tumor is a neoplasm with a variable biological behavior. This neoplasia may have different cytomorphologic types, variable response to chemotherapy and aggressiveness. Amaral and colleagues in 2007 described a cytological classification system to sorting this neoplasia into 3 subtypes: plasmocytoid, lymphocytoid, and mixed, this classification has been used by many Brazilians veterinary pathologists to try to predict the biological behavior in cases diagnosed. The main objectives of this research were comparing the cytological and histopathological diagnoses, and obtain more information about chemotherapy response in the different classifications.

Material and Methods

Dogs with clinical and cytological diagnosis of TVT were studied. The diagnosis results of cytopathology and histopathology were compared and all cytological diagnoses were classified by Amaral (2007). The response of the treatment with vincristine sulfate was evaluated in the three subtypes.

Results

Fifty-two dogs had cytological diagnosis confirmed by histopathology. These neoplasias were classified in 44,24% (23 cases) as plasmocytoid, 34,61% (18 cases) as lymphocytoid and 21,15% (11 cases) as mixed. Forty-six cases treated by conventional chemotherapy were collected. Plasmacytoid tumors represented an average of five to six sessions (5.37), linfocytoid and mixed patterns, four to five sessions (4.6 and 3,65 respectively). Cases of chemotherapy resistance were only observed in TVT classified as plasmocytoid.

Conclusion

Based on the high correlation between cytology and histopathology we suggest that cytopathology can be used as a final diagnose in well differentiated TVT. The classification can be inferred as a possible prediction in the chemotherapy response.

Effect of electrochemotherapy on inflammatory infiltrate, necrosis and mitotic index in dogs with squamous cell carcinoma

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Introduction

Electrochemotherapy (ECT) is an antitumor therapy that has currently been used in animals and humans with cancer, promoting substantial tumor remissions. However, little is known about the inflammatory infiltrate, necrosis and mitotic index pattern induced by this therapy. The aim of this study was to evaluate the effect of ECT on local inflammatory infiltrate, necrosis and mitotic index in canine cutaneous squamous cell carcinomas (SCC).

Material and Methods

Six cutaneous samples of SCC were collected from dogs before ECT (D0) and 21 days later (D21). ECT was accomplished by using the electroporator LC® BK-100 model in association with intravenous bleomycin plus doxorubicin protocol

Results

Prior to ECT, the samples showed mixed (n=3), lymphoplasmacytic (n=2) and neutrophilic (n=1) inflammatory infiltrate. In addition, all the samples exhibited microscopic necrosis. The majority of them were classified as grade II (necrosis over 50%) (n=4). The mitotic index ranged from two to nine mitotic figures per 40x high power fields. After treatment (D21), the inflammatory infiltrate was predominantly mixed (n=4). There was a decrease in necrosis intensity in one lesion, since its grade changed from II to I (necrosis less than 50%). Moreover, there was a decrease in mitotic index in five lesions.

Conclusion

This study suggests that ECT with bleomycin plus doxorubicin induces changes in the inflammatory infiltrate and reduces the mitotic index. Additional studies are needed to investigate the effect of ECT in canine cutaneous SCC, particularly with regard to the role of the inflammatory infiltrate in tumor microenvironment.

Employment of electrochemotherapy in feline cutaneous squamous cell carcinoma

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Introduction

Electrochemotherapy consists of treatment procedure that combines the employment of antineoplastic agents with electroporation (Serša et al. 2006). This study aims at assessing the feasibility, effectiveness and safety of electrochemotherapy in feline cutaneous squamous cell carcinoma, in order to contribute to optimizing its treatment with consequent improvement in the prognosis for patients affected by the disease herein addressed.

Material and Methods

This study included thirty-nine felines with cutaneous squamous cell carcinoma. Electrochemotherapy was administered using intravenous bleomycin sulfate, at the dose of 15U/m2 of body surface area and electric pulses (electroporation) over the entire tumor extension using specific devices. The entire procedure was repeated monthly until complete macroscopic neoplastic remission was achieved.

Results

Thirty-one tumors submitted to the protocol showed complete regression; seven showed partial regression; only one case did not respond satisfactorily. No adverse events and/or complications were observed for this procedure.

Conclusion

Several protocols are used to treat feline cutaneous squamous cell carcinoma. Such procedures show expressive disparity in relation to efficacy, treatment duration, patient recovery period, safety and burden. Additional studies are being developed in the search for more effective and safer therapeutic alternatives (Ferreira et al. 2006). When results were compared to information available on relevant bibliography, it was concluded that electrochemotherapy is an applicable, effective and safe protocol to treat feline cutaneous squamous cell carcinoma.

Evaluation of large-scale gene expression analysis of benign and malignant mammary tumors in female dogs

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Introduction

Mammary tumor is the most frequent tumor of female dogs and close to 50% of these tumors are malignant. The clinical implications of benign mammary tumors are still unknown, while malignant tumors are implicated in the risk of metastases. The purpose of this study was to evaluate the large-scale gene expression profile of benign tumors (BT), malignant tumors (MT) and normal mammary glands (N).

Material and Methods

Gene expression of fresh tissue from BT (simple and complex adenoma, n=15), MT (simple carcinoma, n=21) and normal mammary glands (n=7) of female dogs was assessed by microarray analysis, using the Affymetrix CanGene 1_0-st platform (Santa Clara, CA). Statistical analyses were performed in the Transcriptome Analysis Console software.

Results

The comparison between BT and MT resulted in 121 differentially expressed genes (14 up-regulated genes and 107 down-regulated genes). While the comparison of BT and N showed 898 differentially expressed genes, of which 359 were up-regulated genes and 539 were down-regulated genes. When comparing normal tissue to MT we found 1010 differentially expressed genes (348 up-regulated genes and 662 down-regulated genes).

Conclusion

We observed in this preliminary study a large number of differentially expressed genes between normal mammary glands (N) and BT and between N and MT. While in the comparison of BT with MT the number of differentially expressed genes was not so expressive. The data suggest an involvement of molecular mechanisms in the carcinogenic process and tumor progression.

Evaluation of lymph node metastasis and its prognostic value in dogs with cutaneous mast cell tumors

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Introduction

Mast cell tumors (MCTs) reportedly metastasize to lymph nodes. This study aimed to investigate and correlate the presence of LN metastasis with disease-free interval (DFI) and overall survival (OS) in dogs with cutaneous MCTs.

Material and Methods

Records from 57 dogs with cutaneous MCTs were evaluated. 81 regional LNs were evaluated by cytology prior to surgery and metastasis were confirmed histologically. MCTs were classified by Patnaik and Kiupel systems; KIT and Ki-67 expression were evaluated by immunohistochemistry. All dogs with LN metastasis received adjuvant chemotherapy. DFI and OS were calculated using the Kaplan-Meier method; associations between variables and LN metastasis were tested using Fisher exact test. Values of $P < 0.05$ were considered significant.

Results

There were 2 grade I, 47 grade II and 8 grade III MCTs. 50 were low grade and 7 high grade tumors. 48 (59,2%) LNs were positive for metastasis. Sensitivity and specificity of LN cytology were 86 and 64%, respectively. A significant association was found for high Ki-67 expression and LN metastasis (p=0.02) and with decreased DFI and OS. Histologic grade was associated with DFI and OS, independently of the presence of LN metastasis. 14 (24,5%) dogs died due to progression of disease; all of them had LN metastasis. However there was no statistical difference in DFI (p=0.36) and OS (p=0.29) in animals with and without LN metastasis.

Conclusion

Cytology proved to be a good method in detecting LN metastasis in dogs with MCTs. These results suggest that LN metastasis and Ki67 are prognostic factors in canine cutaneous MCTs.

Evaluation of sonographic appearance and histologic diagnoses of splenic disorders in dogs: a retrospective study of 43 cases

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Introduction

Splenic disorders in dogs are very common in clinical practice, especially in older animals. Clinical signs are usually nonspecific and ultrasonography may reveal different patterns of splenic lesions. This study aimed to evaluate the ultrasonographic appearance of splenic lesions and their histopathologic diagnosis in dogs.

Material and Methods

Forty-three dogs referred to a veterinary hospital were selected. The inclusion criteria were dogs undergone ultrasound examination and splenectomy followed by histopathological analysis. Information about histopathologic diagnosis, race, gender, age and ultrasonographic appearance were obtained from the patient's clinical records. Descriptive statistics was done for the clinical information and the Fisher's exact test or chi-square were used to analyze the association between ultrasonographic appearance and histopathological diagnosis.

Results

The mixed breed dogs were the most affected (37%) and considering all the animals, there was a prevalence of females (70%). The average age of patients was 9.8 years. Neoplastic disorders corresponded to 60% of all cases and hemangiosarcoma was the most prevalent malignancy (38.5%). Other malignancies were lymphoma (23%), hemangioma (15.4%), metastasis (7.7%), fibrohistiocytic nodules (7.7%) and undifferentiated sarcoma (7.7%). Nodular hyperplasia was the most common non-neoplastic disorder (71%). The splenic sonographic appearance such as heterogeneity and nodularity were not associated with malignancy disease ($p>0.05$).

Conclusion

Our results identified hemangiosarcoma as the most common splenic malignancy. The ultrasonographic appearance was not sufficient to identify malignancy disease. Histopathological examination is necessary for definitive diagnosis.

First evidence of a synergistic interaction between EGFR and HSP90 immunoexpression in dog malignant mammary tumours

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Introduction

In canine mammary tumours (CMT) several reports have been published on single epidermal growth factor receptor (EGFR) immunohistochemical evaluation and a few in heat shock protein 90 (HSP90) immunoexpression.

Material and Methods

In order to better understand the clinical significance of the two proteins concurrent expression in CMT, 33 female dogs with malignant CMT were studied by immunohistochemistry, comparing concurrent expression of EGFR and HSP90 with characteristics of tumour aggressiveness.

Results

High EGFR immunoexpression revealed a statistically significant association with mitotic index ($p = 0.014$), nuclear grade ($p= 0.007$), histological grade of malignancy ($p = 0.005$), lymph node metastasis ($p = 0.012$) and overall survival time ($p = 0.011$). High HSP90 immunoreactivity was statistically significantly associated with tumour size ($p = 0.025$), mitotic index ($p = 0.009$), nuclear grade ($p = 0.004$), histological grade of malignancy ($p = 0.002$), lymph node metastasis ($p < 0.001$), clinical stage ($p = 0,003$) and overall survival time ($p< 0.001$). Tumours with simultaneous high immunoexpression of EGFR and HSP90 were statistically associated with characteristics of higher aggressiveness: high mitotic index ($p = 0.013$), nuclear grade ($p = 0.005$), high histological grade of malignancy ($p = 0.01$), presence of lymph node metastasis ($p <0.001$), clinical stage ($p<0.001$) and overall survival time ($p <0.001$). A positive and statistically significant association between COX-2 and EGFR immunoexpression ($p = 0.01$) was also observed.

Conclusion

Present results open perspectives for a combined use of selective inhibitors of EGFR and HSP90, in the context of malignant CMT treatment.

Gene expression of DLADQA-1 in naturally occurring canine transmissible venereal tumours

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Introduction

The canine transmissible venereal tumour is a highly prevalent canine infectious disease in many parts of the world, including Brazil. It is the oldest cancer lineage known in nature and one of the most remarkable features of CTVT is its ability to evade immune rejection, as an allogeneic graft. The major histocompatibility complex antigens play a key role in the recognition of tumour cells by the immune cells. However, the mechanisms whereby this cancer is able to avoid destruction by the host immune system remain incompletely understood. The aim of this study was to evaluate gene expression of DLADQA-1, a classical MHC-II gene, in spontaneous CTVT in dogs.

Material and Methods

Tumour samples were obtained from 20 dogs with spontaneous CTVT from Brazil at two different time points: before and three weeks after starting the chemotherapy. The gene expression analysis was performed by RT-qPCR method.

Results

There was no gene expression of DLADQA-1 in CTVT samples at both time points. Studies have shown many human and animal tumours do not express or alternatively down regulate the expression of MHC class I and II antigens by some mechanism of deleting or mutating the MHC genes or by down regulating these genes by genetic and epigenetic mechanisms. These results suggest that CTVT cells may have acquired it to escape immunological surveillance.

Conclusion

These observations provided preliminary investigation into one of the MHC-II genes present in CTVT and further studies could provide additional information whereby this cancer is able to escape the immune detection as a successful parasite.

Ki67 and FoxP3 immunoexpression in dogs with cutaneous lymphoma.

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Introduction

Cutaneous lymphoma represents 1% of skin tumors in dogs and is classified in epitheliotropic and nonepitheliotropic. Ki67 is a nuclear protein used as marker of cell proliferation and FoxP3 is a transcription factor of regulatory T cells (Treg) and may be associated with tumor progression and aggressiveness. The aim of this study was to analyze the expression of Ki67 and FoxP3 in cutaneous lymphomas.

Material and Methods

Immunohistochemistry was performed in 12 tumor samples of dogs with cutaneous lymphoma (5 epitheliotropic, 7 nonepitheliotropic) using CD20 and CD3 markers for immunophenotyping. For the analysis of FOXP3 and Ki67, 1,000 lymphocytes were evaluated in the tumor and considered as positive at least 10% of labeled cells.

Results

It was found 3 epitheliotropic T-cell and 2 B-cell and 6 nonepitheliotropic T-cell and 1 B-cell. There was a significant difference in the expression of Ki67 ($p=0.001$) between epitheliotropic (24.0±13.5%) and nonepitheliotropic (51.4%±22.5). Just one nonepitheliotropic T-cell was positive for FoxP3.

Conclusion

The Ki67 expression is related to the malignancy for cutaneous lymphoma. According the results, nonepitheliotropic cutaneous lymphoma has a higher grade of malignancy than epitheliotropic. Regarding FOXP3, in human medicine, low expression is associated to some clinical manifestations and a worse prognosis in cutaneous lymphoma, but there isn't study in dogs. Considering the high expression of Ki67, low FOXP3 expression could be associated with advanced stage of disease or even low expression of FOXP3 might be expected since unlike humans, dogs with cutaneous lymphoma express CD8 rather than CD4.

Neoadjuvant response to glucocorticoids are related to prognosis of canine mast cell tumours

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Introduction

Despite advances in prognostification and treatment of canine mast cell tumours (MCTs), glucocorticoids (GCs) remain the most used drugs. The objective of this study was to evaluate the response of canine MCT to the use of GCs in the neoadjuvant setting, and access the impact of this response in the overall survival (OS) of these patients.

Material and Methods

The efficacy of MCWF to restore revert neutropenia was determined by comparing the duration of neutropenia in the control group versus the three MCWF treatment groups.

Results

Partial remission ocurred in 64.5% of cases (40/62), while 35.5% (22/62) had stable disease. Response was moderately correlated with a Patnaik and Kiupel low-grade, cordonal distribution, low Ki-67 (cut-off value of 6,5%) and KIT membranar pattern. Patients with stable disease after GCs reached a median survival of 427 days, while those with partial response did not reach the median (p=0,02).

Conclusion

Clinical responses can be obtained in a significant percentage of dogs with MCTs submitted to neoadjuvant treatment with GCs, especially those with well-differentiated tumours. Measurable responses are associated with increased OS.

Preliminary clinical safety investigations from dogs treated with adjuvant immunotherapy for melanoma

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Introduction

Strategies to activate the immune system have been studied to recognize cancer-specific antigens and eliminate neoplastic cells preferably without side effects. Our hypothesis is that exposing dogs with autologous tumor lysates vaccine it will be evokes an antitumor immunity with rare adverse events. This study assessing clinical features from vaccinated melanoma bearing dogs.

Material and Methods

Dogs with malignant melanoma, and disease progression without prior chemoterapy treatment were eligible after owner's consent. Tumor formations were collected surgically and enzimatically digested following by freezing cells. ATL vaccine was prepared after alternating cycles of freeze-thaw to cause cell death and exposure of antigens plus BCG (immunomodulator) which was added at decreasing doses to each dose of vaccine. The vaccine was apllied by intradermic injection away from tumor site and close to lymph node, and doses are repeated 21-30 days. Side effetcs were evaluated in the application site and systemic effects were observed in short-term (one hour) and in long-term (> seven days).

Results

Thirteen dogs (nine oral/two digit/two skin melanoma) received at least two doses of vaccine being the total of 28 doses applied and most dogs are still under treatment. In short-term no dog showed systemic changes and in the long-term assessment one dog developed intradermal abscesses in application site.

Conclusion

The preliminary clinical safety results from this study showed that the use of autologous tumor lysates+BCG vaccine proved be well tolerated by animals. The data from efficacy of vaccine is underway, since animals are still under treatment.

Pro-apoptotic effects of metronomic chemotherapy in canine mammary carcinomas

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Introduction

The therapy for canine mammary carcinomas (CMC) remains a challenge in veterinary oncology. Metronomic chemotherapy (MC) is a therapeutic option for tumors in dogs and there are no RESULTS on the response of CMC to this therapy. This study evaluated the effects of MC to induce tumor cells apoptosis in CMC.

Material and Methods

Were used 28 bitches with mammary carcinomas, clinically staged, divided into two groups: control and treated. The bitches in the control group (CG, n = 14) underwent bilateral mastectomy and regional lymphadenectomy. The bitches in the treated group (TG, n = 14) underwent the same surgical procedure associated with postoperative MC with cyclophosphamide (15 mg/m2) and piroxicam (0.3 mg/kg), either orally, once daily, for 28 days. Mammary tumors were classified and graded. Metastasis research in regional lymph nodes was performed by immunostaining with cytokeratin. The MC response was evaluated by apoptotic index (AI) obtained by caspase-3 immunostaining. Statistical analysis was performed using Spearman's correlation coefficient with Graphpad Prism version 6.0 ($p < 0.05$).

Results

The quantification of caspase-3 immunostained cells showed that the AI was significantly higher in the TG when compared to CG (TG: 64.5 ± 51.93 cells per hpf and GC: 34.4 ± 17.18 cells per hpf).

Conclusion

With these results we can affirm that MC is able to increase the rate of tumor cells apoptosis of CMC. Thus, this therapeutic modality becomes an option for control and treatment of these neoplasms in dogs.

Prognostic significance of clinical substage in dogs with multicentric lymphoma treated with 19-week CHOP chemotherapy

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Introduction

Despite canine lymphomas are the most common hematopoietic tumor in dogs, survival times are variable even when they are treated with CHOP based protocols. Many studies have been carried out looking for reliable prognostic factors but results are conflicting. The aim of this study was to identify possible prognostic factors in dogs with multicentric lymphomas that have undergone 19-week CHOP chemotherapy.

Material and Methods

Information was obtained from medical records of 48 dogs with multicentric lymphomas. Age, breed, sex, histologic type, immunophenotype, clinical staging and survival time were registered. All dogs were treated with 19-week CHOP protocol. The survival study was assessed by Kaplan-Meier survival curve analysis using Log-rank test.

Results

Only clinical substage was a significant prognostic factor. The median overall survival was 294 days in dogs with clinical substage "a" (n=24) and 84 days in dogs with clinical substage "b" (n=19) (p=0,01). The most commom histologic type was diffuse large B cell lymphoma (DLBCL) (n=34), however, there were no notable differences in survival between dogs with DLBCL vs non-DLBCL (n=13). The median overall survival did not varied by breed (pure-bred vs mixed breed), age (<8 vs ≥8 years old), sex (male vs female), imunophenotype (B vs T) and clinical sage (III vs IV).

Conclusion

Results suggest that clinical substage "b" dramatically reduces survival in canine multicentric lymphoma. Larger studies are needed to a better understanding of the influence of clinical substage on survival in dogs with multicentric lymphoma.

Regulatory T cells (T regs) in peripheral blood of dogs with cutaneous and multicentric lymphoma

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Introduction

Cutaneous lymphoma is a rare neoplasm in dogs that shows aggressive biological behavior while multicentric lymphoma is the most common hematopoietic neoplasm, with variable biological behavior. Regulatory T cells (T regs) are a specialized population of T lymphocytes that have the function of suppressing immune system response against cancer and pathogens, resulting in T cell dysfunction in humans and dogs. Elevation of these cells in cancer patients may promote tumor development. However, with respect to lymphoma in dogs, few studies are available. Thus, this study aimed to assess quantitatively the Tregs in peripheral blood of dogs with cutaneous and multicentric lymphoma.

Material and Methods

For this study, 12 healthy dogs, 15 dogs with cutaneous lymphoma and 15 dogs with multicentric lymphoma were used. Tregs from peripheral blood were measured by flow cytometry (FoxP3+CD4+) (BILLER et al., 2007).

Results

The results showed that dogs with cutaneous and multicentric lymphoma had higher percentage of Tregs (14.85 ± 1.398 and 11.30 ± 1.161, respectively) compared to the control group (5.67 ± 0.89), with significant difference ($p<0.05$). However, there was no statistical difference of Tregs between groups with multicentric and cutaneous lymphoma.

Conclusion

This study suggests that dogs with cutaneous and multicentric lymphoma have a greater percentage of Tregs cells in peripheral blood than healthy dogs, as well as in other neoplasms already described. This fact may contribute to the poor outcome of this disease and future studies aiming to develop therapies directed against Treg cells may be promising in the adjuvant treatment of dogs with cutaneous and multicentric lymphoma.

Retrospective study of oral melanoma in 19 dogs

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Introduction

Melanomas are one of the most frequently diagnosed malignant neoplasms of the canine oral cavity. May present aggressive biological behavior infiltrating locally and progressing to metastatic lymph nodes and lungs. The purposes of this study were to review overall survival and disease free survival of dogs with oral melanoma (OM).

Material and Methods

Diagnostic records of canine OM between 2012 and 2015 were reviewed. Owners were contacted by e-mail to access clinical outcome and survival. Animals with incomplete record or lack of survival data were excluded. The oral site affected, stage at the moment of diagnosis, overall and disease free survival were analyzed.

Results

The group consisted of 9 female and 10 male with mean age of 12 years of which 88.8% were purebred. Maxilla was affected in 35.3% of the cases, lips and mandibula 23%, palate 10.5% and larynx 11.7%. According to the WHO classification 38.5% were stage III, 21% stage II, 15.4% stage IV and 10.5% stage I. The overall survival was 8.4 months and disease free survival after surgery was 6.1 months.

Conclusion

Canine OM is a challenging disease because of high recurrence, metastasis and low survival. Most of the cases were classified as stage III showing an overall survival of 8.4 months.

Thalidomide treatment in canine mammary gland neoplasms presenting distant metastasis

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Introduction

Mammary gland neoplasms are the most frequent neoplasm in female dogs, and around 50% are malignant. Thalidomide has immunomodulatory and anti-angiogenic properties and has been proposed in the treatment of several neoplasms. The aim of the present study was to evaluate the overall survival of canine mammary gland neoplasms presenting lung metastasis treated with surgery, chemotherapy and thalidomide.

Material and Methods

A prospective analysis of female dogs diagnosed with clinical stage V mammary gland neoplasms was performed. Animals were treated with surgical excision of the neoplasm and chemotherapy with 4 cycles of carboplatin (300 mg/m2, IV, every 21 days), followed by thalidomide at the dose of 10 mg/kg, PO, once a day without interruption.

Results

The survival rate of mixed breed dogs was analyzed by the Log-Rank test and was not significantly different from pure breed dogs (p = 0,253).

Conclusion

Prognosis in stage V mammary gland neoplasms is poor and efficient treatments are limited. Survival rates in animals presenting distant metastasis have been described as 13.6% one year after mastectomy. Treatment with thalidomide was well tolerated by all patients and the proposed treatment was considered beneficial.

Thyroid carcinoma associated with Horner Syndrome in a dog

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Introduction

The most common clinical presentation of thyroid carcinomas is palpable formation in the cervical region. Common clinical manifestations are dysphagia and respiratory changes caused by compression of tumors. The diagnosis is carried out in most cases through histopathological examination, surgical excision is the convetional treatment. The aim of the study was to describe a thyroid carcinoma case.

Material and methods

The Veterinary Hospital received care for a dog, mixed breed, 12 years, not sterilized, with swelling in the neck and difficulty swallowing and increase of 6 months. Physical examination observed was miosis, enophthalmos, eyelid and lip ptosis, 3rd eyelid protrusion in the left eye, respiratory stridor, dyspnea mixed with inspiratory predominance, head lateralization and swelling in the left cervical region measuring 8x11 cm.

Results

Radiography showed the presence of cancer with lung metastasis, other tests showed no changes. The diagnosis of thyroid follicular cell carcinoma was confirmed by histopathological examination. Due to metastasis and the invasive nature of the tumor it was not possible to perform surgical resection and the owner decided not to do chemotherapy. The treatment consisted for pain relief.

Conclusion

The thyroid carcinoma can cause Horner syndrome (HS) resulting from the interruption or loss of sympathetic innervations to the eye and its annexes. The thyroid carcinoma is a tumor that affects a small percentage of dogs, however, has significant importance, since it is a malignant tumor that reduces the expectation and quality of life of affected dogs.

Transmissible Venereal Tumor: new possibilities to adjuvant therapies with Cox-2 inhibitors

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Introduction

The Transmissible Venereal Tumor (T.V.T.) is a neoplasia with worldwide distribution. Different biological behaviors, response to chemotherapy and aggressiveness have been reported, moreover, veterinary oncologists have been describing the increase of the numbers of resistance to the conventional chemotherapy with vincristine sulfate and doxorubicin, for these reason new approaches to treat this neoplasm are required. Cox-2 Inhibitors have been used in the last decades as an adjuvant therapy to treat cancer with good results. The aim of this study was to compare the responses between treatments to T.V.T., the conventional treatment with vincristine sulfate and conventional treatment combine to meloxicam.

Material and Methods

Were evaluated 23 cases of animals diagnosed with TVT and subsequently treated with conventional therapy with vincristine sulfate (16 cases) and vincristine sulfate associated with meloxicam (5 cases).

Results

The evaluated cases showed differences between the groups of animals treated with conventional therapy and adjuvant therapy. In the animals treated with conventional therapy, 81.25% (13 cases) demonstrate that were required at least 4 chemotherapy sessions, while 100% (5 cases) using adjuvant therapy with meloxicam, regression was obtained with up to 4 chemotherapy sessions.

Conclusion

The over expression of COX-2 has been correlated to the increase of angiogenesis, inhibition of apoptosis, suppression of the immune system response, greater invasion capacity and metastasis. Conventional chemotherapy in combination to coxibs could bring a new possibility to decrease the number of chemotherapy sessions or maybe extend the survival of our patients with resistance to the conventional chemotherapy treatment.

Transmissible venereal tumor: evaluation of lactate dehydrogenase as tumor marker in dogs

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Introduction

Canine transmissible venereal tumor (TVT) is a round cell tumor. Tumor markers such as lactate dehydrogenase (LDH) are substances produced in response to tumor development, which are present in blood and other biological fluids. The concentrations of these substances in body fluids should reflect the extention of the tumor, response to the treatment and disease progression. LDH is an enzyme that helps with the catalysis in the last step of glycolysis, where the pyruvate is reduced to lactate. This enzime is often increased in serum levels in cancer patients because the neoplastic cell a high rate of glycolysis compared to the healthy cell. So, the purpose of this study was to demonstrate the effectiveness of this enzyme as a biomarker in TVT cases.

Material and Methods

Blood samples of twelve animals affected by TVT and treated with conventional chemotherapy were analyzed weekly with complete blood count by automatic analyzer and the serum LDH enzyme by using biochemical reagents UV-lactate pyruvate methodology.

Results

In all animals the LDH enzyme accompanied the tumor progression. Among the twelve dogs, five (41,66%) of theme revealed a decrease of this enzyme when the treatment was effective and decreases the tumor size, and the other seven (58,34%) animals demonstrated an increase due to concomitant illness and discontinuation of chemotherapy, thereby increasing tumor size.

Conclusion

Thus, one can demonstrate that the LDH enzyme may be used as a biomarker to assess tumor progression TVT and contribute to its premature diagnosis in cases with resistance to chemotherapy.

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Activation of the PI3K/AKT/mTOR pathway and its role in the prognosis of dogs with cutaneous mast cell tumor

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Introduction

The PI3K/AKT/mTOR pathway is related to proliferation, protein synthesis, survival and motility of cells. Mutations in this pathway have been associated with carcinogenesis and prognosis in different tumors, however this relationship has not been reported in canine mast cell tumor (CMCT). The objective of this study was to evaluate the immunoreactivity of PI3K/AKT/mTOR pathway and its role in the prognosis in CMCT.

Material and Methods

46 CMCT were selected for immunohistochemical staining using the following antibodies: p-AKT-Ser-473, p-AKT-Thr-308, p-mTOR-Ser-2448, p-S6K1-Thr-389 and p-4EBP1-Thr-37/46. Immunohistochemical evaluation was performed considering the location (nuclear, citoplasmatic, nuclear-citoplasmatic), percentage of positive cells (0: <5% positive cells, 1: 6-25% positive cells, 2: 26-50% positive cells, 3: 51-75% positive cells, 4: >75% positive cells) and intensity of immunostaining (1: weak, 2: moderate, 3: strong). A score system was obtained multiplying the intensity by the percentage of positive cells: high, moderate, low. Immunostaining results were associated with clinical parameters, histologic grade and several reported prognostic factors.

Results

All the CMCT presented positivity immunoreactivity with the antibodies of this pathway, as described by AMAGAI et al., 2013. A significant association ($p<0.05$) between high score of p-AKT-Thr-308 and p-S6K1-Thr-389 immunoreactivity was defined by multivariate analysis with parameters related to poor prognosis like overall survival less than 6 months, rapid growth, IV/V stage, Ki67 over 23, mitotic index >5, histopathologic grade III, high grade, KIT III and CMCT >3 cm.

Conclusion

Suggesting its role in carcinogenesis and poor prognosis of this neoplasm similar to described by Al Saad et al., 2009, KIM et al., 2011 e FAHMY et al., 2013.

Agarose cell block as a complementary diagnostic method in canine skin formations

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Introduction

The high frequency and aggressiveness of neoplastic diseases in dogs justifies the demand for faster, less invasive, and cheaper diagnostic methods, seeking adequate surgical and therapeutic approach. In this study, we seek to evaluate the appropriateness of the use of agarose cell block technique as a complementary tool to cytologic smears conventionally used in the diagnostic of cutaneous formations, comparing the results obtained from smears and cell blocks, alone and in association with the histopathological diagnosis.

Material and Methods

Skin formations were sampled by fine-needle-aspiration of dogs assisted in the UNIP and UMESP Veterinary Hospitals. To perform the agarose cell block, 70% alcohol was added to cytological sample, proceeding to centrifugation at 200rpm, removal of the supernatant liquid with inclusion of 2% agarose and another centrifugation. The sample was histologically processed and stained by hematoxylin and eosin.

Results

From 41 samples of skin formations, smears showed higher sensitivity and specificity, corresponding respectively to 91.7% and 100%, compared to 90.9% and 83.3% obtained from the agarose cell block. The association of cytological methods reduced the inconclusive samples to 14.6% and increased the sensitivity of the procedure to 96.5%.

Conclusion

Agarose cell block method provides cellular representation and preservation of spatial relationship between them, contributing to the diagnosis. The best results were derived from smears and cell block association, reducing the number of false-negative results and raising the cyto-histologic correlation, highlighting the importance of cytology in the routine of veterinary oncology and usefulness of cytoinclusion for oncologic diagnosis.

Applicability of microvascular density in canine melanomas - preliminary results

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Introduction

Many studies aim to improve the classification of canine melanomas (CM) using different prognostic factors. The microvascular density (MVD) has been used in humans to correlate tumor angiogenesis with survival. In dogs, this prognostic factor has significance in mast cell tumors and mammary tumors. In canine melanomas, only two studies investigated the role of MVD as a prognostic factor, obtaining discrepant results. A prognostic factor widely used in CM is the mitotic index (MI). This study aimed to correlate MVD with MI from CM.

Material and Methods

Twenty-one (n=21) canine malignant melanomas were used and classified according to WHO classification. The melanocytomas and malignant melanomas were differentiated according to degree of pigmentation, ulceration, nuclear atypia, pleomorphism, junctional activity and inflammatory reaction. The MVD was obtained by CD31 immunostaining through the average of microvessels in three hot spots (hpf), not being differentiated according to the anatomical location. The MI was obtained by the average number of mitotic figures per 10 hpf. Spearman correlation coefficient was carried out between DMV and MI ($p < 0.05$).

Results

No correlation was observed between MVD and MI in the analyzed tumors.

Conclusion

In this study, oral and cutaneous melanomas were grouped and maybe stratification of the samples by anatomical location presents different results. With these results, it was not possible to affirm that MVD can be used as a prognostic factor. Studies with a larger number of samples are in progress, as well as evaluation of other parameters such as overall survival and proliferation index.

AR and CAV-1 in the canine prostate carcinogenesis

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Introduction

Androgen receptor (AR) and caveolin-1 (CAV-1) play an important role in the growth, invasion and metastases of prostate cancer in humans. Dogs spontaneously develop tumours, including prostate cancer, and are considered a good model for comparative oncology. Due to the limited information on AR and CAV-1 in the canine prostatic carcinogenesis, this study aimed to investigate these genes in malignant canine prostatic lesion.

Material and Methods

AR and CAV-1 gene expression were evaluated by qRT-PCR analysis in 10 normal prostates, 9 proliferative inflammatory atrophy (PIA) and 17 PCa of formalin-fixed, paraffin embedded tissue. Kruskal-Wallis or Mann-Whitney U tests was applied to compare AR and CAV-1 transcription levels among the samples. $p<0.05$ was considered as significant. Spearman test was used evaluate the correlation of genes expression.

Results

AR expression was down-regulated in PCa compared to normal samples ($p=0.0349$). A significant difference in AR levels was observed between PIA and normal prostatic tissue ($p=0.0159$). Our results revealed high expression of CAV-1 in PCa samples compared to normal samples ($p=0.0329$). Additionally, there was a significant difference in CAV-1 expression between PCa, PIA and normal prostate tissue ($p=0.0490$). No statistic correlation was observed between AR and CAV-1 gene expression ($R= 0.151$, $P= 0.378$) in all samples.

Conclusion

Changes in AR and CAV-1 gene expression are associated with metastatic human prostate cancer and androgen ablation therapy. We suggest that loss AR expression and CAV-1 overexpression are involved on canine prostate carcinogenesis.

CD10 as an early biomarker of feline mammary tumors development

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Introduction

About 90% of feline mammary tumors are malignant. In humans, overexpression of the cell membrane-associated enzyme (CD10) has been reported as an invasion and metastasic factor of breast carcinoma. The objective of present study was to examine CD10 expression in normal, dysplastic and neoplastic feline mammary tissue by comparing CD10 staining intensity to assess its usefulness as a biomarker in research and diagnostic.

Material and Methods

CD10 expression was studied by immunofluorescence on routinely processed tissues. Sixty-one mammary gland full-thickness tissue samples were taken intraoperatively from mature queens during mastectomy. Samples were stained with hemotoxylin-eosin, immunolabeled with anti-CD10 monoclonal antibody, then visualized using light, confocal microscopy and scanning cytometry, respectively. Histologically tumors were classified as adenoma, adenocarcinoma and dysplastic.

Results

CD10 immunopositive reaction was demonstrated in the cytoplasm of stromal and epithelial neoplastic, dysplastic and normal cells with different intensity. The mean±SEM CD10% expression were evaluated at level 18.45±2.76 (A), 28.82±1.95 (A-C): 29.18±2.43 (GI), 37.14±5.40 (GII), 26.08±2.38 (GIII) and 17.92±2.73 (D) with no significant differences ($P>0.0001$). The expression in all affected tissues was significantly higher ($P<0.0001$) than normal tissues (N) 14.31±1.92. No MIF significant differences in all samples confirmed the cross-reactivity of this antibody with the feline antigen

Conclusion

Differences in the distribution and staining intensity of CD10-positive cells suggest a number of potential roles for this protein in the pathogenesis and clinical tumor behavior. CD10 expression demonstrated in the present study suggests that CD10 might be an early marker of feline mammary tumors development which would make it an important novel prognostic factor.

Cell block immunocytochemistry and liquid based cytology for lymphoma diagnosis: higher accuracy and less unsatisfactory results.

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Introduction

Cancer is an important cause of death among dogs, and its proper diagnosis is fundamental to therapeutic and prognostic definition. Liquid Based Cytology (LBC) and cell block (CB) together may improve canine lymphoma diagnosis, allowing immunophenotyping and further classification. We aimed to assess inter-rater reliability and unsatisfactory rate between conventional cytology and LBC; and to compare accuracy between conventional cytology and LBC with CB immunocytochemistry, in order to validate the method to lymphoma diagnosis. All procedures had ethical approval.

Material and Methods

Fine needle aspiration samples of enlarged lymph nodes from 54 dogs were fixed in preservative solution for LBC (Surepath™, Becton Dickinson, Tripath Imaging, Burlington, NC, USA). They were processed according to manufacturer instructions, with cell block production (CB). Slides were submitted to immunocytochemistry with anti-CD79a, anti-Pax5, anti-CD3 and anti-Ki67. An additional conventional slide (Diff-quick stained) was produced from same lymph node. Two veterinary pathologists classified the samples as POSITIVE, NEGATIVE or unsatisfactory for canine lymphoma, in a double-blind experiment.

Results

Results of 19 cases were compared to histopathology in order to achieve accuracy, comparing conventional cytology and LBC together with CB immunocytochemistry. 30 dogs were positive to lymphoma, 80% had B cell lymphoma and 20% T cell lymphoma. LBC inter-rater reliability was moderate (k=0.434). LBC together with CB immunocytochemistry presented an accuracy of 89.47% compared to 68.42% from conventional cytology. Moreover, the unsatisfactory rate was reduced from 11.76% (conventional) to 3.71% (LBC).

Conclusion

LBC and CB together are an accurate method to diagnose canine lymphoma, reducing unsatisfactory rate, but it demands trained observers.

Comparative analysis of proliferation indexes and NANOG expression in canine mast cell tumors

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Introduction

Cutaneous mast cell tumors (MCTs) represent almost one third of malignant skin tumors in dogs. Due to the difficulty of predicting their biological behavior and defining treatment protocols, MCTs have caused several therapeutic frustrations. Histopathology, mitotic index and Ki67 are the main criteria for prognostic formulation in MCTs. NANOG is a pluripotency factor expressed by normal and cancer stem cells. It is considered a prognostic marker and a potential therapeutic target for several human tumors. The aim of this study was to compare the immunohistochemical expression of NANOG with Ki67 and mitotic indexes in canine cutaneous MCTs.

Material and Methods

NANOG expression, and Ki67 and mitotic indexes were evaluated in 48 samples of MCTs from 41 animals (12 grade I, 26 grade II and 10 grade III). The percentage of positive cells for NANOG and Ki67 were evaluated in 5 hot spots and mitotic count was performed in 10 hotspots using the x40 objective.

Results

All samples were positive for NANOG but its expression was not correlated with Ki67 ($p=0.8236$; $r=-0.0417$) or mitotic index ($p=0.2371$; $r=-0.2697$).

Conclusion

Although NANOG is related to pluripotency, its expression does not correlate with proliferative activity in canine cutaneous MCTs.

Comparison of expression of MMP-2 and MMP-9 in feline injection-site (FISS) and non-injection site sarcomas (non-FISS)

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Introduction

Matrix metalloproteinases (MMP) are the proteolytic enzymes degrading type IV collagen and play an important role in tumor invasion (1). The aim of the study was to compare the expression of MMP-2 and MMP-9 in feline injection-site (FISS) and non-injection site sarcomas (non-FISS).

Material and Methods

The study included 19 cases of feline sarcomas (11 cases of FISS and 8 of non-FISS), verified histopatologically. The presence of MMPs (both MMP-2 and MMP-9) in neoplastic tissue was investigated by immunohistochemistry on formalin-fixed, wax-embedded sections of samples collected from cats. The light microscopy was used to evaluate the immunoexpression of MMPs in semi-quantitative scale (2).

Results

The immunoexpression of MMP-2 was positive in 64% and 50% cases of FISS and non-FISS, respectively. The expression of MMP-9 of FISS was positive in 91% (strong intensity in most of cases). The expression of MMP-9 of non-FISS was observed in 62,5% of cases (strong intensity only in 1 case). Immunoexpression of MMP-9 was higher in FISS than in non-FISSs. There was no difference in the expression of MMP-2 in both types of sarcomas.

Conclusion

According to our knowledge, this is the first study comparing the immunoexpression of MMP-2 and 9 in FISS and non-FISS. This study demonstrates that MMP-9 can be involved in high invasiveness of FISS and suggests that this marker is a possible indicator of biological malignancy and targets for future therapeutic strategies.

Coordinated expression of keratins 7 and 20 in canine and feline mammary carcinomas

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Introduction

The keratins form a group of 20 acidic or basic proteins, constituents of the epithelial cytoskeleton with individualized expression throughout the tissues. Its presence may be useful in histogenic characterization in carcinomas of unknown primary site. In this study we evaluate the pattern of expression of keratins in breast carcinomas of canine and feline females using immunohistochemical procedure.

Material and Methods

Canine and feline mammary carcinomas excised at UNIP and UMESP Veterinary Hospitals were submitted to histological processing, classification and tumor graduation. This was followed by immunostaining with the use of mouse anti-keratin human 7 and 20 monoclonal antibodies, respectively, OV-TL 12/30 and K20-8 clones, both DAKO® with 1: 100 dilutions; DAB LSAB + detection system. The measurement of immunostaining was performed by mixed scores, integrating intensity and extension of the marking.

Results

32 cases were evaluated, predominating tubular and solid carcinomas, belong to different degrees of malignancy. As for immunophenotyping, there was predominance in dogs, of the double negativity, CK7- / CK20-, and in cats CK7+/CK20- corresponding respectively, to 75% (15/20) and 58,3% (7/12) of the samples. Solid and anaplastic carcinomas showed double negativity in the eleven cases analyzed.

Conclusion

The predominant immunophenotypic pattern was the double negativity in dogs, as opposed to that reported in the literature, and the isolated positivity for CK7 in cats, which suggests its use as preferential immunohistochemical profiles in breast carcinomas in these species. Such characterization shows to be particularly useful in the search of primary site of metastatic carcinomas of unknown origin in dogs.

Effects of engineered Bacillus anthracis toxin on canine osteosarcoma: in vitro studies.

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Introduction

The osteosarcomas represent one of the most common malignancies diagnosed in dogs. This study was created to evaluate the therapeutic potential of reengineered Bacillus anthracis toxin, activated by urokinase (uPA) and matrix metalloproteinase (MMP) in preclinical trials in osteosarcomas dogs. The uPAs and MMPs are overexpressed proteases in several tumor types, in aggressive metastasis and are rarely present in normal cells. Therefore, the anthrax toxin was reengineered to be activated only by the uPA and/or MMPs, acting selectively in tumors.

Material and Methods

Researchers constructed mutated versions of protective antigen (PA), in which the cleavage furin site with protease present in the cell membrane was replaced with a cleaved sequence selectively only by MMPs (PA-L1) or uPAs (PA-U2), that when overexpressed on the surface of tumor cells, leading to internalize FP59 (a modified version LF with higher toxicity than LF), resulting in several direct cytotoxicity to tumor cells. The toxins were tested in canine (D17) and human (MG63) osteosarcoma cell lines commercially available and in primary canine osteosarcoma cell line (CL3).

Results

Our results of optical density showed that in three cell lines the toxin are effective to cell death, more in FP59 than LF (MG63 – 0.246 for control, 0.074 for LF and 0.010 for FP59; CL3 – 0.228 for control, 0.104 for LF and 0.014 for FP59; D17 - 0.236 for control, 0.225 for LF and 0.021 for FP59), as expect.

Conclusion

We concluded that this anthrax toxin it is a potential drug therapy for the treatment of canine osteosarcoma.

EGFR superfamily expression and its relation to histological grade in ductal carcinomas in situ of the canine mammary gland

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Introduction

Mammary carcinogenesis involves the development of ductal carcinomas in situ (DCIS) evolving towards invasive carcinomas, associated to changes in the expression of epidermal growth factor receptors and adhesion molecules. The objective of this study was to correlate the expression of Egfr, Her-2, Her-3, and Her-4 with histological grade in DCIS of the canine mammary gland.

Material and Methods

Samples obtained from bitches submitted to surgical excision, sent to the Laboratory of Comparative Pathology of the Federal University of Minas Gerais, Brazil, for histopathological analysis and followed by histological grading of DCIS. Immunohistochemistry for Egfr, Her-2, Her-3, and Her-4 was performed.

Results

Histological grade of DCIS presented a moderate positive correlation with Her-2 and cytoplasmic Her-4 expression (r=0.3487, p=0.0163; r=0.3732, p=0.0210, respectively). Her-2 expression was higher in high grade DCIS when compared to low grade DCIS (score=2,031+0,897; score=1,333+0,899, respectively, p<0,05). Positive moderate correlations were observed between membrane Her-4 and nuclear, cytoplasmic, and membrane Her-3 expression in high grade DCIS (r=0.5307, p=0.133; r=0.4898, p=0.0242, respectively). Positive strong correlations between membrane Her-4 and cytoplasmic and membrane Her-3 expression in low grade DCIS (r=0.700, p=0.0358). Nuclear staining for Her-4 was not observed, regardless of histological grade.

Conclusion

Neoplastic progression was directy related to higher expression of Her-2 and Her-4. Furthermore, the observed correlation between membrane Her-4 and Her-3 may be related to higher activity of Her-3 when associated to another receptor of the superfamily, as described in murine mammary tumors. Neoplastic progression may be associated to loss of Egfr and Her-3 expression in neoplasic epitelial cells

Evaluating the intratumoral microvascular density and Ki67 expression in canine soft tissue sarcomas

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Introduction

Cutaneous soft tissue sarcomas (STS's) are a heterogeneous group of mesenchymal tumors that shows a high local recurrence rate. Anti-angiogenic therapies such as metronomic chemotherapy have shown to be promising neoadjuvant treatment to unresectable tumors. However, few studies evaluated the microvascular density (MVD) in canine STS's. This study aimed to evaluate the MVD and proliferative index in canine STS's.

Material and Methods

Thirty-one (n = 31) cutaneous STS's samples were used and graduated (grade I, II and III). The MVD was evaluated using immunohistochemistry (IHC) against von Willebrand factor (DAKO, polyclonal, 1:1000) and the cellular proliferation was evaluated using Ki67 antibody (DAKO, MIB-1; 1:50). IHC analysis was performed using ImageJ software. Five fields at 20X magnification was used for vascular area; Ki67 positive cells were scored and mitotic figures counted.

Results

We identified ten tumors showing grade I, ten tumors grade II and eleven tumors grade III. There was no significant difference among the DMV and histological grade, mitotic index (MI) or Ki67 staining (P>0.05). However, it was observed a tendency of higher MVD in tumor showing necrosis (MVD mean = 0.014) compared to those without necrosis (MVD mean = 0.007). We identified a positive relation between the tumor grade and Ki67 staining (P=0.008) and the histological grade and MI (P=0.01).

Conclusion

Tumors with grades I and II showed less positive Ki67 cells compared to grade III tumors. Our results suggest that the STS's could be target for antiangiogenic therapies and the Ki67 staining showed a positive relation with tumor grade and mitotic index.

Evaluation of fibroblast growth factor 18 (FGF18) presence in animal ovarian tumors

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Introduction

FGF18 is an important regulator of ovarian function, acting, e.g. as a regulator ofestradiol secretion, follicular atresia, and apoptosis processes, however, its role in cancer is poorly studied. Thus, the aim of this study was to evaluate the presence ofFGF18 in ovary tumors from different animal species and its possible role in these neoplasms.

Material and Methods

Forty-five tumor samples from four animal species (canine, feline, equine, and bovine) which wereobtained fromsurgery and necropsy procedures at associated institutions were analyzed. The samples were classified as tumors of epithelial origin: rete ovarii (2), adenoma (2), cystadenoma (5), adenocarcinoma (3); germ cell tumors: dysgerminoma (8); gonadal stroma tumors: granulosa cell tumors (15), Malignant granulosa cell tumors (6), malignant granulosa and theca cell tumors (1) and luteoma (2); tumors from another origin: hemangiosarcoma (1). Immunofluorescencewas applied to identify FGF18 in the samples whilebovine's ovarieswere used as positive controls.

Results

The immunostaining for FGF 18 varied among the different tumors and species, such as granulosa cell tumors in different species, canine luteoma and canine dysgerminoma, which showed pronounced staining. The stainings were predominantly mild in tumors of the epitheliumsurface.

Conclusion

Taken together, considering literature reports, FGF18 appears to exert arole in ovarian cancer, mainly as a stimulant factor for stroma development and for the process involved in thedevelopment of thetumoral microenvironment.

Fibroblast growth factor 18 (FGF18) in mammary tumors of dogs and cats.

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Introduction

Among the breast lesions, tumors are considered the most predominant, with high prevalence in female dogs and cats. Recent studies state that FGF2 and FGF8 are present in women breast tumors. Our previous results found FGF18 in ovarian tumors. Thus, we investigated the presence of FGF18 in canine and feline breast tumors and their role in tumor development, so that these findings could improve the diagnosis, prognosis and treatment of such injuries in the future.

Materials and Methods

Immunofluorescence was applied on histological sections of normal and neoplastic canine and feline breast tissue, totaling five cases analyzed for any kind of tumor.

Results and Discussion

Benign tumors of female dogs did not show fluorescence signal, as well as all types of queen tumors. In the other hand, malignant tumors of emale dogs demonstrated signal in myoepithelial and epithelial components. This result indicating that, besides there are different elements involved in the tumoral process, these vary according to species and malignancy. As for tumors in female cats, it is suggested that another modulator element is involved, so that tumor characteristics differ considerably from those of female dogs, with respect to the main proportion of the epithelialcomponent. Besides that, canine normal breast also got stroma signal.

Conclusion

More studies are necessary to define the real FGF18 function in malignant breast tumors in female dogs, because the signal was present in both normal breast, and in malignant tumor, but the difference was in the place where the signal appeared, indicating that FGF18 modulates tumoral parenchyma.

FOXP3 immunoexpression in feline gastrointestinal lymphoma

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Introduction

Gastrointestinal lymphoma comprises a common hematopoietic neoplasia in cats. FOXP3 is a forkhead transcription factor family member, implicated in T-cell regulation, activation and differentiation, and could be implicate in tumour aggressiveness and progression. The aim of this study was to investigate FOXP3-positive cells in feline gastrointestinal lymphoma.

Material and Methods

Immunohistochemistry was performed in 47 tumour samples of cats with gastrointestinal lymphoma for PAX-5 (Life-Technologies), CD3 (Dako) and FOXP3 (clone:157B/F4-Oxford). For FOXP3 analysis, 1,000 infiltrating lymphocytes were evaluated in the tumour microenvironment, and considered as positive at least 10% of labeled cells.

Results

It was found T-cell lymphoma in 89% and B-cell in 11%. The median of survival time was 24 and 5 months, respectively. Only 3 animals were positive for FOXP3 (two T-cell and one B-cell). One of the cats with T-cell lymphoma showed moderate expression of FOXP3 and had a longer survival, but other 3 animals with negative FOXP3 had a similar survival. The cat with B-cell lymphoma had expression of 12.9% and had a lower survival.

Conclusion

According Roncador et al. (2005) the FOXP3 expression in human lymphomas was rare and it was only detected in the reactive T-cell background and not in tumour cells, the same was observed in the present study. Our data suggest that FOXP3 expression was not related to overall survival and therapeutic response in patients with B and T-cell gastrointestinal lymphoma and this finding differ of dogs with small-cell intestinal lymphoma (Maeda et al. 2015), when a high FOXP3-positive cells density was worse in overall survival.

Frozen Section Biopsy Technique: Key findings in the Veterinary Oncology

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Introduction

The frozen section Biopsy (FSB), is an indispensable tool to assist the surgeons with intraoperative diagnosis in human medicine. Its use has not been well investigated in Veterinary Medicine. The purpose of this study was to evaluate the importance of the FSB in the diagnosis of different neoplasms in small animals.

Material and Methods

Fragments of different types of lesions in dogs and cats were used on the FSB technique to analyze differentiation between neoplasms, inflammatory, cicatricial and degenerative process.

Results

When 285 fragments were assessed by FSB investigation, it came to the result that 266 cases were neoplasms and only 19 were non-neoplastic cases. A total of 239 cases were clear margins and 6 close margins, 21 cases were considered involved margin. In 5 cases it was detected satellite growth, 6 cases the margins were not evaluated, 29 cases had lymph node metastasis and were later confirmed through paraffin histopathology evaluation. The most common type of tumors was Mastocytoma (76), Squamous Cell Carcinoma (23), Soft tissue Sarcoma (13) and Vaccine-Associated Sarcoma (12). A total of 20 cases were reevaluation of margins due to incomplete removal of close or involved margins by the surgeon in a previous surgery procedure.

Conclusion

We have confirmed that the FSB as well as its importance in the human intraoperative medicine, is also an important tool for veterinarian surgeons to assist intraoperative neoplasm surgeries and diagnosis.

HER-2, EGFR, Cox-2 and Ki67 expression in lymph node metastasis of canine mammary carcinomas: association with clinical-pathological parameters and overall survival

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Introduction

Studies about canine mammary tumors based on single molecular markers probably cannot accurately account for the heterogeneity of this disease, and the investigation of multiple molecular alterations in primary tumors and their metastases, in conjunction, has assumed great importance for the understanding of mammary tumor progression.

Material and Methods

In the present study, we selected 54 primary mammary carcinomas with lymph node metastasis (T1,2,3N1M0), 29 primary mammary carcinomas without metastasis (T1,2,3N0M0), and 25 canine lymph nodes metastasis to evaluate the immunohistochemical expression of HER-2, EGFR, Cox-2 and Ki67 and its association with clinical-pathological parameters and overall survival.

Results

Our results found a concordance between the expression of HER-2 (K coefficient: 0.250), Cox-2 (K coefficient: 0.571), and Ki67 (K coefficient: 0.397) and a discordance between EGFR expression (K coefficient: -0.195) in primary mammary carcinomas and paired lymph node metastasis. Furthermore, a high Ki67 index (>24%), large tumor size and the presence of angiolymphatic invasion in canine primary mammary carcinoma with lymph node metastasis plus the presence of extracapsular extension in lymph nodes metastasis were also related to worse prognoses and shorter overall survival ($P<0.05$).

Conclusion

In conclusion, our study demonstrates that primary mammary carcinomas with high expression of HER-2, Cox-2 and Ki67 also show high expression of these markers in paired lymph node metastasis. Moreover, the expression of these molecular markers in lymph nodes metastasis did not demonstrate a prognostic relevance.

Immunoexpression of Galectin-3 in canine tumors.

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Introduction

Galectin-3 is one of the most studied galectins and participates in several biological processes like cell proliferation, adhesion, tissue remodeling and apoptosis. Moreover, it plays key role in cancer development and progression. The expression of this protein is a prognostic marker for some human tumors, such as melanomas and cervical carcinomas. The purpose of this study was to investigate the immunoexpression of Galectin-3 in canine neoplasms.

Material and Methods

Fifteen tumors were selected for the study (osteosarcoma, melanoma, trichoblastoma, hemangioma, squamous cell carcinoma, Sertoli cell tumor, mammary comedocarcioma, mammary solid type carcinoma, mammary mioepithelioma, hemangiossarcoma, hemagiopericytoma, tricolemoma, fibroma, mast cell tumor and lipoma). A primary mouse polyclonal anti-Galectin-3 antibody (A3A12, ab2785, Abcam) was applied. For negative controls, primary antibody was replaced with normal mouse IgG under the same conditions.

Results

All tumors included in this study showed Galectin-3 positive cells, with variation in intensity and percentage of positive cells between tumor types. Both cytoplasmic and nuclear positivity were observed, in neoplastic and tumor-associated non-neoplastic cells.

Conclusion

It is known that Galectin-3 functions are related to location and despite of been synthetized in the cytoplasm, it shuttles between cytoplasm and nucleus. Furthermore, it can also be found in cell surface and biological fluids, and is expressed by fibroblasts and macrophages, corroborating our findings. Complementary studies are needed to evaluate the importance of this protein with respect to prognosis and treatment.

Immunohistochemical evaluation of KIT, Ki67 and VEGF expression in canine mast cell tumor

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Introduction

Mast cell tumor (MCT) is the most common cutaneous cancer in dogs; its biological behavior is evaluated by histopathology and Ki67 and KIT immunomarkers. Recent studies have correlated an increased expression of the VEGF with poor prognosis. The aim of this study was to evaluate the expression of Ki67, KIT and VEGF as prognostic factors in canine MCT.

Material and Methods

Thirty-four tumor samples were evaluated, graded and subjected to immunohistochemistry for KIT, Ki-67 and VEGF markers.

Results

By Patnaik et al. (1984) graduation, 11.8% of the cases were grade I, 58.8% grade II and 29.4% grade III. 23.5% of the dogs died from the tumor, 7 of which were grade III and 1 grade II. The mean survival time was 1074 days in grade I, 937 in grade II and 113 in grade III ($p<0.00001$). In assessing KIT standards, 23.5% were KIT1, 32.4% KIT2 and 44.1% KIT3. For Ki67 the mean of positive cells was 1.06% grade I, 2.68% grade II and 4.71% grade III. In VEGF the staining intensity was light (1+) in 51.6%, mild (2+) in 32.2% and intense (3+) in 16.2%. The median survival time (days) for KIT were KIT1 975, KIT2 626 and KIT3 606 ($p=0.328$); for cell proliferation, Ki67 <1% - 1074, Ki67<7% 622 and Ki67>7% 73 ($p=0.002$); and VEGF (1+) 647, (2+) 871 and (3+) 1074 ($p=0.343$).

Conclusion

We demonstrated that increased expression of Ki67 are related to a worse prognosis in canine MCT, however, KIT and VEGF expression was not significantly correlated with prognosis in this series.

Immunohistochemical markers in the diagnosis of primary liver tumors in dogs

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Introduction

Primary liver tumours in dogs are relatively rare, representing 0.6–1.5% of all tumours in dogs. With regard to histopathology, canine hepatic neoplasms are classified as hepatocellular adenomas and carcinomas, cholangiocellular adenomas and carcinomas, mixed hepatocellular and cholangiocellular carcinomas, and hepatic carcinoids. In some cases, it is necessary to perform the differential diagnosis among primary liver lesions using a panel of immunohistochemical markers. The aim of the present study was to assess and validate the immunomarkers glutamine-synthetase, HSP-70, glipican-3, agrin and arginase in primary liver tumors in dogs.

Material and Methods

Immunohistochemistry was performed in 34 primary liver tumours, being 7 hepatocellular adenomas (HCA), 17 hepatocellular carcinomas (HCC), and 10 cholangiocellular carcinomas (CC).

Results

All samples of HCC were moderately to strongly positive for glutamine-synthetase and arginase immunostaining. On the other hand, all samples of HCA and CC were negative for glutamine-synthetase and arginase, respectively. HSP-70 immunomarker showed weak to moderate positivity in 10/17 of HCC samples and was negative in HCA and CC samples. The Glipican-3 marker was negative in all samples. Agrin showed positive staining in the sinusoidal endothelial cells in HCC samples, but not in cancer cells. In CC, agrin was positive in the basal membrane around the tumor glandular structures.

Conclusion

These data showed a sensitive and specific panel for the diagnosis of primary liver tumors in dogs using the immunohistochemical markers glutamine-synthetase, HSP-70, arginase and agrin.

Mastocytosis in family the cachorro-do-mato (Cerdocyon thous) captives: diagnosis, clinical and prognosis

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Introduction

A study in family of Cerdocyon thous held captive, revealed a mastocytosis framework. Intends to report the case in this species, focusing carcinogenic factors, diagnosis, prognosis and conservation.

Material and Methods

The histopathological diagnosis was established under Kiupel et al. (2011) and immunohistochemistry for c-Kit and Ki-67 for Fonseca-Alves et al (2015).

Results

Results showed that 9 (100%) were diagnosed with canine mast cell tumors, being 6 females and 3 males. The most reported skin topography was followed by lymph node and spleen. The microscopic analysis of three (33%) cases observed 2 (22%) were diagnosed as grade II mast cell tumors (low-grade) and 1 (11%) grade I (low-grade). The reported symptoms were: diarrhea, emesis, rash, blemishes and skin nodules. The blood count and biochemical analysis showed leucopenia and eosinophilia and elevation of liver enzymes in the profile. The immunostaining in case 30030 revealed focal and diffuse cytoplasmic labeling for c-Kit and moderate immunostaining for Ki-67, characterizing the case as progression with poor prognosis. Case 30032 in the cell membrane was immunostained for c-Kit and ki-67 negative, which favors the prognosis, while the 30031 showed no immunostaining. They came to death 6 (66%) possibly due to the systemic mast cell degranulation. Only 1 (11%) had to determine the cause of death microscopically diagnosed with respiratory failure.

Conclusion

The common gastroenteritis in 100% suggests mast cell migration trend to the gastrointestinal tract in these wild canids. Familial mastocytosis diagnosed instigates genetic future studies more enlightening. As predisposing factors suggests the puliciose and chemical containment, and therefore should be avoided.

Overview of regional oncology in wildlife: implications in medicine conservationist, comparative research and only health

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Introduction

Wildlife act as sentinels of ecosystem. Knowing the degree of similarity in the genesis and human tumor progression in relation to wildlife generates treatment opportunities and conservation.

Material and Methods

It conducted a retrospective survey in the period (1962-2015) parsing it cancer cases in necropsy files from the Zoological Park of São Paulo Foundation.

Results

The results indicated that 27,500 deaths in 53 years, totaling 513 cases (2%) supported the cancer spread in (216F: 186M) and 111 cases of indeterminate sex predilection. Of these, 237 (46%) reached mammals; 211 (41%) of the birds; 61 (12%) reptiles and 4 cases (1%), amphibians. Regarding histogenic classification, we obtained: 59 cases (11%) carcinomas distributed in 44 (74%) adenocarcinomas, and 14 basal cell carcinomas and (24%) squamous; 37 (7%) sarcomas, which 13 (35%) fibrosarcomas, 9 (24%) inconclusive, 7 (19%) hemangiosarcomas, 6 (16%) mesotheliomas and neurofibrosarcomas, and 2 (5%) mixossarcomas; 3 (0.6%) melanoma; 45 (8.2%) hematolymphoproliferative which 9 (20%) leukemias, 32 (71%) lymphomas and 4 (8.9%) mastocytoma and 2 cases (0.4%) nephroblastomas totaling 146 malignant therefore represents (28%) of cases. Benign neoplasms corresponded 27 (5%) cases distributed in 10 (37%) lipomas and fibromas, 6 (22%) papillomas, 4 (15%) histiocytomas and adenomas, 3 (11%) leiomyomas, 2 (7%) polyps and hemangiomas respectively. Hence, 173 cases (34%) corresponded neoplasms and 75 cases (15%) non-neoplastic. Not referred cases for histopathology were called indeterminate and accounted for 265 (52%).

Conclusion

The carcinogenic analysis in progress suggest the participation of agents such as papilloma virus, Helicobacter pylori, Retrovirus and deserve attention concerning preventive management.

Prevalence of neoplasms associated with Pteridium aquilinum intake in cattle submitted to examinations in the pathology department of UDESC, Lages, Brazil

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Introduction

Neoplasms that affect large farm animals have become routine in veterinary clinics. Santa Catarina has a huge Pteridium (aquilinum) arachnoideum infestation and neoplasms due to its ingestion by cattle are common, resulting in squamous carcinomas of the upper digestive tract and bovine enzootic hematuria, characterized by hemorrhages and bladder tumors. This study aimed to assess the prevalence of these neoplasms in Santa Catarina plateau region, Brazil.

Material and Methods

The collected data originated from the Animal Pathology Laboratory CAV / UDESC records and consisted of diagnostics of necropsy samples and material sent to the laboratory from 1983 to 2014.

Results

Five hundred and fifty-three (553) necropsy procedures were performed during the period evaluated involving intoxication by P. arachnoideum. Of these, 284 (51%) consisted on bleeding diathesis, 186 (34%) squamous carcinomas, and 83 (15%) bladder tumors.

Conclusion

The high prevalence of neoplasms in cases of poisoning by Pteridium arachnoideum is due to the abundant plant in the pastoral areas of the region. These animals were in areas with high and/or low food supply. Taking into account the loss of productivity of these affected areas, an early intervention is necessary in these cases.

Retrospective study of canine mammary gland diseases from the Laboratory of Histopathology of Los Llanos University – from 2004 to 2011

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Introduction

The canine mammary gland is frequently affected by primary or secondary neoplasms. However, there is scarce information about these processes in Colombia. Thus, our aim was to describe demographic and histopathological characteristics of canine mammary gland tumor samples from the Veterinary Pathology Laboratory of University of Los Llanos, Colombia.

Materials and methods.

In this retrospective study, mammary gland disease cases were selected from a database of clinical histories from 2004 to 2011.

Results

From 335 total cases, 47 (14.03%) were consistent with mammary gland diseases. Of those, 20 (42.55%) were histopathological specimens and were evaluated further. Animal origin was mostly from urban areas (n=16;80%; Chi-square,p=0.0139). Age distribution was more prevalent for geriatric patients (n=12;62.5%; Chi-square,p=0.0106), older than 7 years. Sixteen cases were diagnosed as neoplasias (80%; Chi-square,p<0.0001), which mainly affected animals from mixed breeds (n=8;40%; Chi-square,p=0.0156), Poodles (n=3;15%) and Samoyeds (n=2;10%). Neoplastic origin was essentially epithelial (n=15;93.75%; Chi-square,p=0.0012). Nine cases corresponded to simple carcinomas (56.25%; Chi-square,p=0.0468), 6 to complex carcinomas (37.5%) and one osteosarcoma. Simple carcinomas were distributed as 7 solids and 2 tubular-papillary (Chi-square,p=0.182), whereas complex carcinomas were set as 5 tubular-papillary and one solid (Chi-square,p=0.2207). Epithelial neoplasias were found as Grade I(n=8;53.5%; Chi-square,p=0.2466), Grade II(n=4) and Grade III(n=3).

Conclusion

Similarly to previous reports, we have found that neoplasias are the most frequent mammary gland disease in dogs, and their incidence is increased in geriatric and mixed breed patients. We believe these findings will encourage additional research of mammary gland disease in Colombia.

Serum expression of c-kit in canine mast cell tumor: a molecular study

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Introduction

The proto-oncogene c-kit encodes a transmembrane receptor, considered important for tumor growth and progression in several cancers. C-kit overexpression associated with a variety of mutations have been described in canine mast cell tumors (MCT). The present work was carried out to study the potential of serum expression of c-kit mRNA as a tumoral marker in MCT, using reverse transcription and quantitative polymerase chain reaction (RT-qPCR).

Material and Methods

This study was performed with 48 animals, 10 without tumoral disease and 38 dogs with MCT. Blood was obtained by venopuncture, and cryopreserved in tubes containing EDTA. The total mRNA was extracted, reverse transcribed in cDNA and submitted to qPCR. All dogs underwent surgical excision of the tumor and the diagnosis of MCT was histologically confirmed and the lesions graduated. The level of c-kit transcript was standardized using the expression of beta2-microglobulin, and the relative expression calculated by DDCT method.

Results

The expression of c-kit was detected in 72,9% of the samples, including all the control samples and 25 from MCT carriers, treated or not, with overexpression in 76% of the cases (p<0,01, Mann-Whitney test).

Conclusion

In humans with mastocytosis soluble KIT levels, in particular, seem to reflect the extent and severity of disease. Here we observed that peripheral blood c-kit expression quantification is a promising method in order to identify MCT carriers, submitted or not to chemotherapy.

Similarities between humans and dogs: Identification of the claudin-low subtype in canine mammary cancer

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Introduction

In humans, claudin-low breast cancer is associated with a poor prognosis. It has been proposed that human breast cancer has strong similarities with canine mammary cancer, but the existence of this subtype was not yet described in canine mammary cancer.

Material and Methods

Here, we investigate the expression of a panel of proteins in canine mammary cancer cell lines CMT-U27 and CMT-U309, using human breast tumor cell lines from a variety of subtypes as controls: MCF-7, Luminal; BT474 and SKBR-3, HER-2-positive; MDA-MB-468, Basal-like triple negative; and MDA-MB-231, BT-549, MDA-MB-157 and Hs578T, Claudin-low triple negative. We also assessed their ability to grow in 3D culture and analysed their morphology.

Results

By western blotting, expression of claudin-3 and E-cadherin was not detected in CMT-U309 cells, nor in claudin-low human breast cancer cells. As expected, cells from other subtypes (Luminal, HER-2 and triple-negative basal-like) were positive for claudin-3. Similarly, to claudin-low human breast cell lines, CMT-U-309 cells had high levels of vimentin and HIF1-alpha. HER-2 expression was only observed in BT474, SKBR3 and CMT-U27 cancer cells. Hs578T and CMT-U309 cells have a fibroblast-like morphology, and both cells lines form fewer colonies when compared to other cell lines. Ongoing tumor analyses are focused on determining the prevalence of this subtype in canine mammary cancer.

Conclusion

This is the first time that a canine mammary cancer line (CMT-U309) was identified as claudin-low. Evaluation of similarities between mammary cancer in humans and dogs can strengthen the bridges between veterinary and human oncology.

Sonic hedgehog expression in diffuse large B-cell lymphomas in dogs

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Introduction

Sonic hedgehog (Shh) signaling is associated with tumor maintenance, growth, and chemotherapy resistance in hematological malignancies. Aberrant expression of Shh in DLBCL in human patients may indicate an autocrine pathway activation. The aim of this study was to identify the expression of Shh in diffuse large B-cell lymphomas (DLBCL) in dogs by immunohistochemistry.

Material and Methods

Thirty-three samples of lymph node from dogs with multicentric DLBCL and 8 lymph nodes from healthy dogs were included in the study. The immunohistochemistry staining was performed as previously described, using anti-Shh (Ab73958, Abcam). Protein expression was scored as negative, low or high depending on the staining signal intensity. Samples with less than 10% of cells stained were considered negative. Data were analyzed using the chi-square test.

Results

All DLBCL samples showed cytoplasmic Shh staining and it was high in 23 (70%) cases. The expression of Shh was positive in 6 of 8 (75%) of non neoplastic lymph nodes, which showed low staining in cytoplasm of follicular cells. Statistical difference was found between groups ($p<0,001$).

Conclusion

In this study, our findings suggest that Shh are aberrant and overexpressed in DLBCL in dogs, as in humans. Further studies are fundamental to elucidate the role of Shh in the carcinogenesis of this malignance.

The histological pattern of canine thyroid carcinoma affects immunohistochemical marker expression

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Material and Methods

Immunohistochemical markers (IGF-1, IGF-1R, VEGF, FGF-2, RARα and RXR) were evaluated in healthy canine thyroid glands and in follicular-compact and compact thyroid carcinomas.

Results

IGF-1, IGF-1R and VEGF expression was higher in fibroblasts and endothelial cells of compact carcinoma than in healthy glands ($p < 0.05$). Compared to follicular-compact carcinoma, compact carcinoma had higher IGF-1R expression in fibroblasts, and higher FGF-2 expression in endothelial cells ($p < 0.05$). RARα expression was higher in endothelial cells of compact carcinoma than in those of other groups ($p < 0.05$). The upregulation of these proliferation- and angiogenesis-related factors in endothelial cells and/or fibroblasts of compact carcinoma compared to healthy glands supports the relevance of stromal cells for oncogenesis.

Conclusion

The differences in the protein expression profiles associated with the histological pattern of thyroid carcinoma suggest the distinct functionality and prognosis of these types of cancer (e.g. compact vs. follicular-compact carcinoma).

The relationship between Caveolin-1, APC, E-cadherin and ß-catenin protein expression in canine prostate carcinogenesis and metastasis

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Introduction

Changes in E-cadherin, -catenin, APC and Caveolin-1 expression in prostate epithelial cells are associated with epithelial-mesenchymal transition and metastatic process. This study aimed to evaluate these proteins in different canine prostatic lesions and APC methylation pattern.

Material and Methods

10 normal canine prostates, 10 proliferative inflammatory atrophy (PIA), 10 prostate carcinoma (PC) and 4 metastases were evaluated by immunohistochemistry. For APC methylation status six normal canine prostatic tissues, six PIA and 13 PC were evaluated by pyrosequencing. Immunohistochemistry protein expression was evaluated by scores: score 1 (< 25% positive cells); 2 (26-50%); 3 (51-75%), 4 (>75%) and the methylation results were evaluated by the percentage. Comparison between diagnostic groups was made using Fisher or Qui-square test for immunohistochemistry and T-test for the pyrosequencing.

Results

Less than 25% of normal prostatic epithelial cells showed CAV-1 expression. PIA and PC showed higher expression (p=0,0003). Normal tissue was score 4 for APC and decreased expression was observed in PC (p=0,006). E-cadherin and β-catenin revealed a moderate to strong membranous staining in 100% of normal epithelial cells, and PIA, PC and metastasis showed lower membranous staining. PC and metastasis showed cytoplasmic expression of E-cadherin and β-catenin. Two PC cases and one metastasis showed nuclear β- catenin staining. Methylation analysis showed no alterations in APC gene.

Conclusion

Our results suggest a loss of membranous E-cadherin and β-catenin in PC samples. The loss of APC and gain of CAV-1 in PC samples indicates the involvement of these proteins in carcinogenic process, but APC down expression is not related to methylation process.

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SURGICAL VETERINARY ONCOLOGY POSTERS

SURGICAL VETERINARY ONCOLOGY		
ABSTRACT ID	**TITLE**	**AUTHOR**
SVO01	Clinical-surgical study, cytology and histopathology of perianal tumors in dogs.	Bruna De Castro Miranda
SVO02	Electrochemotherapy combined to surgical resection for the treatment of canine mast cell tumors	Jessica Yumi Asano Reimberg
SVO03	Electrochemotherapy in oral tumors: a multicentric retrospective study of 61 cases	Jennifer Ostrand Freytag
SVO04	Evaluation of splenic masses in dogs: a retrospective study 2014/2015	Claudia Ronca Felizzola;
SVO05	Metronomic chemotherapy as adjuvant treatment for cutaneous mast cell tumor in dogs.	Bruna De Castro Miranda
SVO06	The use of tumor marker CA 15 -3 in the evaluation of dogs with mammary tumor – partial result	Liane Ziliotto;
SVO07	Thermographic characterization of canine soft tissue sarcoma – preliminary study	Patrícia Ferreira de Castro
SVO08	Thermographic characterization of subcutaneous canine mast cell tumor (mct) – preliminary study.	Samanta Rios Melo

Clinical-surgical study, cytology and histopathology of perianal tumors in dogs.

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Introduction

Tumors of the perianal glands are common in dog, often emerge from circumanal glands and less commonly of glands of the anal sac. The focus of this study was to determine the incidence of perianal tumors in male dogs and paraneoplastic hypercalcemia, was performed cytological and histopathological study of these neoplasms.

Material and Methods

The study included 46 male dogs with perianal tumors, 8months-16 years old. Previously the surgical procedure was performed laboratory tests: complete blood count, kidney and liver function, abdominal ultrasound, electrocardiography, serum calcium, cytological evaluation by fine-needle aspiration.

Results

The highest occurrence of perianal tumors were 30,45% on mongrel patients (14/46).The aspiration cytology was inconclusive in only 22% of patients. Histopathological examination revealed 52.38% of tumors as adenocarcinomas of circumanal glands, 42,87% as adenomas circumanal glands and 4.75% as cystadenomas. No patient had tumors of origin of the anal glands bag. As the hypercalcemia 6 patients (13%) patients had adenocarcinoma of the frame, but returned to normal serum calcium 72 hours after surgical excision of the formation. There was no hypercalcemia paraneoplastic as often referred in the literature and is considered a rare condition.

Conclusion

The cytological evaluation by fine-needle aspiration had good correlation with histopathology. Hypercalcemia is considered a rare condition being resolved after excision of the neoplasm.

Electrochemotherapy combined to surgical resection for the treatment of canine mast cell tumors

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Introduction

Mast cell tumor is considered the most common malignant cutaneous neoplasm in dogs, representing 11 to 27% of all diagnosed skin tumors. Electrochemotherapy involves the application of anticancer drugs attached to electrical pulses, which increases the permeability of the tumor cells to the action of the drugs. In similar previous studies, the efficacy of this technique was observed for local control of mast cell tumors in 85% of cases. The aim of this study was to evaluate the relapse rate in animals treated with surgical excision associated with application of electrochemotherapy for extension of surgical margins.

Material and Methods

23 dogs diagnosed with mast cell tumor in the period of 2012-2015, underwent surgery associated with electrochemotherapy (bleomycin sulfate 15U/m², 1000V/cm, 8 pulses 100 µs each, 1 Hz). All dogs had compromised surgical margins to histopathology.

Results

Of the 23 animals, 19 (82.6%) had no recurrence until the date of publication of this study. Four animals (17.4%) relapsed, and the mean duration of disease-free interval was 5 months. Two animals (8.7%) have died and 1 (4.3%) related to the disease. Eighteen animals (78.3%) had grade II mast cell tumor. The median survival achieved by the date of publication of this study was 13 months.

Conclusion

Surgery with wide safety margins is the treatment of choice for mast cell tumor, but 22-54% of the cases present recurrence. In this study, the recurrence rate was 17.4%, proving the applicability of electrochemotherapy associated with surgery, especially when surgical excision with wide margins is not possible.

Electrochemotherapy in oral tumors: a multicentric retrospective study of 61 cases.

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Introduction

Electrochemotherapy, an emerging therapy in human medicine, is becoming more popular in veterinary medicine. It is the combination of electropermeabilization and drugs with reduced permeability in the cytoplasmic membrane. Presents predominantly selective action to cancer cells and, when established in oncology conduct conservative procedures are possible, especially in areas of difficult surgical planning.

Material and Methods

This study is a retrospective evaluation of 60 cases of oral tumors treated with Electrochemotherapy of three centers specialized in oncology in Brazil (Vet Cancer - Sao Paulo, Oncopet - Londrina, Covent - Maringa), between 2012 and 2015.

Results

Sixty two tumors were treated: 35 (56.5%) melanomas, 8 (12.9%) squamous cell carcinomas and the other 19 (30.6%) included fibrosarcomas, osteosarcomas, mast cell tumors, plasmacytomas, hemangiosarcomas, ameloblastomas and lymphomas. Most patients were stage III (30%) and IV (46.7%); For non melanomas median survival was 663 days; objective response of 92% and median response duration 663 days. In melanomas cases: 22.9% were stage III and 57.1% IV; 100% of objective response; median survival of 264 days and median response duration 309 days; survival rate after one year was 36.4% in melanomas. Deaths due to lack of control of oral tumor was 9 (36%) for non melanomas and 7 (20%) for melanomas. In all cases where surgery was also performed, it was conservative.

Conclusion

These results support the idea that the Electrochemotherapy expands possibilities for patients with oral cancer, providing good local control of the disease even with more conservative surgical procedures.

Evaluation of splenic masses in dogs: a retrospective study 2014/2015

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Introduction

The splenic neoplasm is common in dogs. The aim of the present study is to retrospectively describe clinical aspects and histopathological characteristics of dogs with splenic masses in two different cities of Brazil (Sao Paulo and Porto Alegre) in 2014-2015.

Material and Methods

This study was performed based on the incidence of dogs with splenic mass in two different oncological services. All dogs had been previously examined, and undergone laboratory and imaging tests before surgery followed by histopathological analysis. Metastasis, reccurrence and survival time were evaluated and statistical analysis of the cases was performed.

Results

Fifty-three dogs without sex prevalence (27 males, 26 females) were studied. 23 different breeds were affected, but malignant tumors are more common in large breeds whereas in the small ones the splenic mass was more related to endocrine diseases. Splenic masses were diagnosed histologically as non-malignant (n=31; 58,5 %) and malignant (n=22; 41,5%). Hemangiosarcoma (HSA) was the most common histological diagnosis (n=13; 24,5%). The non-malignant masses consisted of nodular hyperplasia (n=18), splenic hematoma (n=3), and splenitis (n=10). Two cases presented splenic hematoma and then developed cardiac HSA. Seven dogs (54%) with HSA had post-surgery disseminated disease and metastasis.

Conclusion

The results corroborate previous findings that hemangiosarcoma is the most frequent neoplasm of the canine spleen. However, in approximately half of the cases benign lesions were histologically diagnosed.

Metronomic chemotherapy as adjuvant treatment for cutaneous mast cell tumor in dogs.

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Introduction

The metronomic chemotherapy changes the tumor microenvironment and suppresses innate characteristics that support the growth of the tumor. The focus of this study was to analyze the use of metronomic chemotherapy with lomustine in the treatment of cutaneous mast cell, to study the side effects and the therapeutic action.

Material and Methods

16 dogs were selected with diagnosis of cutaneous mast cell tumors, they were divided into two groups. Group 1 was treated with vinblastine with a dose of 2 mg/m² and prednisone for 12 sessions and Group 2 received metronomic chemotherapy with a dose of 2.84mg/m²/VO of lomustine for 4 months. Side effects were evaluated according with VCOG-CTCAE criteria.

Results

62.5% (5/8) of the patients of VP group showed some type of side effect. The most frequent side effect was leukopenia, occurring in four patients (4/8). 25% (2/8) of the patients of LP group had some kind of side effect, only one patient experienced adverse events of grade III. No one developed side effect of hepatotoxicity. Didn´t differ adverse events between the VP and LP groups, in both of them, Fisher's test ($p<0.05$). 12.5% of the patients died on account of mast cell tumors. The median survival from the VP group to date are of 240.5 days (120-525 days) while the LP group the survival average was 210 days (89-365).

Conclusion

The metronomic chemotherapy is practical, well tolerated and has few side effects.

The use of tumor marker CA 15 -3 in the evaluation of dogs with mammary tumor – partial result

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Introduction

The mammary tumors are one of the most common neoplasms in dogs, ranging from 50% to 70% of all tumors in a population, with a great impact on veterinary medicine. The epidemiological characteristics already identified are: age, hormonal exposure and race. The tumor markers are biological macromolecules, mostly proteins or its part, present in the tumor, in blood or other body fluids. The neoplastic cell growth leads to its appearance or changes in the markers concentrations. The antigen CA 15-3 has been used in the women breast cancer follow-up, showing up as the most sensitive marker. The aim of this study was to evaluate the effectiveness and viability of the tumor marker CA 15-3 in dogs with mammary tumors, looking for a new method for early diagnosis of the tumor and the metastasis disease.

Material and Methods

Was rated the serum of five animals with mammary neoplasms from the Veterinary Teaching Hospital (Unicentro) clinical routine, and these forwarded for analysis. Samples were taken from patients prior to mastectomy and after 10 to 20 days after mastectomy.

Results

In the pre-average mastectomy samples and the standard deviation of the tumor marker found was 1.48 ± 0.35 U/mL. The samples post-mastectomy were 0.82 ± 0.14 U/mL.

Conclusion

With these results we observed that the tumor marker CA 15-3 presents important variation in two moments and can be considered as an aid to the diagnosis of mammary neoplasia in dogs and new investigate should be done for its use in the early diagnosis of metastasis and prognostic

Thermographic characterization of canine soft tissue sarcoma – preliminary study

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Introduction

Soft tissue sarcoma (STS) describes different types of mesenchymal origin tumor with poorly defined margins. Thermography is noninvasive imaging diagnostic method approved for use in human oncology medicine. The focus of this study was to characterize the STS based on thermographic images.

Material and Methods

We used Thermal Camera FLIR T650SC to obtain infrared images of six dogs with STS and analyzed by Flir Quick Report Software the following parameters: middle point of the tumor (TP), a peripheral point away from the tumor (NTP), elliptical area encompassing the tumor (TA) and elliptical area of equal size to TA, as far as possible from the tumor beyond surgical safety margins (NTA).

Results

The mean temperature of all tumors in middle point and elliptical area was 33.82ºC and 33.37ºC and for non-tumor tissue (NTT) 34.07ºC and 33.83ºC, respectively. The ratio was the same of tumors were warmer (3/6) or colder (3/6) than NTT. There was difference of more than 1ºC from tumor and NTT for point and area in 83% of cases (5/6) and more than 2ºC in 33% (2/6); considering only the area range was 0.33 to 3.32 degrees, and 66% (2/3) of recurrent tumors had this difference of more than 3ºC. As regards the histopathological analysis 50% (3/6) was classified as histological grade II, 33% (2/6) grade III and 17% (1/6) grade I and 50% of the cases (3/6) it was tumor recurrence.

Conclusion

The thermal difference between STS and NTT showed us that thermography could be a new tool in surgical planning of STS.

Thermographic characterization of subcutaneous canine mast cell tumor – preliminary study

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Introduction

Studies indicate that majority of subcutaneous mast cell tumors (SMCT) have favorable prognosis, lower rates of recurrence and metastasis. The focus of this study is to characterize SMCT based on thermography and histopathological patterns.

Material and Methods

We analyzed 10 thermographic images of canine SMCT, with Thermal camera FLIRT650 and Flir-ResearchIR Software. We measured mean temperatures of central tumor point (SpT); and of healthy skin - away from the surgical margin (SpNT); tumoral area (TA) and non-tumoral area (NTA) mean temperature.

Results

Treatment was based on mastectomy (MAST=36/42 bitches, 15/16 cats) and/or chemotherapy (CHEMO =14/34 NI, 6/8 IMC and 13/16 cats). Protocols included (doxorubicin=8, doxorubicin+cyclosphophamide=6, doxorubicin+carboplatin=6, cats received only doxorubicin). Mean number of CHEMO session for NI, IMC and cats were 4 (1 to 6), 3,3 (1 to 9) and 3,4 (1 to 6), respectively. Overall survival (OS) was correlated to short survival in IMC compared to NI patients (Log Rank Test $p<0.001$). OS between bitches and cats was statistical significant (Log Rank Test , $p=0,014$). At the end of follow up, 17/42 of dogs were alive. All IMC and 10 NI patients (41,8%) died from causes related to cancer. Survival mean time, in months, was 6,25(+2,8) for NI(MAST), 7,5(+7,6) for NI(MAST+CHEMO) and 4(+3,9) for IMC. Fifteen cats had died at the end of study, 68,7% related to cancer, survival mean time was 6,2 (+6,7).

Conclusion

Preliminary findings demonstrate new possible tool for surgical planning, invasiveness and activity for the SMCT. We found that these tumors can be more aggressive and invasive then published in current literature. Further studies will include Ki67, KIT and VEGF-A marking for SMCT.

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VETERINARY CANCER EPIDEMIOLOGY POSTERS

VETERINARY CANCER EPIDEMIOLOGY		
A B - STRACT ID	**TITLE**	**AUTHOR**
VCE01	A cross-sectional study of mammary neoplasia in 212 female dogs	Fernanda Duarte Malatesta Ferreira de Almeida
VCE02	A retrospective multicentric study of oral cancer in dogs – 330 cases	Priscila Pedra Mendonça
VCE03	Association of canine mammary neoplasia with urban air particulate matter	Fernanda Duarte Malatesta Ferreira de Almeida
VCE04	Epidemiological analysis, diagnosis and treatment of uterine neoplasias in the canine and feline species	Kilder Dantas Filgueira
VCE05	Non-venereal genital neoplasias of female dogs: a retrospective study	Kilder Dantas Filgueira
VCE06	Ovarian neoplasias in dogs: ten-year study	Kilder Dantas Filgueira
VCE07	Prevalence of cancer and others organic disorders in dogs with increased body weight	Thais Andrade Costa Casagrande
VCE08	Retrospective study of clinical and histopathological aspects of feline mammary tumors	Verônica Mollica Govoni
VCE09	Thyroid gland carcinoma in 11 dogs: A retrospective study.	Bruna Fernanda Firmo
VCE10	Transmissible venereal tumor: retrospective study (2010-2015) of 127 dogs	Thais Almada Nobre de Mello

A cross-sectional study of mammary neoplasia in 212 female dogs

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Introduction

Mammary tumors are the most common neoplasms of female dogs and have shown considerable increase in the last years. We aimed to evaluate the epidemiological behavior of canine mammary tumors in dogs from a Veterinary Oncology Clinic in São Paulo city, Brazil.

Material and Methods

The study includes 212 dogs. Data were obtained retrospectively from medical records between 2008 and 2011. We included all dogs with histologically diagnosed mammary tumor or clinically diagnosed inflammatory carcinoma. An epidemiological survey was applied with social-demographic data, routine of life and clinical patient records. Data were submitted to Chi-square test and was tested by SPSS 20.0 software for Windows

Results

The mammary cancer diagnosis was confirmed upon histopathological evaluation of 198 cases, with the exception of 13 cases where the diagnosis was clinic, since those were inflammatory carcinoma suspect cases. It proved to be a condition that affects females at old age (9.58±2.64years), predominantly purebreds (poodles-22.2%), presents high morbity and mortality rates (59.4%), delayed diagnosis, occurs more often in non-neutered females (63.4%), with no association with type of diet, pseudopregnancy and use of contraceptives. Tubulo-papillary and complex carcinoma subtypes comprise 60% of the cases, followed by solid carcinoma. Solid carcinoma displayed a more aggressive behavior, increased invasiveness, lower survival rate and worst prognostic. Complex carcinoma presented lower number of metastases ($p<0.001$).

Conclusion

This data strengthens the literature, where the progression of canine mammary tumors is influenced by the hormonal status, clinical staging at the diagnosis and histological type of the neoplasia.

A retrospective multicentric study of oral cancer in dogs – 330 cases

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Introduction

The frequent occurrence and high morbidity and mortality of oral cancer in dogs justifying characterize risk populations. The aim of this study was to evaluate the epidemiological profile of dogs with oral cancer.

Material and Methods

Retrospective, prospective and multicenter study with oral cancer dogs in two dental centers and two cancer services (2010-2013). Assessing the variables gender, reproductive status, age, life stage, breed, baseline weight, dog size, location and tumor size and more frequent histological types and clinical staging.

Results

303 medical records were analyzed. Malignant neoplasms (67%) were more frequent, malignant melanoma (43%) in small and medium-sized animals; among benign neoplasms (20%), was ameloblastoma (49,2%) in medium and large. No breed or gender preference and unneutered were more involvement by malignancy. Average age of malignant neoplasms dogs was 11.22 years and 9.53 in benign. Average weight dog with malignant cancer was 17,13kg and benign tumor dogs with 22,4kg. The most frequent site was jaw (35.5%), and the average malignant tumor size was 3,52cm and benign 1,53cm. Patients with malignant neoplasm, geriatric have higher odds of death and the stage IV was the most frequent. Senile and geriatric patients, small and unneutred females are more affected by malignant tumor, geriatric patients at higher risk of death from malignant tumor.

Conclusion

There was a greater chance of involvement of unneutered animals by malignant neoplasms, showing that castration may be a protective factor for malignant oral cancer, particularly in spayed females, further studies should be conducted to explain this feature.

Association of canine mammary neoplasia with urban air particulate matter

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Introduction

Mammary tumors have shown considerable increase in the last years. Reviews suggests that urban air pollution is associated with malignancies. Among pollution sentinels, the dog has been adequate for presenting level of pollution exposure similar to its owner. We evaluated the epidemiological behavior of canine mammary tumors and the association with atmospheric particulate matter (PM10) in dogs from a Veterinary Oncology Clinic in São Paulo city, Brazil.

Material and Methods

This study includes 212 dogs. Data were obtained from medical records between 2008 and 2011 and supplemented with telephone call. Exposure of pollution was estimated by PM10 annual average provided by CETESB (Governmental Agency of Pollution Control). Pollution exposure buffers were stratified through spatial analysis. Data were submitted to Chi-square test. Alpha was set at 5% and in PM10 10%.

Results

PM10 associated with poor prognosis (p=0,07). Spatial analysis showed a predominance of cases in the south area of the city (68,4%), greater concentration on medium exposure streets (51,4%) and poor prognosis (p=0,005) in animals subjected to high exposure.

Conclusion

Epidemiological studies also associate the increase in breast cancer incidence in women living in different cities from different continents with vehicle-emitted air pollution levels. The relation between cancer and air pollutants is physiopathological suggested by induced mutagenesis; however, this association still needs to show causal evidence. We concluded that mammary cancer from dogs living in São Paulo presented aggressive behavior, late diagnosis, and urban air pollution exposure may be associated with its development.

Epidemiological analysis, diagnosis and treatment of uterine neoplasias in the canine and feline species

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Introduction

Uterine neoplasias are rare in dogs and cats, with a frequency below 1% of all tumors that affect both species. Usually, detection is incidental, because such neoplastic proliferations are generally asymptomatic. In this sense, the objective was to establish a profile of uterine tumors of dogs and cats.

Material and Methods

Data were collected retrospectively through medical records from the Veterinary Hospital of UFERSA, between the years 2004 to 2014. For each patient data were collected relating to species, age, breed, use of contraceptives, histological classification and treatment. The data was distributed in medium and frequencies.

Results

In the period analyzed, four (50%) bitches and four (50%) queens were affected. The average age of females was nine years. Four (50%) patients had defined breed and other (4/50%) were mongrel. In most (7 / 87.5%) of animals analyzed applied exogenous progestin. Histopathology was detected four (50%) leiomyosarcomas, two (25%) leiomyomas, one (12.5%) adenocarcinoma and one (12.5%) adenomyoma. For five (62.5%) patients were established surgical sterilization and three (37.5%) there was no therapeutic adoption.

Conclusion

Advanced age and the use of exogenous progestational substances could justify the genesis of uterine tumors in both species. Histopathology was essential for the differentiation of the various morphological patterns and different biological behaviors of assessed uterine neoplasias. Surgical sterilization is the treatment of choice, supporting the therapy used in the majority of cases in this study.

Non-venereal genital neoplasias of female dogs: a retrospective study

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Introduction

The transmissible venereal tumor (TVT) is the most frequent genital neoplasm in dogs, native to tropical areas. However, other neoplasias may affect the genitalia of female dogs. The aim of this study was to establish a profile of non-venereal genital neoplasias in female dogs.

Material and Methods

The Information was obtained from medical records, of the Veterinary Hospital of UFERSA, from 2009 to the first semester of 2015. For each patient data were collected relating to age, breed, duration, growth rate, region committed, histological classification and treatment. Data were distributed as frequencies.

Results

Six female dogs were affected. The average age was seven years and six months old. Four (67%) animals hada defined breed and two dogs (33%) were mongrel. The average period of evolution of the lesions corresponded to eight months. The growth rate varied from low (50%) to fast (50%). Three (50%) cases involved the vagina, two (33%) the vulva and one (17%) the uterus. Histopathology was detected in two cases (33%) fibroleiomiomas, and also the plasmacytoma (1 / 16.75%), myxoma (1 / 16.75%), mast cell tumor (1 / 16.75%) and leiomyoma (1 / 16.75%). In four (67%) patients, surgical therapy was established and in two (33%) there was no therapeutic procedure.

Conclusion

Older age and a possible breed bias could justify the genesis of tumors, which mainly affect the external genitalia, in a relatively short time. The diagnosis based only by the anatomical location leads to erroneous diagnoses and treatments. Histopathology was essential to differentiate from ordinary canine TVT.

Ovarian neoplasias in dogs: ten-year study

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Introduction

The ovarian tumors are uncommon in dogs. In general, detection is incidental for routine sterilization of surgical practice. The aim of this study was to establish a profile of a decade of ovarian neoplasias in female dogs.

Material and Methods

Information was obtained from medical records of the Veterinary Hospital of UFERSA, between the years 2006 to 2015. For each patient such data was collected: age, breed, contraceptive use, disposal unilateral or bilateral lesion, histological classification and treatment. Data was distributed in mean and frequencies.

Results

In the reporting period, six dogs were affected. The average age was seven years. Four (67%) patients had defined breed and the rest (2/33%) were mongrel. Four (67%) females were not applied exogenous progestin and two (33%) the drug was used. Most (4/67%) patients had tumor only unilaterally, with the more involved the right ovary (3/75%). Histopathology, two (33%) papillary adenomas, one (16.75%) papillary adenocarcinoma, one (16.75%) granulosa cell tumor, one (16.75%) leiomyosarcoma and one (16.75%) granulosa cell tumor associated with papillary adenoma were detected. For all (100%) patients ovariectomy was established.

Conclusion

Older age and a possible racial bias could justify the genesis of neoplasias, unlike the use of exogenous progestin. There was a trend for the commitment of only one gonad. Histopathology was essential to differentiate the morphological patterns and biological behavior of tumors examined. The ovariectomy is the treatment of choice, supporting the therapy used in this study.

Prevalence of cancer and other organic disorders in dogs with increased body weight

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Introduction

The deleterious effects of obesity are well known in humans and most recently in animals. An important factor is the chronic inflammatory process, produced by the change of cytokines from adipose tissue. Comorbidities affect almost all organic systems, leading to the orthopedic disorders, hypertension, immunosuppression, increased insulin resistance, dyslipidemia, skin diseases, cancer and lower reproductive efficiency.

Material and Methods

Thus, this study aimed to assess the prevalence of cancer and other organic disorders in patients with increase body weight, by the analysis of clinical records from patients treated between September 2009 and November 2014. The selection of overweight animals was performed by Body Condition Score, which scores 6-7/9 were considered over ideal weight and 8-9/9 obese. The charts were evaluated for the main owner complaint and diagnosis obtained by anamnesis, clinical examination and laboratory examination and/or imaging test.

Results

2500 medical records were reviewed and 374 animals were considered overweighted (305 classified as overweighted and 69 as obesity). Only 6.7% and 22.24% of pet owners with overweight and obesity, respectively, reported as the main complaint weight gain, which indicates that this change still seems to be less important to most of people. Oncological disorders were the most prevalent in overweight animals (21.65%) followed by dermatological (17.38%) and orthopedic disorders (11.28%). Endocrine disorders were the most prevalent in animals with obesity (25.4%), mainly by the presence of dyslipidemia. Secondly orthopedic disorders appear as the most prevalent (16.4%), followed by oncological (13.23%) and skin disorders (10.05%). Obesity patients also had more diseases association in the different systems.

Conclusion

Obesity can be related to organic disorders, including cancers.

Retrospective study of clinical and histopathological aspects of feline mammary tumors

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Introduction

Feline mammary neoplasms are the third most common reported tumor in this species with a mean age at diagnosis of 10–12 years. Feline mammary carcinomas are frequently associated with an aggressive clinical behavior, it has been reported that between 80% and 96% of mammary tumors in cats are malignant. The aim of this study was to perform a retrospective study of clinical and histopathological parameters in feline mammary tumors.

Material and Methods

We selected 52 felines with mammary tumors in the Public Veterinary Hospital from ANCLIVEPA, between 2012 and 2015. Data were collected from medical records and the histopathological grading was performed according to WHO criteria.

Results

The average age of the patients was 11 years; 96.2% were females; 73.1% were crossbreed and 26.9% were Siamese; 69.2% were castrated; 28.9% had history of long-term progestin treatment. The tumors were presented as single mass in 69.2% of the cases and 48.1% were ulcerated. The most prevalent histopathological subtype was tubular carcinoma (28.8%), followed by solid carcinoma (18.6%) and tubulopapillary carcinoma (11.9%). Of the total, 36.5% had metastasis. The treatment used in 96.2% of the animals was radical mastectomy, being associated with chemotherapy with doxorubicin or carboplatin in 23.1% of patients.

Conclusion

The study showed several clinical and histopathological features consistent with those described in literature. Additionally, the use of contraceptives was demonstrated as potential risk factor for breast tumors development in cats.

Thyroid gland carcinoma in 11 dogs: A retrospective study.

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Introduction

Thyroid tumors are uncommon in dogs, accounting for 1.2% to 3.8% among neoplasms. There are no Brazilian data of this disease in dogs.

Material and Methods

This retrospective study reviewed the pathological records of dogs referred for thyroid neoplasia between 2004 and 2014. Data extracted included age, breed, sex, histopathological type, vascular and/or lymphatic invasion, survival and outcome.

Results

Of 3317 pathological cases 11 (0,33%) were of thyroid carcinoma in dogs. The median age was 8,91 years (range 5-13 years). Regarding the breed three (27,28%) were mongrel dogs, two (18,18%) Labradors Retrievers, one (9,09%) Australian Cattle Dog, one (9,09%) Boxer, one (9,09%) Cocker, one (9,09%) Siberian Husky, one (9,09%) Maltese, one (9,09%) Pinscher. Eight (72,73%) were females and three (27,27%) were males. The histopathological type of thyroid neoplasia was 6 (54,55%) follicular-compact thyroid carcinoma. Three (27,27%) compact (solid) thyroid carcinoma. And 2 (18,18%) was undifferentiated thyroid carcinoma subtype giant cells. Seven (63,63%) cases had vascular and/or lymphatic invasion. This 3 (50%), 2 (66,66%) and 2 (100%) were follicular-compact thyroid carcinoma, compact (solid) thyroid carcinoma and undifferentiated thyroid carcinoma respectively. The median survival time were 17,75 months (range 1,5-31 months). Regarding the outcome 5 (45,46%) were euthanized, 3 (27,27%) was alive and 3 (27,27%) had no information.

Conclusion

Thyroid carcinoma had low incidence (0,33%), the median age were 8,91 years, there are no breed predisposition, females were more affected. The follicular-compact thyroid carcinoma was the more common type and high level of vascular and/or lymphatic invasion was found. Survival time varied widely.

Transmissible venereal tumor: retrospective study (2010-2015) of 127 dogs

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Introduction

Transmissible venereal tumor (TVT) may have external genitalia localization and infrequently present extragenital primary lesions or metastasis.

Material and Methods

Retrospective study of five years evaluated TVT localization, incidence of metastasis and chemotherapy protocol. Patient that were lost in follow-up had treatment considered incomplete when received at least four doses, and lost when received less than four.

Results

Data was obtained from 127 dogs (84 females, 44 males), mainly mongrel breed (58/84 and 37/44), external genitalia localization (82/84 and 32/44) and only one male dog confirmed with spleen metastasis. Extragenital localization in female was nasal (1/84). Male dogs presented nasal (7/44), cutaneous (2/44), genital, cutaneous and conjunctival (1/44), systemic (1/44). After confirmative cytology, weekly chemotherapy with vincristin (0.5-0.7mg/m2) intravenously was performed until absence of neoplastic cells, except one dog with vincristin resistant spleen metastasis, which was treated with doxorrubicin (30mg/m2) intravenously every 21 days. Medical discharge was observed in 23 female dogs (age 4.9±3.3years; weight 15.3±9.9 kg; total doses 9.3±4.1) and 16 male dogs (age 4.8±2.4 years; weight 19.9±8.2 kg; total doses 7.2±1.7), without significant difference, respectively ($p=0.7$; $p=0.07$; $p=0.1$). Significant difference was observed ($p=0.001$) between female (95.6%) and male (50%) leucopenic patients. Incomplete treatment was observed in 21.4% of female and 34% of male dogs. Treatment loss occurred in 51.2% female and 29.5% dogs.

Conclusion

The incidence of visceral metastasis was rare. The high number treatment loss or incomplete treatment, contributes to chemotherapy resistance and therapeutic failure with tumor persistency and possibility of metastasis.

WVCC meeting during the 2nd WVCC in Paris, 2012.

Sandi Strother (VCS), Julia Maria Matera (ABROVET), Barbara MacGhee (VCS), Johan de Vos (ESVONC), Maria Lucia Zaidan Dagli (ABROVET), Ruthanne Chun (VCS), Laura Garrett (VCS).

THANK YOU!

Hope you enjoyed the 3rd WVCC!

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3rd WVCC 2016

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