Proceedings of the 2010 Annual Congress of the European Society of Veterinary Oncology

18th – 20th March 2010

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Turin
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OUTLINE PROGRAMME

Venue: Principi di Piemonte****, Via Gobetti, 15 - 10123 Turin

Thursday 18th MARCH 2010		**PRE-CONFERENCE SYMPOSIUM "INTRA-THORACIC TUMOURS"**
1230	**REGISTRATION**	
	Welcome	**Prof Paulo BURRACO**
1300 – 1700	Dr Federica ROSSI	Imaging of Intra-thoracic Tumours
	Prof Jolle KIRPENSTEIJN	Thoracoscopy
		Surgery of Intrathoracic Tumours in Dogs and Cats
	Dr Nadja SIGRIST	Peri-Operative Care for Intra-thoracic Tumour Management

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Thursday 18th MARCH 2010 **MAIN CONGRESS**

1830-2000 **Welcome Reception and registration**

Sponsored by Pfizer Animal Health

Friday 19th MARCH 2010 **MAIN CONGRESS**

0730	**REGISTRATION**	
0800	**Welcome**	
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		METRONOMIC CHEMOTHERAPY
		Sponsored by Pfizer Animal Health
0930-1230	**ESVONC Junior Research Award – abstracts**	
1240-1355	**LUNCH (on your own)**	
1400-1515	**ESVONC Junior Research Award – abstracts (continued)**	
1515-1600	**Master Class**	**Dr Miriam KLEITER & Prof Jolle KIRPENSTEIJN**
		WHEN SURGEON AND RADIOTHERAPIST WORK TOGETHER
		Sponsored by Pfizer Animal Health
1630-1720	**General Abstracts**	
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Saturday 20th MARCH 2010

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		SENTINAL LYMPH NODES AND MARGINS OF EXCISIONS
		Sponsored by Pfizer Animal Health
0800- 0930	**General Abstracts**	
1230-1330	**LUNCH (sandwiches)**	
1330-1425	**Keynote speaker**	**Prof Rainer STORB**
		BONE MARROW TRANSPLANTATION IN DOGS
		Sponsored by Pfizer Animal Health
1430-1600	**General Abstracts**	
1600	End of Scientific Session	
1630-1800	**Annual General Meeting**	
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1930	**Reception** *(part sponsored by Alcyon Italia)*		
2000	**Gala Dinner** *(part sponsored by Merial)* *Salone della Feste, Hotel Principi di Piemonte*		

FUTURE CONGRESSES

2011 Glasgow, Scotland 24^{th} – 26^{th} March

2012 Paris, France **2nd World Veterinary Cancer Congress** 22^{nd} – 24^{th} March

2013 Portugal March 2013

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KEYNOTE SPEAKERS

Prof Deborah W. Knapp, DVM, MS, DiplACVIM

Dolores L. McCall Professor of Comparative Oncology
Veterinary Teaching Hospital, School of Veterinary Medicine,
University of Purdue

Dr. Knapp received her BS degree from North Caroline State University and her DVM degree from Auburn University in 1983. She worked in small animal practice in Anchorage, Alaska and Wilmington, North Carolina before pursing residency training in Veterinary Medical Oncology at Purdue University. Dr. Knapp became board certified in Veterinary Medical Oncology by the American College of Veterinary Internal Medicine, earned a MS degree from Purdue University with her thesis work focused on the antitumor effects of nonsteroidal anti-inflammatory drugs. This was followed by a Post Doctoral Research Fellowship in Cancer Pharmacology. Dr. Knapp joined the faculty in the Dept. of Veterinary Clinical Sciences at Purdue University in 1990.

Dr. Knapp is currently the Dolores L. McCall Professor of Comparative Oncology at Purdue University, West Lafayette, IN. She is Chief of the Oncology Service, and Co-Director of the Purdue Comparative Oncology Program. She serves on the Executive Committee of the NCI-designated Purdue Cancer Center, and is a member of Purdue's Oncological Sciences Center. The research work her group performs is internationally recognized and is aimed at improving the outlook for pet animals and humans with cancer.

Prof Giuseppe Viale

Professor of Pathology and Chairman, Director of the Department of Pathology and Laboratory Medicine
European Institute of Oncology and
University of Milan School of Medicine
Milan, Italy

Prof Viale graduated from the University of Milan in 1976. He became specialist in pathology in 1979, and is now full professor of pathology at the University of Milan School of Medicine. He has been director of the Division of Pathology and Laboratory Medicine at the European Institute of Oncology in Milan since 1994. A member from 1987, he was elected Fellow of the Royal College of Pathologists (FRCPath) in 1997.

Scientific interests include the validation of predictive and prognostic markers in breast cancer, the abnormal expression of oncogenes and tumour-suppressor genes in human malignancies, derangements in cell-cycle control and apoptotic pathways, and the role of angiogenesis in tumour progression and response to therapy.

He is the chairman of the Central Pathology Office and of the Biological Protocol Working Group of the International Breast Cancer Study Group (IBCSG), Lead Pathologist of the Breast International Group (BIG), and Co-chair of the Translational Research Committee of the BIG2-98 trial (Trans-TAX).

He is also an active member of many international societies, organizations and committees such as the AJCC working group for the TNM classification of breast cancer, the Pathology Sub-study Group of the Intergroup Exemestane Study (PathIES), the Executive and Steering Committees of the ALTTO trial (acting as co-chair of the International Pathology Committee and responsible for the Central Pathology Laboratory for non-US Centers), the FFPE Working Group of the Breast International (BIG) and National Cancer Institute (NCI)- sponsored breast cancer Cooperative Groups, the Early Breast Cancer Trialists' Collaborative Group Steering Committee, the Translational Research Committee of the HERA trial, and he is a Founding Member of the International Sentinel Node Society.

Prof Viale has authored 310 articles in peer-reviewed international Journals and 36 chapters in books.

Prof Rainer Storb

Program Head of Transplantation Biology,
Fred Hutchinson Cancer Research Center, Seattle,
Professor of Medicine,
University of Washington,
Seattle, USA

Rainer Storb, MD, a native of Germany, attended the University of Freiburg Medical School. After two years clinical training in Essen and Munich and three years research in Paris, Dr Storb travelled to Seattle in 1965 on a Fulbright Fellowship to work in the Division of Hematology at the University of Washington with Dr E Donnall Thomas. It was here that Dr Storb participated in the birth of the Seattle marrow transplantation program.

For the next 43 years, he worked to develop new concepts in transplantation biology and apply them to patients. Their studies from the 1960s and 1970s included the demonstration of peripheral blood stem cells for allogeneic transplantation, the importance of in vitro histocompatibility typing for outcome of related and unrelated transplants, the definition of immunologic recovery after marrow transplantation, the development of conditioning programs for transplantation, uncovering the nature of graft-host tolerance, developing strategies of treating and preventing graft-vs-host disease, and studies on hematopoietic engraftment. Many transplantation protocols currently in use have been directly extrapolated from his studies. One practical example of his work translated from preclinical studies into the clinic concerns the novel use of combination drug therapy to prevent graft-versus-host disease, which occurs when donor bone marrow reacts against the patient after transplantation. Dr Storb's formulated drug schedule is now the "gold standard" in use at centres worldwide. His work applied to patients with aplastic anaemia has defined and improved treatments and increased the long-term survival of this patient group to greater than 90 percent. His current studies to develop protocols for establishing chimeric grafts, where the marrow is part donor and part patient, uses transplant regimens which have little toxicity and allow for the treatment of genetic and malignant diseases in both old and young patients in the outpatient setting. In these transplants, cures of malignancy are achieved through an allogeneic graft-vs-tumour effect rather than through the high-dose cytotoxic radiochemotherapy previously used.

Dr Storb has more than 1400 publications. He has won numerous including the Alexander von Humboldt Award, the Joseph Steiner Award, the Gustav Carus Prize of the German Academy of Natural Sciences, the Meyenburg Prize, the Henry M. Stratton Medal and the E. Donnall Thomas Prize from the American Society of Hematology, the Joseph H. Burchenal Clinical Research Award from the American Association for Cancer Research, the Don Metcalf Lecture Award from the International Society of Experimental Hematology, The Jacqueline Seroussi Memorial Foundation for Cancer Research Award and the American Society of Blood and Marrow Transplantation Lifetime Achievement Award. Throughout the years, Dr Storb has trained over 130 researchers in his laboratory, who are now raising the standard of hematopoietic cell transplantation biology research throughout the world.

Bone Marrow Transplantation in Dogs: The State of the Art

Rainer Storb, M.D.

"It should be noted that marrow grafting could not have reached clinical application without animal research, first in inbred rodents and then in outbred species, particularly the dog"
(E. Donnall Thomas, the Nobel Prizes, 1990).

Besides humans, only one other mammal has that rare combination of unusual genetic diversity and a widespread, well-mixed gene pool: dogs. Accordingly, over the last four decades, canine studies have laid the groundwork for successful treatment of human patients with malignant and nonmalignant hematological diseases by hematopoietic cell transplantation. Specifically, high-dose conditioning programs for transplant have been worked out as have been issues of tissue typing to select the best-matched donors. The optimal immunosuppression necessary to prevent complications such as graft-versus-host disease after transplantation has also been established in canine studies. Most recently, the canine work has resulted in the development of a radical new approach for hematopoietic cell transplantation from both related and unrelated donors which has minimal toxicity and is safe enough to be administered in the ambulatory care setting. This approach utilizes the donor's immune T-cells rather than high-dose cytotoxic therapy to eradicate underlying malignant diseases. Canine models of nonmalignant diseases have shown that this new approach can also be used to treat hereditary hemolytic anemia and certain autoimmune diseases. This new treatment approach has been successfully translated clinically to treat >1,500 human patients with malignant and nonmalignant hematological diseases.

Scientific Abstracts

The abstracts as published in these proceedings have not been subjected to extensive peer-review and therefore should not be quoted in publications. The abstracts may be considered as Personal Communications and referenced as such, with permission of the individual authors.

RESIDENTS' ABSTRACTS

The ESVONC Junior Research Award aims to encourage members in the earlier years of their career in veterinary oncology to pursue a period of scientific investigation in the field of veterinary oncology**.**

The award is sponsored annually by Pfizer Animal Health.

ANTI-TUMOUR ACTIVITY OF A NOVEL DNA BINDING AGENT (SJG-136) IN CANINE NEOPLASIA.

Maria Mellinas-Gomez

M Mellinas-Gomez, A Stell, S Baines, J A. Hartley & D E. Thurston

Royal Veterinary College,

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Introduction

SJG-136 is a novel sequence-selective DNA cross-linking agent that causes minimal distortion of the helical structure such that the cross-links persist and are not repaired. SJG-136 is an effective cytotoxic agent in rodent and human tumour cell lines. Our hypothesis is that SJG-136 is effective in killing canine cancer cells in vitro.

Material and Methods

14 canine cell lines were exposed to SJG-136 at various concentrations for 1h and 96h. Inhibition of growth was investigated using SRB and MTT assays to calculate the concentration causing 50% inhibition (IC50). Formation of inter-strand cross-links was measured using a modified single cell gel electrophoresis (COMET) assay to calculate the concentration causing 50% decrease in Comet tail moment (XL50).

Results

A potent cytotoxic effect was seen in all cell lines. Cytotoxicity at 1h and 96 h was positively correlated. Low (< 1nM) IC50 values were seen for mast cell tumour (C2), oral melanoma (KMeC), mammary carcinoma (CFMg35), haemangiosarcoma (DEN), osteosarcoma (D17), and a connective tissue tumour (A72). DNA cross-links were identified in 5 cell lines, with a linear increase in cross-link formation as drug concentration increased. The IC50 and XL50 for 5 cell lines were positively correlated with increasing IC50 being associated with increasing XL50, and there was no repair of DNA inter-strand cross-links over 48 hours post-exposure incubation.

Conclusions

This preliminary data suggests that SJG-136 might be a useful cytotoxic agent for the treatment of canine tumours, particularly melanoma, mast cell tumour and mammary carcinoma.

ASSESSMENT OF THE MYOEPITHELIAL CELL LAYER INTEGRITY IN CANINE MAMMARY TUMOURS. CORRELATION WITH HISTOLOGICAL GRADE AND PROLIFERATION INDEX

Raquel Sánchez-Céspedes

R Sánchez-Céspedes, Y Millán, S Guil-Luna, A Espinosa de Los Monteros, JC Reymundo, J García-Monterde & J Martín de las Mulas

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Introduction

Histological evidence of malignancy in canine mammary tumours does not invariably imply a malignant clinical course. In human breast cancer, an intact myoepithelial cell layer highlights in situ carcinomas.

Material and Methods

Seventy four proliferative lesions of the mammary gland (2 dysplasias, 8 benign tumors, and 64 carcinomas -1 in situ, 32 grade 1, 23 grade 2, and 8 grade 3-) were studied. The percentage of tumour nodules surrounded by a single layer of calponin-positive, spindle cells was evaluated in 8 microscopic fields per case at 20X magnification and charted as type I (≥90%-100%), type II (>70%-<90%) and type III (≤70%). Proliferation index was expressed as the percentage of MIB-1 antibody-positive cells. Data were analyzed using non parametric Mann-Whitney "U" test. $P<0.05$ was considered statistically significant.

Results

Calponin expression type I lesions (37.8%) included all dysplasias, the in situ carcinoma, 62.5% of benign and 32.8% of malignant tumours, type II (32.4%) were 37.5% of benign and 32.8% of malignant tumours and type III (29.7%) were malignant tumours exclusively. Malignant tumours type I included 46.9% grade 1 and 21.7% grade 2 carcinomas. The majority of carcinomas grade 3 (75.0%), and more than half of carcinomas grade 2 (56.5%) were type III. The level of calponin expression (types I&II versus III) correlated with histological grade of carcinomas ($p=0.00001$) and proliferation index ($p= 0.04$).

Conclusions

The immunohistochemical expression of calponin may be usefully applied for the evaluation of the myoepithelial cell layer integrity and seems to represent a marker for the biological behaviour of canine mammary tumours.

CANINE MAMMARY TUMOURS IN A POPULATION OF ENGLISH SPRINGER SPANIELS IN SWEDEN

Patricio Rivera

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[2] Department of Animal Breeding & Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden; [3]Broad Institute of Harvard & MIT, Cambridge, MA, USA; [4] Department of Medical Biochemistry & Microbiology, Uppsala University, Uppsala, Sweden

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Introduction

This study describes case history, signalement, pathological findings and survival in a Swedish population of female English Springer Spaniel (ESS) dogs with or without mammary tumours (MT).

Material and Methods

Information on 488 females ESS was retrieved from questionnaires, pathology reports and medical records. The distribution of various clinical and pathological variables and survival time were compared between dogs with or without MT and between dogs with malignant or benign tumours.

Results

In all, 299 dogs had MT (61%). The mean age of MT diagnosis was 6.9 years (50% CI 6.8-7.0 years). Benign MT was found in 122 dogs (41%), 55 dogs had malignant MT (18%) and 122 dogs had MT with unknown histopathology (41%). The proportion of MT was higher ($p=0.01$) in intact female dogs than in spayed dogs. Dogs with benign MT had longer survival time after diagnosis compared to dogs with malignant MT or MT with unknown histopathology ($p=0.018$). 110 of the treated dogs (38%) presented new tumours or had recurrence after surgery.

Conclusions

This study describes clinical characteristics of MT in a high-risk breed. Results suggest that histological subtype and reproductive status appears to be associated with development and survival time after diagnosis of MT affected dogs.

CANINE MEDIUM MACRONUCLEATED CELL/ MARGINAL ZONE LYMPHOMA: DESCRIPTION OF 16 CASES (2001-2008)

Paola Valenti

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Introduction

Canine medium macronucleated cell/marginal lymphomas (MZL) are indolent lymphomas arising from the marginal zone of B-cell follicles. The aim of this retrospective study is to gain informations about clinical features, treatment options and outcome of dogs with MZL.

Material and Methods

16 dogs with a cytological/ histopathological diagnosis of MZL. Clinical, laboratory and follow-up data were collected.

Results

The splenic form predominated (50.0%, 8 cases), followed by nodal (31.2%, 5 cases) and disseminated (18.8%, 3 cases) subtypes. The great majority of dogs had stage IV disease (62.5%). Among splenic MZL, 4 dogs underwent splenectomy and adjuvant chemotherapy, 3 dogs were treated by means of chemotherapy, and 1 dog underwent surgery only. The 3 longest survivals were recorded in dogs with splenic MZL treated by means of surgery and adjuvant chemotherapy. All dogs with nodal MZL were treated with systemic chemotherapy; 4 (80.0%) achieved complete remission and 1 partial remission. Two out of the 3 dogs with disseminated MZL had the shortest survival. During the disease course, 2 (12.5%) dogs transformed into high-grade centroblastic lymphoma. Median survival for all dogs was 549 days.

Conclusions

Anatomic origin of MZL may delineate tumors with distinct biological behavior in dogs. Splenic MZL appears indolent and may benefit from splenectomy, possibly followed by systemic chemotherapy. Nodal and disseminated MZL seems to be clinically aggressive, possibly requiring dose-intense chemotherapeutic strategies.

EFFECTS OF A SMALL INTERFERING RNA (SIRNA) AGAINST THE EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) IN A FELINE LARYNGEAL SQUAMOUS CELL CARCINOMA CELL LINE (SCCF1).

Gurå T Bergkvist

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Introduction

RNA interference is a highly specific way of targeting proteins at the post transcriptional level utilising siRNA. The EGFR has in recent years been identified as a major driver of oncogenic pathways in Head and Neck Cancer in man and therapeutics specifically targeting this protein have recently been licensed. Feline oral squamous cell carcinomas have also been shown to express EGFR.

Material and Methods

The SCCF1 cell line was transiently transfected either with siRNA directed against the feline EGFR or a scrambled control sequence. Total RNA was extracted and protein lysates were obtained. The mRNA levels were analysed by Real Time PCR using the Comparative Cp method and protein levels were evaluated by Western blot. The ability of transfected cells to undergo proliferation was assessed in parallel with an EGFR inhibitor, gefitinib, using standard assays.

Results

Real Time PCR showed a 40-50% reduction in EGFR mRNA levels when compared to scrambled control for up to 72 hrs after transfection. Western blot analysis revealed a marked reduction in EGFR protein at 48 and 72 hrs compared to scrambled controls. EGFR targeted cells showed statistically significant reduction in proliferation ability at 72 hrs when compared to scrambled control, mock transfected, and untreated cells ($p<0.001$, One-Way ANOVA). The effect of EGFR knockdown was comparable to treatment with 10µM of gefitinib.

Conclusions

Targeting the EGFR in SCCF1 cells with siRNA results in specific reductions in both EGFR mRNA and protein levels. Targeting the receptor produces marked reduction in tumour cell proliferation in the SCCF1 cell line.

EFFECTS OF PROGESTERONE, MIFEPRISTONE AND ONAPRISTONE ON PROLIFERATION AND APOPTOSIS OF THE PROGESTERONE RECEPTOR-POSITIVE CANINE MAMMARY CARCINOMA CELL LINE CMT-U27

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Introduction

Cell lines provide a useful tool to investigate the effect of progesterone and antiprogestins on human breast cancer cells. However, there are no such studies using canine mammary cell lines. The aim of this work was to determine whether the progesterone receptor positive canine mammary carcinoma line CMT-U27 is suited for the investigation of the effect of progesterone and antiprogestins on proliferation and apoptosis.

Material and Methods

CMT-U27 cells were incubated with 10-6M P (24h, 48h, 72h) alone and after incubation with 10-6M Mifepristone or 10-6M Onapristone (24h, 48h, 72h). Incubations with 10-6M Mifepristone, 10-6M Onapristone or vehicle were used as controls. Formalin-fixed, paraffin-embedded cells were labelled with immunoperoxidase for the Ki67 antigen (MIB-1) and for cleaved Lamin A. A proliferation index (PI) and apoptotic index (AI) based on the percentage of positive cells was assessed for each incubation condition.

Results

Progesterone alone or in combination with Onapristone consistently produced no effect on the PI, while incubation with mifepristone alone or in combination with progesterone was variably associated with an increase of the PI. Progesterone application resulted in a clear decrease of the AI compared to non-treated cells. Mifepristone, Onapristone or Mifepristone combined with progesterone variably decreased the AI, while the combined treatment with Onapristone and progesterone variably reverted the inhibitory effect of progesterone on the AI.

Conclusions

Data from this preliminary experiment suggest that the CMT-U27 cell line is well suited for the study of the effect of progesterone and antiprogestins on canine mammary carcinoma cells.

EVALUATION OF THE 'HEALTH RELATED QUALITY OF LIFE QUESTIONNAIRE' IN VETERINARY CANCER PATIENTS PRESENTING TO A REFERRAL CANCER CENTRE

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Introduction

Health related quality of life (HRQoL) assessment has been studied extensively in human medicine. Limited research in animals has been undertaken and there is no standard HRQoL evaluation for veterinary oncology patients. The aim of this study was to assess the practicality, usefulness and robustness from a pet owner's and clinician prospective of a questionnaire for the assessment of HRQoL in canine and feline cancer patients.

Material and Methods

The owners of patients referred to the Royal (Dick) School of Veterinary Studies Oncology Service from August to November 2009 were asked to complete two questionnaires prior to their pet's consultation: a HRQoL questionnaire entitled Cancer Treatment Form and an evaluation of this form entitled Minitest for Owner. The clinician also completed a Minitest for Clinician form to evaluate the questionnaire following the consultation.

Results

116 questionnaires were collected from the owners of 99 dogs and 17 cats. 96% (111/116) of the Cancer Treatment Forms, 72% (84/116) of the Minitest for Owner forms and 100% (116/116) of the Minitest for Clinician forms were completed. Where completed, 98% (82/84) of owners felt that the Cancer Treatment Form reflected their pet's quality of life and 81% (66/81) felt the Cancer Treatment Form made them feel more involved in their pet's treatment. 99% (81/82) of owners found the Cancer Treatment Form detailed and complete enough and 95% (105/111) of completed Cancer Treatment Forms were found to be valuable by the clinician.

Conclusions

The questionnaire was well received by both owners and clinicians. Minor improvements incorporated for future clinical trials.

EVALUATION OF THE NECESSITY OF CLINICAL STAGING IN MAST CELL TUMORS (MCT):A RETROSPECTIVE STUDY ON A REFERRAL POPULATION OF 237 DOGS (1998-2008).

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Introduction

The purpose of this study was to evaluate the utility of performing full clinical staging in dogs with a primary cutaneous / subcutaneous MCT if there is no evidence of metastasis to the local lymph node (LN).

Material and Methods

237 dogs with cutaneous MCTs that presented to the QVSH, Cambridge over a period of ten years were evaluated retrospectively. Clinical staging consisting of aspiration of the palpable loco-regional LN, hematology, biochemistry, thoracic radiographs and abdominal ultrasound were performed in all cases. LN were considered positive for metastasis based on cytological findings.

Results

19 dogs were excluded because of incomplete clinical staging. Mean age of presentation was 7.4 years. There were no significant differences between sexes. Labradors were over represented comprising 31% of cases. 29.2% of the cases (n = 69) had local LN involvement at presentation and 6.2% (n = 17) also had evidence of distant metastasis. Only one dog had evidence of lung metastasis without local LN involvement.

Conclusions

Age, breed and sex results were consistent with previous studies. All dogs with detectable distant metastases also had LN involvement. The case with pulmonary metastasis without LN involvement had clinical and radiographic findings suggestive of a concurrent primary lung tumour. These findings suggest that FNAs of loco-regional LN should be performed on all dogs with primary MCTs but it is debatable whether a complete clinical staging should be performed as standard in the absence of LN involvement unless there are other clinical signs that justify it.

GENE EXPRESSION PROFILING AND PATHWAYS ASSOCIATED WITH HIGH METASTATIC POTENTIALS IN CANINE OSTEOSARCOMA: IN VITRO STUDY

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Introduction

Dogs with osteosarcoma die from metastatic disease making local control of the primary tumor alone or in combination with chemotherapy often inadequate. To gain more insights into the metastasis promoting processes, we analyzed gene expression profiles of a canine osteosarcoma cell line with high metastatic capacity to the lung (HMPOS) and its primary parental cell line (POS).

Material and Methods

These cell lines have shown to produce metastatic lesions in mice models where HMPOS inoculated mice developed macrometastases more rapidly compared to POS. These findings were confirmed by an in vitro anchorage independent growth and tritium-thymidine proliferation assays. A canine-specific cDNA microarray representing 20,313 gene transcripts were used to profile these cell lines. Quantitative real-time PCR (QPCR) was performed to validate the overall expression data.

Results

Significance Analysis of Microarray (SAM) analysis revealed 278 genes differentially expressed at a False Discovery Rate of 10%. Pathway analyses revealed gene enrichment for the integrin, Wnt, cadherin, apoptosis, angiogenesis and p53 signaling to be deregulated. MetaCoreR analysis on gene regulatory networks revealed 3 transcription factors p53, SP1 and c-myc to play a 'central hub' connecting the differentially expressed genes. Decorin was selected for further investigation in primary tumors using QPCR and immunohistochemistry. Appendicular OS with high decorin mRNA expression had significantly lower DFI. Decorin protein expression was observed scattered throughout the primary tumors while the metastasis have higher expression towards the peripheries.

Conclusions

Future work will be directed towards investigating the targets and/or pathways revealed by the present study to develop targeted therapies against canine OS metastasis.

IDENTIFICATION OF CANCER STEM CELLS IN CANINE GLIOMA AND RESISTANCE TO THERAPEUTIC MODALITIES.

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Introduction

We have previously isolated and characterised cancer stem cells from canine osteosarcoma. Recent reports have identified the presence of cells with cancer stem cells properties in vitro in human brain tumours. In this study we aim to identify cancer stem cells in a canine glioma cell line and evaluate their sensitivity to common chemotherapeutic drugs, such as doxorubicin and mitoxantrone, and to x-irradiation.

Material and Methods

Cells from the canine glioma cell line, J3T were magnetically sorted to identify cells with the cell surface protein marker CD133, indicative of a cancer stem cell population. Cells were also grown in a serum-free selective culture system to identify the presence of neurospheres. CD133 positive cells were then examined for the presence of genes characteristic of embryonic stem cells. These putative cancer stem cell populations were treated with a range of common chemotherapy drugs and x-irradiation and their sensitivity evaluated.

Results

Neurospheres and CD133 positive cells were identified and shown to have increased resistance to common chemotherapeutic drugs and x-irradiation. The presence of stem cell associated genes Nanog, Oct4 and STAT3 indicates the identified cells are representative of cancer stem cells.

Conclusions

We have identified cancer stem cells in the canine glioma. Significantly, we have shown that putative cancer stem cells show a greater resistance to common chemotherapeutic drugs and x-irradiation. In future studies we will analyse DNA damage signalling pathways to identify the mechanism of cancer stem cell resistance.

INVESTIGATING CD44 AS A POTENTIAL CANCER STEM CELL MARKER IN CANINE TUMOURS

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Introduction

CD44 is a cell surface glycoprotein which acts as a hyaluronate receptor. Its physiological functions relate to cell-cell and cell-matrix adhesion. Aberrant expression is recognised in many neoplastic disorders, particularly in association with invasion and metastasis. The cancer stem cell hypothesis proposes that tumours are maintained by a population of cancer stem cells (CSC) which must be eradicated to prevent disease relapse after treatment. Cells expressing high levels of CD44 have been identified as candidate CSC in a variety of human tumours. This study sought to investigate CD44 expression and its potential as a CSC marker in canine cancer

Material and Methods

CD44 expression in several canine cancer cell lines was determined by flow cytometry. Cells with low and high levels of CD44 expression were sorted from a canine mammary carcinoma cell line, REM134, and examined for differences in growth characteristics, colony forming ability, drug sensitivity and cell cycle profile.

Results

All cell lines tested expressed CD44. CD44-high cells demonstrated enhanced growth in both adherent and low-density serum free ("tumoursphere") conditions. However, there were no differences between the two populations' sensitivities to doxorubicin or NF-kappaB pathway inhibitors. Moreover, whilst most CD44-low cells were in a resting phase of the cell cycle, most CD44-high cells were actively dividing. Upon proliferation in culture, both populations gave rise to progeny with a full spectrum of CD44 expression.

Conclusions

CD44 in canine cancer cell lines is associated with proliferation, but may not be a true CSC marker. Further work will assess its expression in primary tumours.

JAK2 MUTATION IN A DOG WITH PRIMARY POLYCYTHEMIA

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Introduction

In human beings, Polycythemia Vera (PV) is characterized by a primary increase of the red cell mass. Recently an acquired recurrent mutation within the JAK2 gene was found in 90% of human patients with PV. This mutation (V617F) changes codon 617, localized in the pseudokinase domain of JAK2, leading to a constitutive activation of the kinase responsible for the PV. The detection of the mutation has become a major diagnostic tool in human medicine. Primary Polycythemia is a rare disease in dogs classified within myeloproliferative syndromes. Clinical signs are non-specific with a chronic progressive course. Diagnosis is based on exclusion of secondary polycythemia causes. The canine JAK2 gene shares strong homology with its human counterpart, particularly within the pseudokinase domain.

Material and Methods

DNA from peripheral blood of dogs with presumptive diagnosis of PV was extracted. Direct sequencing of the exon of the pseudokinase domain was performed.

Results

We found the presence of 3 base changes located within 2 consecutive codons in one dog. This mutation is the counterpart of codons 617 and 618 mutation in human patients. These mutations were respectively V617F and C618L. By PCR products subcloning, we demonstrated the coexistence of 2 sequences: 1) the wild type sequence and 2) the three mutations present on a single mutant.

Conclusions

The apparent conservation of one identical mutation (JAK2 V617F) between humans and dogs makes it highly likely that a similar molecular pathogenesis could explain polycythaemia in some dogs.

LARGE INFILTRATIVE LIPOMAS IN DOGS: A RETROSPECTIVE STUDY OF 22 CASES.

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Introduction

Lipomas are benign mesenchymal tumors comprised of adipocytes and most common in dogs. In a number of cases, dogs are presented with large and partly infiltrative disease, impairing body function The aim of the retrospective study was to report the clinical presentation, epidemiology, diagnostic and therapeutic approach, treatment outcome and recurrence rates of such large lipomas in dogs in which additionally diagnostic imagine by CT or MRI were performed.

Material and Methods

The records of all canine patients diagnosed with large lipoma between 2002 and 2009 were reviewed. Data recorded included epidemiologic data, clinical staging, size, localization, diagnostic imaging (CT or MRI), complications after surgery and recurrence rate. Kaplan-Meier analysis was used for outcome analysis. Cox forward univariate analysis was used to evaluate patient variables.

Results

22 dogs meeting inclusion criteria entered the study. In 10 dogs, a CT examination and in 12 dogs, a MRI examination was performed. The masses were relatively homogenous and infiltrated the surrounding structures in twelve cases. 19 dogs underwent surgical excision (DFI 482 days) and five dogs did not undergo any further treatment. Two dogs with infiltrative disease were treated with postoperative radiation therapy (10 x 4 Gy). (DFI 94 and 621 days). The local recurrence rate in these dogs was 6%. Seroma formation as a common complication in very large lipomas developed present in 4 cases.

Conclusions

Imaging tools like MRI and CT allows visualisation of the full extent of a lipoma and facilitate an appropriate preoperative planning.

PERI-ARTICULAR HISTIOCYTIC SARCOMA IN BERNESE MOUNTAIN DOGS: A RETROSPECTIVE INVESTIGATION OF THE PREVALENCE OF THIS TUMOUR IN ASSOCIATION WITH PREVIOUSLY DISEASED JOINTS.

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Introduction

Histiocytic sarcoma complex is commonly found in Bernese Mountain Dogs (BMD), and a genetic association has been unravelled in this breed. Peri-articular histiocytic sarcoma (PAHS) is a sub-entity of this histiocytic sarcoma complex. The hypothesis of this study is that PAHS in BMD will be more frequently encountered around previously diseased joints compared to normal joints.

Material and Methods

Data were compiled from a European internet questionnaire (www.bmdhealthsurvey.eu), and the medical records of two pathology labs. Statistical analysis was performed through Chi-square tests and logistic regression analysis, with significant results assumed at $p< 0.05$. Effect Size was analyzed by means of Nagelkerke R^2.

Results

Data from 1550 BMD were obtained, of which 697 had a completed questionnaire and were used for statistical analysis. 22 BMD were identified with PAHS. A significant association between previous joint trauma and the development of PAHS around the same joint was demonstrated for the left elbow ($p=0.026$), right elbow ($p=0.035$), left stifle ($p=0.018$), and right stifle ($p=0.023$). Effect Sizes (R^2N) for these joints were 0.621, 0.651, 0.721, and 0.499 respectively.

Conclusions

Significant association in combination with reasonably high Effect Sizes indicate a causal relation of previous joint trauma and the development of PAHS in elbow and stifle joints of European BMD. However, studies with larger numbers of dogs with PAHS should be performed to make this conclusion more powerful. Future investigations on PAHS carcinogenesis, e.g. combining genetic predisposition and chronic arthritis, may lead to the development of early-detection programs for PAHS in BMD with known joint pathology.

PI3K/AKT/PTEN PATHWAY IN THE PATHOGENESIS OF FELINE MAMMARY TUMORS AND ITS CORRELATION WITH EXPRESSION OF ERA AND HER2, HISTOLOGICAL GRADING AND CLINICAL FOLLOW-UP.

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Introduction

AKT is activated and amplified in several human tumors, such as breast cancer, conferring poor prognosis and resistance to endocrine therapy and chemotherapy. In breast cancer the activation of AKT is mediated mainly by EGFR1, HER2/neu and oestrogen receptor alpha (ERα). The purpose of this study was to investigate by IHC and WB analysis the relationship between Her2, ERα and the PI3K/AKT/PTEN pathway in feline mammary carcinomas (FMC) and to correlate IHC results with histological malignancy and disease free period.

Material and Methods

IHC against p-AKT, HER2, ERα and PTEN was evaluated by IHC on 27 FMC (12 benign lesions, 4 metastasis and 2 normal mammary glands).WB anti AKT, p-AKT, HER2, PTEN and p-PTEN was performed on 6 FMC cell lines. PCR products corresponding to sequence of feline AKT were automatically sequenced.

Results

Eighty five percent of FMC and 100% of metastasis revealed positivity to p-AKT by IHC. Western blot analysis revealed that all FMC cells lines had p-AKT activation in correlation with expression of HER2 and p-PTEN reduction. Gene sequencing f-AKT cDNA revealed a homology of 98% with canine and human AKT sequences.

Conclusions

IHC results demonstrated that AKT activation was correlated to HER2 positivity, histological malignancy and recurrence while there was not correlation with ERα expression. These data demonstrate that AKT activation presents the same relationship with HER2 and PTEN status seen in human breast cancer confirming that f-AKT plays an important role in the pathogenesis of FMC and its value as a suitable natural model in comparative oncology.

THE DEVELOPMENT OF DELIVERY SYSTEMS FOR SMALL-INTERFERING RNA TARGETING CANINE TELOMERASE

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Introduction

Telomerase is largely responsible for tumour immortalisation, and is considered a near universal tumour marker and therapeutic target. We have demonstrated that targeting the canine telomerase RNA template (cTR) gives the most consistent reduction of telomerase activity using siRNA, but a successful siRNA anti-cancer therapy relies on an efficient delivery system. Here we explore two delivery methods for delivery, cellular delivery and the use of Polypropylenimine dendrimer (PPIG3) nanoparticles using in vivo imaging technologies.

Material and Methods

For cellular delivery, we developed a series of endothelial cell lines expressing fluorescent labels. Cell trafficking was explored using in vivo optical imaging. For nano-medicine delivery, we engineered a cTR siRNA expression construct that could be delivered wrapped in PPIG3 dendrimers. The system was validated using in vitro transfections and then utilized in vivo in a NOD-SCID mouse model of Haemangiosarcoma.

Results

We have validated cellular delivery of a fluorescent tag to tumour cells in vivo. We have demonstrated that cTR siRNA construct can be delivered to cells using the PPIG3 technology.

Conclusions

We can traffic therapeutic molecules to tumour cells but we need to validate whether we are getting sufficient target modulation for therapeutic benefit.

UROKINASE PLASMINOGEN ACTIVATOR PROTEIN EXPRESSION AND ITS PROGNOSTIC VALUE IN CANINE MAMMARY TUMOURS

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Introduction

Although urokinase plasminogen activator (uPA) has been associated to aggressive behaviour and poor prognosis in human breast cancer, there is no information on its expression in canine mammary tumours (CMT).

Material and Methods

Immunohistochemistry analysis was performed in 119 CMT (24 benign and 95 malignant) to investigate uPA expression and its relationship with clinical and histopathological parameters. uPA expression evaluation was based on the percentage of neoplastic and intratumoural and peritumoural stromal cells (10% cut-off) with cytoplasmic staining according to scoring methods used in human breast cancer studies. Dogs with malignant mammary tumours (MMT) were submitted to a 2-year follow-up. The Kaplan-Meier method was used to compute overall survival (OS) and disease-free interval (DFI) and to construct the survival curves.

Results

uPA expression was significantly higher in stromal (P<0,001) and neoplastic cells (P=0,003) from MMT compared to benign. In MMT, uPA stromal overexpression was significantly associated with tumour size (P=0,011), invasion (P=0,002), histological grade (P=0,005), Ki-67 expression (P<0,001), nodal status (P=0,002) and distant metastasis (P=0,014). uPA overexpression in neoplastic cells was weakly associated with tumour size (P=0,043). uPA overexpression in stromal cells was significantly associated with lower OS (P=0,002) and lower DFI (P=0,009).

Conclusions

This is the first study of uPA protein expression in CMT and the first evidence that uPA overexpression in tumour stroma is associated with prognosis, as previously reported in human breast cancer studies. Therefore, this study suggests that uPA expression in tumour stroma may be a useful prognostic indicator in dogs with MMT.

USE OF FLOW CYTOMETRIC TECHNIQUES TO IDENTIFY CANCER STEM CELLS IN CANINE TUMOURS

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Introduction

The cancer stem cell hypothesis proposes that a specific subpopulation of "cancer stem cells" (CSC) is responsible for maintaining tumour growth. As well as the cardinal properties of self-renewal and multilineage differentiation capacity, these CSC might be expected to share features with normal tissue stem cells, in terms of both phenotype and behaviour. This study sought to identify candidate canine CSC subpopulations using flow cytometry.

Material and Methods

Established cell lines representing a range of common canine tumours were examined for the expression of stem cell-associated surface markers including CD34, CD117, CD44 and CD133. Cells were tested for stem cell-like functions known to confer resistance to chemotherapeutic agents, through activity of membrane pump transporters (such as P-glycoprotein or ABCG2, demonstrated by efflux of Rhodamine 123 and Hoechst 33342, respectively) or intracellular enzymes (aldehyde dehydrogenase). These tests were then used to examine cells derived directly from spontaneous canine tumours.

Results

Established cell lines showed an "all-or-none" staining pattern rather than subpopulations of cells positive for surface markers. Functional assays required careful interpretation - Hoechst in particular gave inconsistent results compared to those with human or feline cells - again, no canine cell line showed a consistent positive subpopulation. Conversely, primary cells and those recently derived from tumours did demonstrate positive subpopulations, which may represent cancer stem cell-like cells.

Conclusions

Flow cytometric methods can be adapted to identify canine CSC or clonally evolved subpopulations. Whilst cell lines are valuable in assay development, distinct CSC-like subpopulations are more reliably identifiable in primary or recently-derived tumour cells.

EXPRESSION OF KI-67, PCNA, AND P27KIP1 IN CANINE PITUITARY CORTICOTROPH ADENOMAS

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Introduction

Pituitary-dependent hypercortisolism (PDH), which is caused by adrenocorticotropic hormone (ACTH)-secreting pituitary adenomas, is a common endocrinopathy in dogs. Dogs with non-enlarged pituitaries harboring a microadenoma have a better prognosis than those with enlarged pituitaries. The aim of this study was to investigate the expression of the proliferation markers Ki-67 and proliferating cell nuclear antigen (PCNA) and the cell-cycle inhibitor p27kip1 in corticotroph adenomas in enlarged and non-enlarged pituitaries.

Material and Methods

The expression of Ki-67, PCNA, and p27kip1 was analyzed by immunohistochemical staining of 17 pituitary adenoma samples harvested during pituitary surgery in dogs with PDH. The labeling index (LI) was calculated by counting the number of immunopositive cells per 1,000 cells.

Results

The mean (± standard deviation) LI for Ki-67 was 8.4% ± 14.2% for the group with enlarged pituitaries and 8.8% ± 5.5% for the group with non-enlarged pituitaries; for PCNA 35.5% ± 12.2% and 37.0% ± 15.5%; and for p27kip1 29.3% ± 22.6% and 42.5% ± 27.9%, respectively. No significant differences in Ki-67, PCNA, and p27kip1 labeling indices were found between enlarged and non-enlarged pituitaries. However, a trend toward significance was observed when comparing the expression of p27kip1 in enlarged pituitaries versus normal pituitary tissue.

Conclusions

It is concluded that Ki-67 and PCNA are not useful as proliferative markers for studying the pathobiology of pituitary corticotroph adenomas in dogs. p27kip1, on the other hand, is an interesting target for further research.

Winning abstract of 2009 Dutch Animal Cancer Foundation Award Basic Sciences

GENERAL ABSTRACTS

MAMMARY TUMOURS IN PET RABBITS. IMMUNOHISTOCHEMICAL FINDINGS

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Introduction

Mammary adenocarcinomas are fairly common in female rabbits over 3 years of age. In this study a series of 15 cases of mammary lesions in pet rabbits were investigated to demonstrate the role of known breast cancer biological phenomena in the progression of rabbits mammary tumours and their aids in therapeutic approach.

Material and Methods

Three hyperplastic mammary glands, 2 simple adenomas, and 10 adenocarcinomas (9 simple tubulo-papillary and 1 mucinous) removed from 15 rabbits. Serial sections underwent ematoxylin-eosin and immunohistochemistry stains with the following panel of antibodies: proliferation-associated antigen Ki67 (clone MIB-1), estrogen receptor-α (ER-α), HER-2, telomerase (TEL) and E-cadherin (E-Cad). Comparisons between malignant and non-malignant (hyperplasia+adenomas) lesions were evaluated.

Results

80% of the non-malignant and 30% of the malignant cases were positive (cut off 10%) to ER-α (Chi square, P=0.06). A significant higher MIB1 index (percentage of positive nuclei) was recorded in carcinomas compared to non-malignant lesions (t-test, p= 0.011). HER-2 positivity (staining in more than 10% of neoplastic cells) was observed in 2/15 cases (one adenoma and one carcinoma). In 7 carcinomas E-Cad showed reduced expression with a cytoplasmic localization in 5 cases.

Conclusions

In the rabbit a lower expression of hormone receptors and high proliferation activity characterizes the mammary carcinomas, in which low is the expression of HER-2 while TEL activation involves more than 50% of the cases. It seems that anti-proliferative and anti-telomerase therapeutic approach should be the most proper in rabbit mammary carcinomas.

TSLC1 TUMOUR SUPPRESSOR GENE EXPRESSION IN CANINE MAST CELL TUMOURS

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Introduction

Tumour suppressor in lung cancer-1 (TSLC1) is a tumour-suppressor gene that encodes an adhesion molecule that expressed by normal human mast cells. Reduced TSLC1 expression is associated with a poor prognosis in several human cancers. A previous pilot study showed that TSLC1 expression by canine MCTs was strongly inversely correlated with tumour grade. This study sought to investigate if TSLC1 expression levels could be used to predict outcome in dogs with intermediate-grade MCTs.

Material and Methods

Intermediate grade MCT samples from 35 dogs with clinical follow-up data were immunohistochemically stained using a rabbit anti-human polyclonal antibody. Samples were then scored based on the intensity of cellular staining across each section. Adjacent sections of the same samples were also stained for Ki67.

Results

Of the 35 dogs, 14 died due to metastatic spread of MCT and had a mean survival time of 76 days (range 19-817days). Twenty-one dogs did not die due to MCT with a median follow-up time of 1452 days (range 988-1947 days). The TSLC1 score was inversely correlated with whether the dogs died due to metastatic disease, ($p=0.058$). Ki67 score did not significantly correlate with survival times in the study ($p=0.381$), but did inversely correlate with TSLC1 expression ($P=0.024$). The age, breed and sex of the dogs did not influence outcome.

Conclusions

TSLC1 is potentially an important tumour suppressor gene in canine MCT, and this tumour may provide an important model for studying this gene in a naturally occurring disease setting.

GENE EXPRESSION, IMMUNOHISTOCHEMISTRY AND GEL ZYMOGRAPHY OF MATRIX METALLOPROTEINASES AND RELATED TISSUE INHIBITORS IN CANINE MAMMARY TUMOURS: PRELIMINARY RESULTS

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Introduction

Matrix metalloproteinases (MMPs) are a family of enzymes involved in the degradation and remodelling of extracellular matrix. Previous studies on matrix metalloproteinases (MMPs) have indicated that they are implicated in cancer invasion and metastases. Only few studies, where the entire gene-protein-enzyme machinery expression and activity have been evaluated in dog are present. Therefore, in the present study MMP2, 9, 14, TIMP1 mRNA and protein pattern expression, and MMP2 and 9 activity (zymography) were measured in canine mammary tumours (CMTs).

Material and Methods

Eleven CMTs, 5 benign and 6 malignant, were considered in the present study. The expression and activities of MMP2, MMP9, MMP14 and TIMP1 were evaluated by immunohistochemistry and gelatin zymography. Gelatinolytic activities of secreted MMP2 and MMP9 were also analysed in pre and post-surgery plasma. Real Time RT-PCR assays were set up by using UPL probes, validated for relative quantification, and gene expression profiles measured.

Results

MMP2 and MMP9 activities were significantly higher in malignant neoplasia than in benign tumours. MMP2, 9, 14 and TIMP1 immunolabelling was observed in both benign and malignant tumour tissues, but it was stronger in the latter. At the mRNA level, an increase of MMP9, MMP14 and TIMP1 (15.5, 2.1, 35.1 mean -fold changes, respectively) was generally observed in tumour specimens.

Conclusions

The immunohistochemical data indicate that MMP2, MMP9 and TIMP1 are highly expressed in CMTs and in association with gel zymography it suggests that the activation of proMMP2 may be an indicator of malignancy. MMP9, MMP14 and TIMP1 induction was also confirmed pre-transcriptionally.

THE USE OF A VACCINE TARGETING TELOMERASE REVERSE TRANSCRIPTASE (TERT) IN DOGS AFFECTED BY MALIGNANT LYMPHOMA

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Introduction

Dog TERT (dTERT) largely confined to tumor tissues can constitute a valid target for translational cancer immunotherapy. The ability of Adenovirus serotype-6 (Ad6)dTERT injections and DNA dTERT electroporation (DNA-EP) to induce immune response against dTERT in malignant lymphoma (ML) dogs has been evaluated.

Material and Methods

Sixteen dogs of various breed, <10 years of age, with generalized lymphoadenopathy, stage III and IV, without systemic signs, with high-grade lymphoma, B-cell type were enrolled. Dogs were treated with cyclophosphamide, vincristine and prednisone chemotherapy for 6-8 weeks to reach a complete remission (CR). Then maintenance chemotherapy was instituted (prednisone and cyclophosphamide or chlorambucil and melphalan) coupled every three weeks with vincristine. During maintenance chemotherapy each dog received two injections of (Ad6)dTERT, two weeks apart. The procedure was completed once every two weeks by a bilateral injection of 1 ml (2.5 mg of dTERT DNA/injection site) followed by electroporation in the tibial muscle for a total of three initial treatments. An additional series of three DNA-EP was performed few months later.

Results

The procedure was able to induce and maintain a dTERT-specific immune response in all animals and remained detectable in absence of autoimmunity or other side-effects. The vaccination can be easily combined with standard chemotherapy regimen.

Conclusions

Both the survival and first relapse times of vaccinated dogs in comparison to a group of historical control dogs similarly treated showed better results. Final conclusions cannot be driven at the moment because most of vaccinated dogs are still alive.* Approved by Ministry of Health, Italy

FLOW CYTOMETRY AND BUFFY COAT EXAMINATION FOR CLINICAL STAGING PURPOSES IN DOGS WITH LYMPHOMA

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Introduction

In dogs with lymphoma, flow cytometry has been established for immunophenotype determination but not for staging purposes so far. Buffy coat smears are also not routinely examined. Therefore, the objective of this prospective study was to show whether these methods may lead to improved clinical staging and prognostic information in canine lymphoma patients.

Material and Methods

Blood and fine needle aspirates from lymph node, liver, spleen, and bone marrow of dogs with multicentric lymphoma and five healthy control dogs were examined via flow cytometry. Light scatter and cell-surface antigen expression properties of lymphocytes/-blasts were determined and the percentages of CD21+ and CD3+ cells assessed. Buffy coat and whole blood smears were examined microscopically and lymphoblasts identified by means of morphologic features.

Results

Samples from 44 dogs with lymphoma (Nine T-cell, 35 B-cell) were examined. In all tissues, the median CD21+:CD3+-ratio of B-cell lymphomas was higher than that of T-cell lymphomas. Evaluation of light scatter and antigen expression properties indicated lymphoma involvement in samples of blood and bone marrow: n=19, liver: n=28, and spleen: n=40. Thirty-one (70%) of the 44 dogs showed neoplastic lymphocytes in the buffy coat examination, whereas only 24 (55%) had lymphoblasts evident in the evaluation of whole blood smears.

Conclusions

Flow cytometry of fine needle aspiration samples obtained from different tissues revealed differences in lymphocyte subset distribution between B-cell and T-cell lymphomas. Buffy coat examination showed a high proportion of positive cases. The ongoing study has to show whether these results possess prognostic significance in canine lymphoma.

IMMUNOHISTOCHEMICAL DETECTION OF APOPTOSIS-RELATED MARKERS IN CANINE LYMPHOMA

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Introduction

Deregulated apoptosis is considered to contribute to lymphomagenesis and a pivotal role in this respect is commonly attributed to Bcl-2 family proteins. Their roles in canine lymphoma are, however, yet poorly explored. The aims of this study were to analyze expression of the anti-apoptotic Bcl-2 family members Mcl-1 and Bcl-x in canine lymphoma over the background of apoptosis and proliferation.

Material and Methods

A panel of 93 archival canine lymphoma samples immunophenotyped, classified and assembled into tissue arrays was analyzed using a standard immunoperoxidase method with antibodies against cleaved caspase-3 and cleaved lamin A, the anti-apoptotic Bcl-2 family proteins Mcl-1 and Bcl-x, and Ki-67. Immunohistochemistry for markers of apoptotic cells was validated using UV-irradiated cultured canine keratinocytes. Immunohistochemistry for anti-apoptotic proteins Mcl-1 and Bcl-x was performed by means of commercially available antibodies selected using canine recombinant proteins and was validated using cultured cells and a panel of normal tissues; preincubation of the primary antibody with canine recombinant proteins served as a negative control.

Results

Immunohistochemical expression patterns of Mcl-1 and Bcl-x in normal canine tissues roughly coincided with those reported for human tissues. Canine lymphomas were found to frequently express Mcl-1 and, to a lesser extent, Bcl-x, across a range of different subtypes. There was no correlation between the expression of these two markers, while there was a good correlation between caspase-3 and lamin A immunolabelling.

Conclusions

Our findings suggest different roles for anti-apoptotic Bcl-2 family members Mcl-1 and Bcl-x in the genesis and maintenance of canine lymphoma.

DOGS WITH HIGH-GRADE MULTICENTRIC LYMPHOMA SURVIVING LONGER THAN 2 YEARS: IS CURE POSSIBLE?

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Introduction

Lymphoma is the most common hematopoietic tumor among dogs. There are no reports focusing on long-term survivors (dogs living beyond 2 years after diagnosis). Aim of the study was to determine the frequency of dogs with multicentric high-grade lymphoma living beyond 2 years, to review their clinical and biological characteristics, and treatment variables.

Material and Methods

Medical records were reviewed to identify dogs with multicentric high-grade lymphoma, being completely staged and treated with conventional chemotherapy. Dogs living >2 years after diagnosis were defined as long-term survivors, the others served as control. Several factors were analyzed to assess an association with dogs affected by lymphoma living >2 years.

Results

Among the 127 enrolled dogs, 13 (10.2%) survived > 2 years with a median survival time of 914 days (range, 740 to 2058 days). Survival rates at 3, 4 and 5 years were 3.9%, 3.1%, and 0.8%, respectively. Favorable prognostic factors, common to 11 of the 13 long-term survivors at diagnosis, were: body weight >/= 10 kg, hematocrit >/= 35%, absence of ionized hypercalcemia, centroblastic lymphoma, immunophenotype B, absence of bone marrow involvement, lymphoma stage I-IV, and not receiving corticosteroids prior to diagnosis. Four (66.7%) out of the 6 long-term survivors succumbing during the study period died from another malignant tumor; 3 of the 4 had osteosarcoma.

Conclusions

The presence at diagnosis of a combination of fortunate variables may help identifying dogs with lymphoma living longer than 2 years, possibly being cured. Second cancers, in particular osteosarcoma, may develop in long-term survivor dogs with high-grade multicentric lymphoma.

WHOLE-BODY POSITRON EMISSION TOMOGRAPHY USING 18F-FLUORODEOXY-GLUCOSE IN COMBINATION WITH COMPUTED TOMOGRAPHY (PET/CT) FOR EVALUATION OF CANINE PATIENTS WITH MALIGNANT LYMPHOMA: FIRST RESULTS.

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Introduction

F-18-fluordeoxyglucose (FDG) PET/CT has found widespread application for staging and monitoring neoplasia in humans. It seems to be the best possibility to characterize and qualitatively visualize vitality of tumor masses and also holds promises for efficient therapy response monitoring in human patients with malignant lymphoma. The purpose of this study is to characterize canine lymphoma by whole-body PET/CT imaging at diagnosis as well as to re-evaluate the patients during and after completion of chemotherapy. An additional goal is to evaluate this technique as a staging modality and to compare it with conventional staging of malignant lymphoma in the dog based on thoracic and abdominal radiographs, bone marrow examination, ultrasonographic and cytologic examinations of abdominal organs.

Material and Methods

Anesthesia was induced with propofol and maintained with TIVA. The dogs were injected intravenously with 300 MBq of human-grade 18FDG. After 1 hour the dogs were placed in ventral recumbancy, and positioned within the PET/CT scanner gantry so that the whole body was scanned.

Results

To date three canine patients with multicentric lymphoma have been included in this prospective study. Two dogs were stage IV and one was stage V. Two dogs achieved complete remission and one dog a partial remission. The PET/CT before chemotherapy showed good visualisation of the tumor masses.

Conclusions

In conclusion the study may demonstrate a promising tool in the evaluation of canine lymphoma patients. Further studies will assess the ability of staging with PET/CT to predict clinical outcome after first-line treatment in dogs with malignant lymphoma.

A C-KIT INHIBITOR (MASITINIB) SHOWS THERAPEUTIC POTENTIAL IN DOG NEURO-FIBROSARCOMA

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Introduction

An 11 year-old, mixed breed, female dog was diagnosed with neurofibrosarcoma in October 2007. The tumour recurred despite two prior surgeries, chemotherapy with doxorubicin and metronomic chemotherapy (endoxan/meloxicam). A strong relationship exists between the pathogenesis of neurofibrosarcoma and both mast cells and Schwann cells. This suggests possible benefits from treatment with targeted c-Kit or PDGFR inhibitors, respectively. Masitinib (Masivet®) is a tyrosine kinase inhibitor (TKI) that potently and selectively inhibits PDGFR and c-Kit, as well as Lyn and Fyn, without inhibiting kinases of known toxicities.

Material and Methods

Neurofibrosarcoma was diagnosed by histological analysis. Mast cell infiltration was evidenced by c-Kit and tryptase staining. Treatment with Masivet® was initiated in April 2009 with 12 mg/kg/day administered orally. No concomitant treatments were administered and the patient was carefully monitored for adverse events.

Results

The tumour was infiltrated by scattered mast cells at the vicinity of blood vessels. Signs of superficial necrosis were visible at day-4 of treatment. At week-3 a reference mass was measured, 5x4cm, which at week-6 had shrunk to 3x2cm and was negligible at week-10. However, newly developed masses may indicate a higher dosage is required to control tumour metastases. No adverse events were reported, with only a minor decrease in protein level.

Conclusions

This represents the first reported case of canine neurofibrosarcoma being treated with the TKI Masivet®. Rapid tumour growth was quickly stabilised followed by a significant decrease in volume; treatment was well tolerated. There is compelling motivation to conduct further preclinical (mechanistic, resistance) and clinical (efficacy and safety) studies.

ANALYSIS OF DRUG RESIDUES IN SALIVA AND HAIR OF DOGS RECEIVING ANTI-CANCER CHEMOTHERAPY: FIRST RESULTS

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Introduction

Therapeutic modalities for the treatment of cancer previously only used in human medicine are increasingly employed in veterinary practice. Recently, residues of cytotoxic drug were demonstrated in urine for differing time periods as well as serum sampled shortly after treatment, whereas 7 days after treatment serum contained no measurable drug concentration. The aim of this study now is the quantitative analysis of chemotherapy residues in saliva and hair following cytostatic treatment in canine cancer patients in order to further characterize the presence or absence of occupational and/or environmental hazards associated with cytostatic therapy in veterinary medicine.

Material and Methods

Quantitative LC/MS/MS methods to measure vincristine, cyclophosphamide, and doxorubicin in canine saliva and hair have been developed. Saliva and hair samples from dogs receiving anti-cancer chemotherapy for the treatment of lymphoma are collected at different time points after treatment and undergo analysis. Hair samples are rinsed and separate analyses of the washing fluid and the hair itself is undertaken.

Results

In one dog, low concentrations of cyclophosphamide were measured in saliva on day 0 and day 1 after treatment (<10 µg/kg, respectively), whereas no saliva residues were detected following treatment with vincristine or doxorubicin. Additionally, cyclophosphamide traces were measured in one hair sample (<10µg/kg), while washing fluid contained no drug residues.

Conclusions

To date only trace amounts of cyclophosphamide have been detected in saliva and hair of canine lymphoma patients following chemotherapy administration. Further studies are warranted investigating this aspect of anticancer treatment in veterinary medicine.

USE OF TOTALLY IMPLANTABLE VASCULAR ACCESS PORT WITH MINI-INVASIVE SELDINGER TECHNIQUE IN DOGS UNDERGOING CHEMOTHERAPY

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Introduction

Vascular ports are totally implantable devices designed to provide repeated access to the vascular system for the delivery of chemotherapy, antibiotics, fluids, parenteral nutrition and blood. Port access is performed by percutaneous needle insertion using a non-coring Huber needle.

Material and Methods

A silicone or polyurethane catheter is inserted up to the junction of the cranial vena cava and the right atrium approaching the external giugular vein with non-invasive Seldinger technique by mean of guidewire, dilator and sheath introducer. A subcutaneous pocket and a tunnel to the venous entrance site is created. The tip of the tunneler is advanced up to the venous entry site. The catheter tip is then threaded on to the end of the tunneler and this is pulled to the port entry site. The catheter is connected to the port. The port is placed in the subcutaneous pocket and the skin sutured.

Results

Vascular ports have been placed in seven dogs receiving chemotherapy. None of these patients showed complications immediatly after implantation or signs of intolerance in the following period. Two of these patients devoleped intermittent fever and anorexia; their port was removed and resulted positive for bacterial infection.

Conclusions

Vascular access ports are useful for patients undergoing long-lasting chemotherapy protocols; these devices allow peripheral veins to be stored and not seriously damaged for repeated infusion of vescicant drugs. The most common complications are: infection, malpositioning of the catheter, thrombosis.The implant is well tolerated and may be left for several months.

BOVINE PAPILLOMAVIRUS AND EQUINE SARCOIDS: EVALUATION OF THE POTENTIAL ROLE OF FLIES AS VECTORS IN DISEASE TRANSMISSION.

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Introduction

BPV-1 is the causative agent of equine sarcoids. The viral sequences detected in sarcoids differs from those detected in cattle infections which indicates the disease is not transmitted via cattle as previously proposed. Infact recent evidence suggests equid to equid transmission of disease, however the mechanism(s) of transmission is not known. Many insects are the primary or intermediate hosts or carriers of diseases. The distribution of lesions and predilection for sarcoid development at wound sites suggests that insects may act as disease transmission vectors.

Material and Methods

All fly captures were performed at the Donkey Sanctuary, Devon, UK. Adhesive paper fly traps were used to catch flies at various locations where sarcoid affected and unaffected donkeys were housed. Three trapping sessions were performed at different times during the summer fly season. Swabs were used to collect sarcoid cells from affected animals at the time of fly capture. Captured flies were identified and individual species pooled. DNA was extracted from both flies and sarcoid samples and subjected to BPV-1 PCR amplification followed by sequencing analysis.

Results

BPV-1 DNA was detected in 50% of the captured fly samples collected. Characterisation of the BPV-1 DNA sequences from the flies indicated that the viral variants detected in the fly samples were the same as those present in the sarcoid tumours of affected animals.

Conclusions

BPV-1 DNA can be detected in flies. This study provides evidence to support the hypothesis that flies may therefore be significant in disease transmission, however further studies are required to confirm this.

THYMIDINE KINASE 1 EXPRESSION IN SERUM AND TUMOUR TISSUE IN DOGS - A NEW PROLIFERATION MARKER

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Introduction

Activity measurement of the S-phase specific enzyme Thymidine Kinase 1 (TK1) in serum is valuable for staging and monitor treatment response in haematological neoplasia in both dogs and humans. Detecting expression of the enzyme in serum and especially tumour tissue would likely expand usefulness of TK1 in solid tumours as well.

Material and Methods

Two TK1 antibodies were established; one polyclonal rabbit/anti-canine (PabC) towards the c-terminal part and one monoclonal mouse/anti-human (MabH) (active-site specific, 97% canine homology). Expression was investigated with immuno-blots in serum from normal (n=3) and tumour bearing dogs (n=5) and in formalin-fixed paraffin-embedded (FFPE) specimens from malignant lymphomas (n=8) and solid tumours (n=9).

Results

Both antibodies showed TK1 specificity in western-blotting experiments. Level of expression corresponded to pre-established TK1 activity. In FFPE tumour samples the PabC gave more background staining, whereas the MabH showed specific and reproducible staining in both lymphomas and solid tumours - especially mammary tumours (n=6).

Conclusions

For the first time, TK1 expression is reported in canine serum and tumour tissue. This will enable clinically relevant, refined grading of lymphomas and also solid tumours. The new antibodies will be compared with other proliferation markers (e.g. Ki-67). As the antibodies work with FFPE specimens it makes them particularly interesting since large archive material can be tested. TK1 has an obvious potential as proliferation marker both via activity measurements and protein expression analysis in the same tumour type. The comparative aspects are also obvious with the high homology to human TK1.

PROGNOSIS FOLLOWING SURGICAL EXCISION IN CANINE AND FELINE MALIGNANT SKIN TUMORS: THE ROLE OF THE HISTOLOGICAL EVALUATION OF MARGINS

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Introduction

Wide surgery represents the best therapy for skin tumors, although hampered by local recurrences after incomplete removal. As the clinical assessment of tumor size rarely corresponds to its actual extension, the completeness of surgery can be effectively determined only by the histological evaluation of excision margins.

Material and Methods

To assess the efficacy of the histological evaluation of margins in predicting the development of local recurrence, 50 canine and feline surgically-removed skin tumors (21 MCTs, 18 soft tissue sarcomas, 11 carcinomas) have been examined. Margins were judged as clean, clean but close (tumor cells < 2 mm from margins) or infiltrated. Histological grade was assessed in sarcomas and MCTs. Tumor recurrence-free intervals (RFIs) at 1 and 2 years were recorded.

Results

Margins were infiltrated in 19/50 cases (38%), clean but close in 9/50 (18%), and clean in 22/50 (44%). Recurrence occurred in 19 cases. Mean RFI was 233 days (range: 31-661). Recurrence rates were 68.42% (infiltrated margins), 44.44% (clean but close margins), and 9.09% (clean margins). The method accuracy improved with the extension of the follow-up period, and was highest for carcinomas (100%), intermediate for sarcomas (89%) and lower for MCTs (78%), due to actual difficulties in distinguishing if mast cells found on margins are normal or neoplastic.

Conclusions

Histological evaluation of margins is a good predictor for tumor recurrence, although more likely to generate false positives in MCTs. Because of generally long recurrence times, post-surgical surveillance should be extended to at least 2 years. Tumor grade influences RFI but not the method accuracy.

CARBOHYDRATE RESPONSE ELEMENT BINDING PROTEIN (CHREBP): A POTENTIAL NEW BIOMARKER IN COMPANION ANIMAL MAMMARY GLAND TUMOURS

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Introduction

Carbohydrate response element binding protein (ChREBP) regulates expression of fatty acid synthase (FASN), a marker of poor prognosis in human breast cancer. Tumours upregulate glucose uptake via the facilitative glucose transporter Glut-1, to support the increased energy demands of the hypoxic tumour microenvironment. ChREBP is itself activated by increased levels of glucose. We propose that ChREBP may link increased glucose uptake and fat synthesis in breast tumours.

Material and Methods

Immunohistochemical detection of ChREBP, Glut-1 and FASN was carried out on a series of 10 canine mammary carcinomas followed by semi-quantitative analysis of expression in malignant versus non-malignant epithelial cells.

Results

FASN was expressed in >40% of epithelial cells in most mammary carcinomas, as well as in normal mammary epithelial cells. In some dogs, epithelial cells from both carcinoma and normal tissue had low levels of expression or were negative for FASN. ChREBP was expressed in the cytoplasm of >60% of epithelial cells in mammary carcinomas and at similar or lower levels in most normal mammary epithelial cells. Glut-1 was not expressed in normal mammary epithelial cells, but there was positive staining for Glut-1 in 0 to 60% of epithelial cells in mammary carcinomas, mainly in degenerating or necrotic cells.

Conclusions

Glut-1 staining in canine mammary carcinoma was consistent with its association with necrosis and tissue hypoxia in human cancers. Despite evidence that there may be upregulation of ChREBP expression in some canine mammary carcinomas, there is no evidence of substantial alterations in FASN expression in this preliminary study.

EVALUATION OF THE STOMACH WALL BY HELICAL HYDRO-CT (HHCT): NORMAL TECHNIQUE AND CLINICAL CASES.

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Introduction
In HHCT, water is used as a neutral luminal contrast with intravenous contrast for the diagnosis of gastric neoplasia in humans. The aim of this study is to describe a technique for HHCT in dogs and to evaluate its usefulness in clinical cases.

Material and Methods
6 normal adult dogs (2 < 10Kg, 2 between 10-25kg and 2 > 25Kg) underwent HHCT to evaluate gastric distension with 3 different volumes of water (10, 20, and 30ml/kg), given via gastric tube. Dogs were scanned in sternal recumbency, 3 mm slice thickness and a pitch 2:1. The images were reviewed using soft tissue and bone algorithms. Once optimal wall distension was found, the tube was withdrawn and the oesophagus aspirated. Intravenous contrast enhanced CT was performed. The studies were reconstructed with slice thicknesses of 1 and 3 mm. Six dogs with gastric neoplasia (5 adenocarcinoma , 1 leiomyoma) underwent HHCT with 30ml/kg of water.

Results
Without HHCT, artifactual thickening of the greater curvature was always present. The administration of 30ml/kg of water achieved optimal uniform gastric distension, with the entire gastric wall being visible as a thin regular line. In the 6 dogs with gastric neoplasia, focal thickening was present with moderate contrast enhancement. The extent of the lesion was easily assessed in all cases.

Conclusions
HHCT is a simple and sensitive technique for assessing the stomach. It is our preferred method for staging gastric tumours and surgical planning.

IMMUNOHISTOCHEMICAL STUDY OF C-ERBB2, CD31 AND P53 IN CANINE MAMMARY GLAND CARCINOMA

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Introduction
The objective of the present investigation was to determine the expression of c-erbB2, CD31 and P53 by using antihuman antibody and their relation with morphologic features and histologic grade.

Material and Methods
In this study, 30 mammary gland tumor samples were studied. The histological diagnosis was made on the basis of the current WHO classification for canine mammary tumors and then tumors were graded histologically in accordance with the Elston and Ellis method for human breast tumors. Immunohistochemical staining for c-erbB2, Cd31 and P53 was performed on canine mammary gland carcinoma tissue.

Results
Results of staining with c-erbB2 antibody was %76.7 negative and %23.3 positive. All samples which were stained for Cd31 marker were negative. Results of Semiquantitative immunohistochemistry assessment of p53 protein with monoclonal antibody Do7, was %36.7 negative, %6.7 one positive, %3.3 two positive, %53.3 three positive. There was significantly difference between histologic grade and P53.

Conclusions
The results of Immunohistochemical staining of c-erbB2 and P53 showed that using antihuman antibody is useful and it would help the pathologist and clinician for more accurate prognosis and treatment in canine mammary carcinoma and More research about c-erbB2 and P53 will be useful for radioimmunotherapy and genetherapy.

ANALYSIS OF C-KIT MRNA EXPRESSION AND MUTATIONS IN CANINE CUTANEOUS MAST CELL TUMOURS

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Introduction

Canine cutaneous mast cell tumour (MCT) is one of the most common neoplasm in dogs. The c-KIT proto-oncogene has been studied due to its involvement in cellular proliferation and MCTs development. In particular c-KIT mutations and aberrant cytoplasmic KIT protein localization had been previously associated with both decreased disease-free and overall survival rates. In the present study, further than protein localization and mutations identification, c-KIT mRNA expression in MCTs compared to non-pathologic tissue samples was evaluated.

Material and Methods

48 MCT samples and margins were collected during surgical intervention. Immunohistochemistry was performed in formalin-fixed paraffin-embedded samples using the CD117 polyclonal primary antibody. Total RNA was isolated from frozen samples and used for the identification of c-KIT mutations in exons 8, 9, 11 and for the gene expression analysis by quantitative Real Time PCR.

Results

For CD117, three staining patterns were recognized: a membrane-associated pattern, a focal cytoplasmic pattern, and a diffuse cytoplasmic pattern. In 4 cases out of 48, c-KIT mutations were identified: 2 internal tandem duplications, 1 deletion and 1 point mutation (S479I). Interestingly, a statistical significant increase of c-KIT mRNA has been observed in grade I and II MCTs towards surgical margins and normal tissue.

Conclusions

In the present study, a prevalence of 8% of c-KIT mutations, already evidenced in literature, has been observed in MCTs. Furthermore, the increased c-KIT mRNA in grade I and II MCTs towards surgical margins and normal tissue confirm the importance of c-KIT as an optimal prognostic biomarker in canine MCTs.

THE ROLE OF THE PI3K/AKT/MTOR SIGNALING PATHWAY IN CANINE TUMOURS

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Introduction

The PI3K/Akt/mTOR signaling pathway has become a promising cancer therapeutic target due to previous findings revealing its important role in the regulation of cell growth, survival and protein synthesis in normal and cancer cells. Herein, we sought to evaluate whether inhibition of PI3K/Akt/mTOR pathway can prevent the progression of canine tumours.

Material and Methods

We examined the effect of an mTOR inhibitor (rapamycin) and a PI3K inhibitor (wortmannin) on 4 canine cancer cell lines derived from canine lymphomas (3132 cells), hemangiosarcomas (SB cells), mammary gland tumours (REM cells) and gliomas (J3T cells), in light of the growth inhibition which was evaluated by cell viability assay and the inhibition of the active (phosphorylated) form of the member proteins of the PI3K/Akt/mTOR pathway by using western blotting techniques.

Results

Our data demonstrated that the mTOR inhibitor, rapamycin, inhibited 50% of the growth of 3132, SB, REM and J3T cells at 1 mM, 10 mM, 10 mM and 20 mM, respectively. The PI3K inhibitor, wortmannin, inhibited 50% of the growth of both 3132 and SB cells at 10 and 40 mM, respectively. Conversely, REM and J3T cells were resistant to wortmannin with 15-20% growth inhibition at 40 mM. Western blotting analysis on 3132 cells revealed that rapamycin reduced the levels of the active form of mTOR downstream targets, including p70S6K, S6RP and 4EBP1.

Conclusions

we demonstrate that the PI3K/Akt/mTOR signaling pathway may offer a target for therapeutic intervention in veterinary oncology.

COMPARATIVE ANALYSES OF HIGH MOBILITY GROUP A (HMGA) GENE EXPRESSION AS TUMOUR MARKER IN CANINE UND HUMAN ORAL SQUAMOUS CELL CARCINOMA

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Introduction

Cancer of the oral cavity is the eighth most common cancer worldwide in men and the fourth most common cancer in dogs. In both species the incidence and mortality rate is generally higher for males than females. In men, more than 95% of the carcinomas of the oral cavity are of squamous cell type, while in dogs these neoplasias cover about 17-25% of the oral malignancies. The identification of factors involved in formation and progression of oral squamous cell carcinomas could be of significant value for the development and evaluation of therapeutic approaches in both species. The high-mobility-group-A proteins (HMGA1, HMGA2) were described in many human cancers as tumormarkers playing a significant role in progression and aggressive behaviour of tumours.

Material and Methods

We analysed the expression pattern of HMGA1 and HMGA2 in human and canine oral carcinomas and healthy tissues via relative real-time PCR (qPCR). Ten healthy reference and ten tumour samples of eleven human patients and four cell lines derived from the primary tumours of four patients were analyzed. The canine samples included eight oral squamous cell carcinoma and two reference tissues. As reference genes, HPRT and GUSB of the respective species were used and the results were analysed via Delta-Delta-CT Method.

Results

Statistical analysis showed in both species a significant up-regulation of the HMGA2 gene while no significant values for HMGA1 could be detected.

Conclusions

Our results indicate that HMGA2 could serve as tumourmarker in both species and be of value for diagnostic and therapeutic approaches.

RESISTANCE TO RADIATION AND CHEMOTHERAPY OF CANCER STEM CELLS DERIVED FROM FELINE BREAST CANCER.

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Introduction

The cancer stem cell theory has challenged the accepted paradigm of tumourigenesis, where any cell in the body has the potential for malignant transformation. In human medicine, breast cancer stem cells have been identified. Naturally occurring cancers in companion animals share many biological features with human cancer, including molecular targets, telomerase biology and tumour genetics. Here, we have shown that cancer stem cells can be isolated for feline mammary carcinoma cell line, and are more resistant to traditional chemo- and radiotherapies.

Material and Methods

Putative cancer stem cells were isolated from feline mammary carcinoma cell line by expression of the cell surface markers CD133 or CD34, and on their ability to form mammospheres in serum free semi-solid media. These different cell populations were assayed for their sensitivity to common chemotherapy drugs and x-irradiation.

Results

Putative cancer stem cells were isolated form feline mammary carcinoma cell line, and show increased resistance to common chemotherapeutic drugs and x-irradiation compared to parental cell line.

Conclusions

Significantly, we have identified cancer stem cells from a feline mammary carcinoma cell line, indicating breast cancer in the cat is a stem cell disease. Here, we show that feline mammary carcinoma stem cells are more resistant to common chemotherapy drugs and x-irradiation. This provides an explanation for why conventional therapies, which often shrink the tumour bulk but fail to completely eradicate it, as they can not effectively target cancer stem cells embedded with in a tumour.

ACCELERATED RADIATION THERAPY WITH CONCOMITANT CARBOPLATIN FOR TREATMENT OF FELINE ORAL SQUAMOUS CELL CARCINOMA

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Introduction

We previously reported a 9 day radiation protocol for oral SCC in cats. Minimal toxicity was seen but overall median survival was only 86 days. We hypothesized that this protocol delivered with concomitant carboplatin would improve tumor control without increasing toxicity.

Material and Methods

Cats were staged with thoracic radiographs, lymph node aspiration, and tumor imaging. Radiation was administered twice daily for 7 treatment days using 3.5 Gy fractions to a total of 49 Gy. Carboplatin (90 mg/m²) was given IV immediately before the first or second treatment, and repeated 4.5 -5 days later for total dose of 180 mg/m² within the 9 day period.

Results

Twenty-nine cats have been treated with tumors of mandible (n=10), maxilla (n=7), tongue (n=8), tonsil (n=3), and cheek (n=1). Acute reactions ranged from grade 0-1 in skin (commonly 1), and from 0-3 in the oral mucosa (commonly 2). Feeding tubes were placed in 23 cats. All but one cat responded to treatment; 12 were judged to be complete. One cat experienced bone necrosis at 3.5 months and one cat appeared to have tongue fibrosis at 2 months post therapy. Median survival was 163 days (range 30-724 days), with a mean of 277 ±50 days. Nine cats are still alive. Significant prognostic indicators found were tumor site (P = 0.0162), tumor response at 30 days (P= 0.0440, and tumor stage (P= 0.0225)

Conclusions

The treatment was well tolerated by all cats in the acute phase. Late reactions may have been seen. The median survival has improved over radiation alone.

ADJUVANT HYPOFRACTIONATED RADIATION THERAPY FOR THE TREATMENT OF CANINE SOFT TISSUE SARCOMAS

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Introduction

Soft tissue sarcoma represents 15% of all canine soft tissue tumours. If curative surgical excision is not achieved, adjuvant radiation therapy has been shown to improve local control. Limited access to veterinary radiation facilities, costs and requirement for repeated anaesthesia limit the application of publishedhyperfractionated regimens. The purpose of this study was to determine the potential benefit of hypofractionated radiation treatment for canine soft tissue sarcomas.

Material and Methods

Data from canine patients with soft tissue sarcomas that received hypofractionated radiotherapy for incomplete surgical excision were collected retrospectively. Data regarding the tumour included histological diagnosis, grade and location. Cases were collected from two centres.All patients were treated using a 4MV linear accelerator and each received a total of 4 doses of 9Gy given at intervals of 7 days.Data were analysed using Kaplan-Meier survival curves

Results

All patients completed the course of therapy and no treatment breaks were required due to acute side effects.Disease-specific survival was 81.4% at one year and 72.2% at two years, comparingfavourably with published fractionation protocols. Local recurrence was 16% andmetastatic rate was 4.9%.

Conclusions

Hypofractionated radiation therapy appears to be an acceptable alternative adjuvant therapy for incompletely resected soft tissue sarcomas.

POSTER ABSTRACTS

COX INHIBITORS AS PALLIATIVE TREATMENT IN DOGS WITH METASTATIC MAMMARY CANCER

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Introduction

The presence of COX-2 receptor by immunohistochemistry, as well as the content of COX-2 measured by enzyme immunoassay in canine mammary tumours (CMT) has been demonstrated. Both are higher in malignant than in benign CMT, and COX-2 levels are related to a poor prognosis.These findings suggest that COX-2 inhibitors, might be useful in the treatment of malignant CMT.

Material and Methods

This is a retrospective study (2000-2009) that includes fifteen female dogs, diagnosed with malignant mammary tumours (non inflammatory mammary carcinoma) with distant metastases in which surgery was not recommended. Eight animals were treated either with firocoxib at 5 mg/kg/24h (n=6) or piroxicam at 0.3 mg/kg/24h (n=2) until euthanasia (cases); and seven animals did not receive any treatment (controls).

Results

Overall survival in cases (range: 13 to 427 days; mean ± SD: 137 ± 135 days) was significantly higher ($p < 0.05$) than in controls (range: 7 to 85 days; mean ± SD: 35 ± 30 days). One dog treated with firocoxib developed diarrhea for a week, which was solved with supportive treatment, and firocoxib was restored afterwards, without further signs of toxicity. Another dog treated with piroxicam, developed a severe gastrointestinal disease after 40 days of treatment, which was the main reason for the euthanasia.

Conclusions

These results suggest that COX-2 inhibitors, especially firocoxib due to the lower toxicity compared with piroxicam, might be useful as a palliative treatment of dogs with metastatic mammary tumours, due to the increased survival times observed. Further studies including more animals are necessary to confirm these findings.

COX-2 EXPRESSION IN CANINE HYPERPLASTIC AND NEOPLASTIC LYMPH NODE TISSUES

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Introduction

Cyclooxygenase-2 (COX-2) is a key-role enzyme in prostaglandin biosynthesis. Its expression has been associated with many human pathologic conditions, and preneoplastic and neoplastic disorders. Several studies have demonstrated the COX-2 over-expression and its prognostic value in feline e canine tumours. COX-2 is over-expressed in human lymphomas. The aim of this study is to investigate the presence of COX-2 over-expression in canine hyperplastic and neoplastic lymphatic tissue disorders.

Material and Methods

Thirty-four canine lymph node tissue samples (13 from hyperplastic and 21 from neoplastic lymph nodes) have been evaluated for COX-2 expression by immunohistochemistry. Scoring was performed according to previous studies and tumours scoring +2 and +3 were recorded as overexpressing COX-2. Statistical analysis was also performed.

Results

COX-2 overexpression was observed in 5/21 lymphomas (24%) with a moderate staining intensity and in 4/13 (31%) lymph node hyperplasias. No significant differences were found between hyperplasias and lymphomas in COX-2 expression.

Conclusions

This is the first study describing COX-2 immunoreactivity in hyperplastic and neoplastic lymph nodes tissues. Even if a wider number of samples needs to be investigated to draw some firmer conclusions, our data suggest a rationale for further investigate COX-2 expression in these neoplasms for prognostic, chemopreventive and chemotherapic implications.

RETROSPECTIVE STUDY OF FELINE SARCOMA

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Introduction

The sarcoma was the second most prevalence cutaneos neoplasia in feline. The mean age of affected cats is ten years. The clinical presentation was a firm regular nodule on the body.

Material and Methods

From October 1998 to February 2006, 47 cats received the diagnosis of feline sarcoma in the SSAS – SVMAS/USP. The records were study and the statistical analysis was performed. The data analyzed were breed, gender, histopathological type, body region affected, mass size, consistency and adherence. The treatment and presence of metastasis were evaluated too.

Results

From the total FS cases (47), 26 (55%) were females and 21 (45%) were males; the mean age was ten years. Domestic short hair represented 40 (85%) of the cases, while 7 (15%) had a defined breed. We observed 31 (66%) mass in toraco-abdominal region; the mass size measure varied from 0.5 to 12,5cm. The predominant type by histopathological exam was fibrosarcoma in 19 (40%) animals, followed by injection-site sarcoma in 13 (28%) of them. The mass surface was regular in 37 (79%) cats and the mass had firm consistence in 30 (64%) of them. The most were attached in 38 (81%) cats. 32 (68%) animals were submitted to surgery. Only 13(28%) animals were submitted to chemotherapy; 5 (11%) showed metastasis and 10 (21%) had presented recurrence.

Conclusions

Face of the large variation of measurement, description and treatment found was propose that a standardization of procedures with a feline that presents a regular and firm mass must be employed, aim to propose the best treatment and prognosis.

EVALUATION OF PROGNOSTIC FOR CANINE MASTOCYTOMA (MCT) THROUGH THE EXPRESSION OF KIT BY IMMUNOHISTOCHEMISTRY AND REAL TIME POLYMERASE CHAIN REACTION (RT-PCR)

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Introduction

C-kit is a proto-oncogene related with survival, proliferation and activation of mast cells. The expression and mutation of the KIT, the c-kit gene product, could be related to the genesis and progression of MCT and the expression of KIT is a potential prognostic indicator for MCT.

Material and Methods

This study correlated the gene expression of KIT, immunohistochemistry and RT-PCR at the rate of recurrence and death of 81 dogs with MCT. In immunohistochemistry was observed patterns staining of KIT, divided into groups according to KIUPEL et al. (2004). For gene expression in RT-PCR, primers were used for KIT and its ligand (c-kit-LIG). We used the Kaplan-Meier method for construction of curves free of death and recurrence.

Results

The expression patterns of KIT-I was found in 9 (11.11%) tumors, KIT-II in 50 (61.73%) and KIT-III in 22 (27.16%). Through the method of Kaplan-Meier wasn't found an association of the KIT pattern staining with death and recurrence rate ($p = 0278$, $p> 005$). There was also no association between the rate of recurrence and death with the gene expression of KIT ($p = 0.289$, $p> 0.05$) and the gene expression of c-kit-LIG ($p = 0.106$, $p> 0.05$).

Conclusions

Although, if we didn't find association of the staining pattern of the KIT in MCT and survival, we suggest a correlation between aberrant KIT localization and increased proliferation activity of MCT. The RT-PCR appears to be a sensible method for quantitative detection of c-kit gene expression in canine MCT, despite these expressions levels don't correlate with prognosis.

ESTRACTION OF NUCLEIC ACIDS FROM STAINED BLOOD AND LYMPH NODE SMEARS: A TOOL FOR RETROSPECTIVE STUDIES ON CLONALITY AND MICRORNA

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Introduction

Studies on spontaneous neoplasms require elevated numbers of homogenous cases with known follow-up, thus retrospective studies are often the only possible approach for most clinical studies. However, in a clinical setup, it's often difficult to sample different kind of material for future potential studies. Aim of this study was to standardize a protocol for DNA and RNA extraction from lymph nodes and peripheral blood stained smears of dogs affected by lymphoid neoplasms and to check the possible use of such material in analysis of clonality and microRNA pattern.

Material and Methods

Smears from lymph node aspirates and peripheral blood of dogs affected by B-cell and T-cell lymphoma and CLL were subjected to direct resuspension and nucleid acid extraction with TRIzol as described by the manufacturer. DNA was subjected to PCR amplification for B-cell and T-cell antigen receptor gene rearrangements. Total RNA was subjected to RT-qPCR for the microRNAs miR-17-5p and miR-181a.

Results

Clonality was successfully detected from the majority of DNA samples extracted from the smears. MiR-17-5p/miR-181a ratio values obtained from lymphomas samples were similar to what already reported for fresh-frozen and FFPE samples.

Conclusions

The method recovers nucleic acids from stained lymph node and blood smears. The quality of sample is suitable for detection of DNA targets, for genotyping and detection of clonality, and RNA targets for microRNAs pattern, with results comparable to fresh-frozen and FFPE samples. This could be useful for retrospective studies on lymphoproliferative disorders, such as lymphoma and leukaemia, in which stained smears are often the only archive material available.

CYTOCHROMES P450 AND ABC-TRANSPORTERS GENE EXPRESSION PROFILING IN DOG OSTEOSARCOMA, MAST CELL, AND MAMMARY GLAND TUMOURS

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Introduction

Members of the cytochromes P450 (CYPs) superfamily of drug metabolizing enzymes play an outstanding role in bioactivation and/or inactivation processes of anticancer drugs and procarcinogens. Likewise, ABC-transporters (ABCTs) are considered of relevant importance in multidrug resistance phenomena. In humans, the presence of CYPs and ABCTs has been demonstrated, in tumour and non-tumours tissues, by using immunohistochemistry. On the other hand, scarce information have been published in the dog. Thus, aim of this study was to evaluate the expression of CYPs and ABCTs in canine neoplasias.

Material and Methods

Surgical biopsies of 25 canine mammary tumors, 40 mast cell tumours, 6 osteosarcoma as well as control samples from pathogen-free Beagles or euthanized dogs were collected. Quantitative Real Time PCR assays for canine CYP1A1, CYP1A2, CYP1B1, CYP2B11, CYP2C21, CYP2D15, CYP3A12, MDR1 (PgP), MRP1, MRP2, MRP3, and 2 housekeeping genes were set up by using UPL probes. Finally, the differential expression of target genes was evaluated between normal and tumour samples.

Results

Generally, CYP1A1, CYP1A2, CYP2B11, CYP2C21 and CYP2D15 mRNA expression was strongly inhibited in tumour specimens compared to the respective normal tissues. CYP3A12 mRNA was increased 5-fold in osteosarcoma, while CYP1B1 was generally over expressed in almost all tumour samples. Among ABCTs, PgP and MRP3 were generally induced in tumour specimens.

Conclusions

Results suggest that CYPs mRNA general inhibition in tumour samples confirm the potential usefulness of gene pro-drug therapy with CYPs involved in anticancer drugs metabolism. On the contrary, CYP1B1 and CYP3A12 could be proposed as potential biomarker of outcome in dog tumours, likewise to humans.

A PRELIMINARY STUDY USING A NOVEL QUESTIONNAIRE TO ASSESS THE QUALITY OF LIFE OF VETERINARY CANCER PATIENTS REFERRED TO LIVERPOOL UNIVERSITY SMALL ANIMAL TEACHING HOSPITAL

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Introduction

Quality of life (QOL) refers to emotional, social and physical well-being. Tumour associated morbidity and treatment related side-effects significantly impact the QOL of human cancer patients. Based on minimal published veterinary research, it is unclear to what extent cancer affects the QOL of dogs and cats.

Material and Methods

Owners of dogs and cats referred with a diagnosis of cancer completed a questionnaire regarding their pet's QOL. Eleven questions assessed behavioural and physical parameters using visual analogue scales (VASs) with 'no impact' (score 0) and '100% affected' (score 100) descriptors at either end of the scale. The attending clinician was blinded to the owner's responses and gave a 'professional' assessment of the pet's QOL for comparison.

Results

Thirty-six dogs and 3 cats with various tumour types were represented in this preliminary study (mast cell tumour, n=10; lymphoma, n=7; sino-nasal tumours, n=5; osteosarcoma, n=4; thyroid carcinoma, n=3; soft tissue sarcoma, n=3 and 1 each of insulinoma, anal sac tumour, osteoma, mammary carcinoma, adenocarcinoma, oral SCC, melanoma and plasmacytoma). Individual QOL scores varied considerably between tumour types and between individuals affected by the same tumour type (range 0-759). Mean total scores grouped by tumour type tended to be low (< 300). Correlation between the owner and professional QOL score was poor (r^2=0.189).

Conclusions

QOL of veterinary cancer patients is good overall based on favourable scores in this preliminary study; in individual patients, however, it may be impacted severely. Discrepancy between veterinary and owner QOL assessment may be addressed through questionnaire design and execution. Patient recruitment is ongoing.

POSSIBLE PHANTOM LIMB PAIN IN 2 DOGS AFTER AMPUTATION FOR OSTEOSARCOMA

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Introduction

Bone neoplasia is an indication for limb amputation in small animals. In human amputees, 60-80% experience phantom limb pain (PLP) after surgery. Chronic knifelike, burning, pricking or throbbing sensations are described; their cause and treatment remains unclear. A single case report describes PLP like symptoms in an amputated cat but this has never been described in dogs despite this being a relatively common surgical procedure. This syndrome may be rare or it may be difficult to identify in canine and feline amputees.

Material and Methods

Chronic pain assessments using a questionnaire (Dog 1) or a 'pain diary' (Dog 2) were performed in 2 dogs that underwent amputation for osteosarcoma and showed post operative signs suggestive for PLP. The dogs did not have discernible stump pain on physical examination. Gabapentin was administered to both dogs.

Results

Brief episodes of vocalising at night, restlessness and periodic attention to the amputation site began 62 days after surgery in dog 1. Impact on quality of life was low based on questionnaire responses. Treatment with gabapentin (10mg/kg PO TID, reducing dose) is ongoing. Brief pain episodes (1-9 episodes/day) described as sudden yelps (0.25-6s duration) began 30 days after amputation in dog 2. Gabapentin (5mg/kg PO TID) successfully reduced the frequency of episodes and was discontinued after 70 days of treatment.

Conclusions

PLP like symptoms may occur after amputation in dogs. The onset of signs is delayed. Gabapentin may be an effective therapy. A prospective study is planned to determine the incidence of this syndrome in veterinary patients.

IS N-3 FATTY ACIDS SUPPLEMENTATION A VALID SUPPORT IN DOGS AFFECTED BY LYMPHOMA TO TREAT CACHEXIA?

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Introduction

Many studies have shown that n-3 fatty acids may play an adjuvant role to prevent cancer cachexia.The aim of this study was to evaluate their efficacy to improve clinical and nutritional conditions in lymphoma dogs treated with chemotherapy.

Material and Methods

Twelve dogs were randomly divided in two groups: the first received a supplementation with 4g of Resurgen® (NBFLanes)/10kg/day, the second group a placebo. In 1g of this product there are 160mg EPA, 90mg of DHA, 70mg of CLA, 600mg of vitamin E and 900mg of beta-catotene.During treatment, each dog underwent a blood work and a clinical and nutritional (body condition score -BCS- and relative body weight -RBW) examination. Data were compared at the beginning of the study, after 4 and 7 weeks by WinEpiscope Cohort study.

Results

At the beginning of the study, 33% of treated dogs exhibited cachexia (BCS≤3), whereas none in placebo group. In supplemented group BCS and RBW had the tendency to increase while in the placebo group there was no difference. Hair conditions in supplemented group improved after 7w while in the placebo did not. There were no differences in other parameters evaluated (appetite, gastro-enteric signs and blood parameters) and in survival time between the two groups.

Conclusions

Use of n-3 fatty acids seems to improve coat and nutritional conditions in cancer patients. Their concentration in the basal diets was not calculated and this could be a confounding factor. Future studies should investigate the improvement of n-3 fatty acids on nutritional conditions, considering specific cachexia markers as TNF-alfa.

CHARACTERIZATION OF THREE NEW CANINE MAMMARY TUMOUR CELL LINES WITH PHENOTYPES REFLECTING AGGRESSIVE CANCER SUBTYPES AND FIRST DESCRIPTION OF A CANINE CELL LINE EXHIBITING ENTOSIS.

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Introduction

Mammary carcinomas (MC) are a heterogeneous group of malignancies. In humans, 5 molecularly defined subtypes with prognostic relevance can be routinely identified using immunohistochemistry for HER2, estrogen and progesterone receptors (ER, PR), cytokeratins (CK) and epithelial growth factor receptor (EGFR) (Sorlie et al. PNAS 2001). Canine MC with IHC patterns comparable to human MC subtypes have been identified. We attempted to establish new canine MC cell lines reflecting two aggressive subtypes (HER2+ or "basal-like") for which the need for new adjuvant treatments is pressing.

Material and Methods

Nine high-grade canine MC were manually dissected and cultured in DMEM, 10% fetal calf serum and antibiotics. Long-term cultures were characterized for HER2, ER, cytokeratins and EGFR expression by immunocytochemistry on cytospin preparations.

Results

Three cell lines were obtained: NA-MC1 was only CK+, NA-MC2 and NA-MC3 were CK+, HER2+, ER+ and EGFR+. Moreover, NA-MC1 (growing in suspension) exhibited entosis, a phenomenon by which a tumour cell is internalized into a neighbouring cell and is then, after a cohabitation step, eventually degraded or released (Le Bot, Nat Cell Biol 2007).

Conclusions

These three new canine MC cell lines, reflecting two different aggressive MC subtypes, are new tools for antineoplastic drug activity assessment.We describe also for the first time a canine cell line exhibiting entosis. NA-MC1 could be used to study this still mysterious phenomenon that is currently suspected to be either a tumour-suppressor mechanism or, on the contrary, a strategy for cancer progression, as entosis could provide a survival advantage to the host cell through nutrient recycling.

CHARACTERIZATION OF CANINE ADIPOSE DERIVED MESENCHYMAL STEM CELLS: A NEW CELL SOURCE FOR TUMOR THERAPY?

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Introduction

Cancer treatment in dogs is still insufficient with regard to prognosis and relapse occurance. Using canine adipose derived mesenchymal stem cells (cAD-MSCs), dogs could benefit of novel stem cell based therapeutic approaches to treat tumor derived disorders like overepression of locally released pathotropic proteins and improved regeneration of damaged tissues. So far the use of cAD-MSCs in dogs has been limited due to the fact that the pluripotent behaviour of these cells has been not yet been satisfactorily characterized.

Material and Methods

Aim of the study was therefore the characterization of cAD-MSCs with regard to pluripotency marker gene and protein expression. In total 12 fat samples were collected from 10 dogs for cAD-MSCs isolation.

Results

cAD-MSCs show strong positive immunofluorescence signals typically expressed by mesenchymal stem cells, whereas the expression of haematopoetic stem cell markers (CD34, CD45) is absent. Gene expression of a marker set of in total 5 pluripotency markers including OCT4 and Nanog was detected in the isolated cells by RT-PCR. Furthermore the ability of cAD-MSCs to differentiate into an osteogenic phenotype was evaluated by positive von Kossa staining and Osteocalcin detection in the extracellular matrix.

Conclusions

The here introduced study demonstrates that canine adipose tissue is a rich and easily obtainable resource for cAD-MSCs. A deeper insight into the identification and differentiation potential of cAD-MSCs may help to promote the future use of these cells in regenerative medicine.

COMPARISON OF CELL PROLIFERATION IN CANINE AND FELINE MAMMARY GLAND HYPERPLASIA AND NEOPLASIA

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Introduction

Ki-67 nuclear antigen is one of the main immunohistochemical (IHC) marker used to evaluate tumor proliferative activity. Studies on canine mammary tumors have revealed an association between proliferative activity and several clinical and pathological variables but similar studies on feline mammary tumors produced contradictory results. The purpose of this study was to investigate and to compare the IHC expression of the Ki-67 antigen in the canine and feline mammary hyperplasia and neoplasia.

Material and Methods

Seventy-five canine and 23 feline surgical specimens of mammary tumors were studied. Tumors were classified according to the World Health Organisation and histological grading of carcinomas was performed according to the Nottingam method. IHC determination of Ki-67 was performed using the streptavidin-biotin-peroxidase complex method.

Results

Comparison between proliferative activity in different histological types of canine mammary tumors showed that the Ki-67 indeks was significantly higher in anaplastic than in tubulopapillary carcinomas ($p<0,05$). Furthermore, the Ki-67 indeks was significantly higher in moderate and poor differentiated canine mammary carcinomas than in benign and well differentiated tumors ($p<0,05$). In feline mammary carcinomas the Ki-67 indeks was significantly higher in poor differentiated carcinomas than in benign, well and moderate differentiated tumors ($p<0,05$). There were no association between Ki-67 expression and presence of necroses or tumor infiltrates.

Conclusions

Ki-67 represents a robust marker of cell proliferation in routinly processed canine and feline mammary tumors. In both species, the Ki-67 index correlate with tumor grade, but only in canine mammary tumors the Ki-67 indeks correlate also with different tumor type.

EVALUATION OF HISTOLOGICAL PROLIFERATION MARKERS TO PREDICT THERAPEUTIC OUTCOME FOR HIGH RISK CANINE CUTANEOUS MAST CELL TUMOURS TREATED WITH VINBLASTINE AND PREDNISOLONE AFTER SURGERY: A RETROSPECTIVE STUDY OF 61 CASES.

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Introduction

Canine cutaneous mast cell tumour (MCT) is characterized by a variable biological behaviour. Among prognostic significant histological markers a high proliferative index, evaluated by Ki-67 antigen immunohistochemistry, is associated with the progression of MCT treated by surgery alone. The aim of this study was to evaluate vinblastine and prednisolone as adjuvant therapy after surgery, for high risk MCT especially for tumours with a high Ki67-index.

Material and Methods

61 dogs with grade I or II MCT with Ki67-index >10%, grade III or metastasized tumours were treated with surgery, and adjuvant chemotherapy with vinblastine, prednisolone and cimetidine. Evaluated prognostic factors for overall survival time were Patnaik's grade, Ki67-index, and presence or absence of tumour infiltration of surgical margins.

Results

Among the 61 tumours, 3 were grade I MCT, 43 grade II, and 14 grade III. Twenty-four dogs had a loco-regional metastasis, and 6 were stage 4. Proliferation index was measured by Ki67 immunohistochemistry in 51 cases. The index was high (>10%) in 79% of these tumours. The median survival time for dogs with grade II MCT was 1923 days, and 1267 days for dogs with grade III tumours (p=0.033). 92%, 82%, and 78% of dogs with MCT with high Ki67 values were alive after 1, 2 and 3 years respectively.

Conclusions

The vinblastine and prednisolone protocol seems a valuable adjuvant treatment for MCT associated with a high Ki67-index. These promising results have to be confirmed in a prospective study comparing surgery versus surgery plus vinblastine in this subgroup of MCT with negative prognostic factors.

FRACTURE/IMPLANT-ASSOCIATED OSTEOSARCOMA: A REPORT OF TWO CASES IN DOGS.

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Introduction

The development of osteosarcoma has been rarely reported in dogs as like as in human and other species as a rare complication of the use of metallic implants. Two cases of osteosarcoma postsurgical development in long bone fractures are described.

Material and Methods

Two dogs, one 8 years old, mixed breed, female and one 5 years old, Rottweiler, female were treated for a fractures of appendicular long bones, associated with marked instability and lateral displacement, with two different principles of fixation.

Results

After different times postsurgery both dogs developed in the fracture sites radiographical destruction of bone, associated with bone masses.

Conclusions

Anatomohistopathological investigations revealed two cases of primitive Osteosarcoma developed at the site of old fracture and around the metallic implants.

CARCINOID OF THE GALLBLADDER IN TWO DOGS.

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Introduction

Carcinoid (neuroendocrine carcinoma) is a very rare tumor that can develop in many body sites, including liver and biliary tree.

Material and Methods

Two cases of carcinoid of the gall bladder are described: a male 10-year-old mixed-breed and a female 12 year-old Siberian Husky. In both cases major hematochemical abnormality was increased serum liver enzymes, including a severe increase in alkaline phosphatase (2060 U/l and 2500 U/l in case 1 and 2 respectively); this was the only liver abnormality in case 2.

Results

In case 1 the gallbladder was moderately enlarged, while in case 2 it was normally sized without cholestasis, and the tumor was incidentally discovered during ultrasound for staging an anal sac glands carcinoma. Gallbladder contained thick bile, and the tumor was visualized only after ursodesossicolic acid therapy; in both cases it consisted of a large (about 3 cm), sessile, well demarked intraluminal mass. Histopathological and immunohistochemical features of the tumors were consistent with carcinoid. In both cases surgery was curative, and serum abnormalities resolved soon after surgery. The male dog died 18 months later for acute pancreatitis without signs of relapse or metastases; the second dog received chemotherapy (carboplatin 300 mg/m2) for a suspect metastasis of the anal sac tumor, and is healthy 2 months after surgery.

Conclusions

Based on literature reports and these two cases, carcinoid seems to be a relatively common gall bladder tumor, that carries a good prognosis after excision, despite hepatic primary carcinoids are aggressive tumors with poor prognosis.

MORE THAN 700 DAYS OF SURVIVAL TIME IN A DOG WITH III STAGE SPLENIC HEMANGIOSARCOMA TREATED WITH METRONOMIC THERAPY.

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Introduction

Median survival time for dog with splenic hemangiosarcoma (HS) treated by surgery plus conventional chemotherapy was described no more than 10 months, even with the addition of immunotherapy. Recently one work in dogs with HS treated with continuous low-dose chemotherapy showed both higher median survival time and disease-free interval compare whith conventional chemotherapy alone. Here we present a case of dog with splenic HS treated, after surgery, with conventional doxorubicin chemotherapy plus continuous oral low dose chemotherapy (cliclophosphamide+meloxican) that is still alive and disease-free after 700 days.

Material and Methods

Eight years old male cocker spaniel was diagnosed for stage III splenic HS, with confirmed metastasis in mesenteric lymph nodes. Two weeks after Splenectomy, the dog was treated with VAC chemotherapy protocol. After 5 cycles of VAC protocol, dog started oral low dose chemotherapy based on cyclophophamide (10 mg/m2/EOD), metotrexate (2.5 mg/m2/twice per week) and meloxicam (0.1 mg/kg/daily). 20 mg capsules of omeprazol, as a gastric protector, were also administrated daily.

Results

After 9 month of oral metronomic therapy, dog developed a moderated cytopenic episode and then oral therapy was discontinued. One month later hemogram normalized and dog came back to the same protocol of low dose chemotherapy, except metotrexate. Currently, more than 700 days after the surgery, dog is still enrolled in this oral therapy, and is doing well, no signs of disease are shown, and non new drug adverse effects are presented.

Conclusions

Continuous low dose oral chemotherapy combined with conventional chemotherapy an be very effective for treated dog with splenic HS.

CYTOLOGICAL AND IMMUNOPHENOTIPICAL EVALUATION OF LEUKEMIAS IN DOGS.

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Introduction

Leukemia is a neoplastic proliferation of hematopoietic cells originating within the bone marrow. An accurate diagnosis is essential for prognosis and therapeutic management of the patient. The aim of the study was to evaluate sensitivity of morphological classification with the results of flow cytometry (FC) and/or cytochemical stains.

Material and Methods

72 dogs were examined at the Veterinary Teaching Hospital of the University of Turin. Complete Blood Count (CBC), flow cytometric and cytochemical analyses (if available) were made on blood or bone marrow samples. Morphological evaluation based on French-American-British classification of acute leukemias was done from 3 observers on slides stained with May-Grunwald Giemsa. Final diagnosis was obtained from cytology, FC and/or cytochemical analyses.

Results

46 lymphoid leukemia (20 acute lymphoid leukemia ALL, 13 chronic lymphoid leukemia CLL, 7 large granular lymphosarcoma/leukemia LGL), 19 myeloid leukemia (17 acute myeloid leukemia AML, 2 chronic myeloid leukemia CML) and 7 undifferentiated leukemia (AUL) were diagnosed. Good agreement (k> 0,85) between observers was found in lymphoid and myeloid leukemias. Cytological sensitivity was low in acute leukemia (ALL 45%, AML 56%, AUL 14%) whereas in CLL and LGL was higher (77% and 86% respectively). When all lymphoid leukemia was considered the sensitivity was high (95%). Specificity was high in both acute and chronic disease (>90%).

Conclusions

Morphological evaluation is useful in the diagnosis of leukemia especially in chronic leukemia. Cytology is able to detect mainly lymphoid origin. Immunophenotyping with monoclonal antibodies allows the characterization of all hematopoietic neoplasia.

PROGNOSTIC EVALUATION OF MAST CELL TUMOR (MCT) IN DOGS, USING DESIGN-BASED STEREOLOGICAL TOOLS. A PRELIMINARY COMMUNICATION.

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Introduction

MCT is the most frequent malignant tumour in dogs and has a variable clinical evolution. Generally speaking, mast cell tumour is morphometrically classified into three grades, i.e. well (G1), intermediate (G2) and poorly differentiated (G3).

Material and Methods

This study aimed correlate the data volume estimating the of mastocytoma, total number of mast cells, mast cell density, cell and nucleus size and their ratio using design-based stereological methods with the recurrence and death rates of these animals by Kaplan-Meier method for construction of curves free of death and recurrence. The stereological tools for these data were fresh weight-converted volume coupled with optical disector, six-line vertical-planar rotator, and PSI, respectively.

Results

Eleven tumour masses were harvested and analyzed, with four tumour of G1, five of G2 and two of G3. Data are presented as mean (Cvobs) (p value). The tumour volume was 51592,73 (2,84) mm^3 (p=0,133). The total number of MCT cells was 6091836364 (2,622396103) (p=0,113). The numerical density of mast cells was 109000 mm^{-3} (0,594973757) (p=0,997). The number-weighted mean volume of MCT cells was 548,9722727 μm^3 (0,331602585) (p=0,677). Number-weighted mean MCT cell nucleus volume was 89,52454545 μm^3 (0,351344507) (p=0,479), and the volume-weighted mean mastocytoma cell nucleus volume by PSI was 118,5473 µm3 (0,273868) (p=0,874). Nucleus-cell-size ratio was 0,164636364 (0,118536966) (p=0,630).

Conclusions

In conclusion, no association of the stereological parameters with the prognostic of MCT was found. Further studies are wanted to find out which classification (morphometrical vs. stereological) is more related to the clinical status of mast cell tumor in dogs.

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF THERAPEUTIC TARGETS IN CANINE BLADDER CANCER

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Introduction

The aim of this study was to characterize the expression pattern of potential therapeutic targets in transitional cell carcinoma (TCC) of the canine urinary bladder.

Material and Methods

Immunohistochemistry was applied onto a series of 18 canine TCC to evaluate COX2, telomerase subunit hTERT, and tyrosine kinase receptors EGFR, KIT and PDGFR, and their expression pattern was correlated to Ki67 proliferation index, histological grading and tumor invasiveness.

Results

COX2 was markedly expressed in all TCCs (100%), hTERT in 10 (55.6%), EGFR in 8 (44.4%), CD117 in 8 (44.4%), and PDGFR in 15 (83.3%). There was no significant relationship between the expression pattern of any of these markers and tumor grading, invasiveness and proliferative activity, except for hTERT index, which was correlated with the extent of tumor invasion and Ki67 index. Nevertheless, simultaneous expression of EGFR, PDGFR and hTERT was more often detected in TCCs with high proliferative activity and muscular invasion.

Conclusions

Beside the confirmation of COX2 activation in canine TCCs, this study has proved evidence of the relevant expression also of the tyrosine kinase receptors EGFR, KIT and PDGFR, and telomerase. The irregularity of the expression pattern of these markers, and their divergency from normal urothelium, suggest a dysregulation of these pathways and advocate a possible efficacy of specific inhibitors in canine bladder tumors.

COMPARISON OF IMMUNOHISTOCHEMICAL METHODS FOR DETECTION OF HER-2 RECEPTOR IN CANINE BREAST CANCER.

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Introduction

Her-2/neu is a transmembrane receptor with intrinsic tyrosine-kinase activity which is believed to play an important role in cancer development. Her-2 can be used as a useful prognostic indicator. Authors compare three different immunohistochemical methods to evaluate Her-2 overexpression in affected tissues.

Material and Methods

a total of 35 formalin-fixed and paraffin-embedded canine breast carcinomas were enrolled in the trial. The Hercep test (Dako®) score system was utilized as gold standard for the comparison of Her-2 different immunohistochemistries: all tests utilised rabbit polyclonal ab (A0485, Dako) as primary antibody but changing the visualization systems: 1) streptavidin-biotin-peroxidase complex technique (LSAB, Dako); 2) horseradish peroxidase antirabbit IgG (Vector PI-1000); 3) horseradish peroxidase antirabbit polymer, biotin free (Mach 4T, Biocare),. All immunostainings were scored according to the Hercep-test producer recommendations (+1,+2,+3) in order to standardize evaluation of intensity of reaction, background and percentage of stained cells of the 3 detection systems.

Results

For 3+ sections all IHC stainings showed an adequate, specific positivity while for 2+ there were some visible difference. The major accordance was found between Hercep Test and Mach 4; while horseradish peroxidase antirabbit IgG highlighted 3+, but much less for 2+ sample. LSAB showed widespreading cytoplasm and nuclear background which could interfere with correct evaluation of the slide.

Conclusions

All test were effective for overexpression(+3) of Her-2/neu with higher sensitivity (and concordance) found in Mach 4.

CROSSTALK BETWEEN PROLIFERATION AND T-LYMPHOCYTIC INFILTRATE IN MALIGNANT CANINE MAMMARY TUMOURS

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Introduction

Mammary tumors are among the most common neoplasms in intact female dog. Despite of the intensive clinico-pathological investigation very little is known about the crosstalk between the T-lymphocytic infiltrate and the proliferation in canine mammary tumours (CMT).

Material and Methods

In the present study we evaluated the proliferation index (PI) and the T-lymphocytes infiltrate in 36 malignant CMT [27 tubulo-papillary carcinomas (simple, n=8; complex, n=19); 7 solid carcinomas and 2 carcinossarcomas], by immunohistochemical detection of Ki-67 and CD3 antigens respectively. CD3-positive cells were counted into 10 HPF inside the tumour, in the periphery of the tumour and in the adnexal non-tumoral mammary gland. For Ki-67, was determined the fraction of positive nuclei, in a total of 500 tumour cells, in the tumour region and in the adnexal non-tumoral mammary gland.

Results

Our results revealed significant statistical associations of Ki-67 and CD3+ T-lymphocytic infiltrate with several clinico-pathologic characteristics that reflected tumour aggressiveness. A positive correlation between CD3 + T lymphocytes in the periphery of the tumour and intratumoral Ki-67 ($r = 0.660$, $p < 0.001$) was observed. The intratumoral Ki-67 was also positively correlated with the Ki-67 of the adnexal non-tumoral mammary gland ($r = 0609$, $p = 0.006$).

Conclusions

Our results suggest that chemical mediators released by lymphocytes located in the tumour periphery may have a potential effect on tumour proliferation. Our results also indicate that growth factors produced by the tumour, might act on the adnexal non-tumoral mammary gland in a paracrine manner, contributing for a high proliferative tumoral microenvironment.

TREATMENT OF A CANINE NASAL CARCINOMA WITH GEMCITABINE AND FIROCOXIB

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Introduction

Most sinonasal tumors are malignant, and they carry a poor prognosis regardless of histologic type. With surgery or different chemotherapy agents-including gemcitabine-, the median survival time is 3 to 6 months, and with radiation therapy 8 to 23 months. COX-2 overexpression is a common feature in nasal tumors. To our knowledge, combination of COX-2 inhibitors and gemcitabine has not been previously reported.

Material and Methods

An 11 yo male WHWT was referred to us with a 5 month history of bleeding in left nasal cavity and no response to several courses of antibiotics/steroids. Previous radiographic studies of nasal cavity showed soft tissue/fluid density on the left nasal turbinate.After biopsy, a diagnosis of transitional carcinoma was obtained. Due to unavailability of radiation therapy we used gemcitabine (675mg/m2) every 3-4 weeks and firocoxib, a COX-2 selective inhibitor, (5mg/kg) daily. CBC was evaluated periodically during the treatment. The patient received a total of 5 gemcitabine doses.

Results

Tumor growth was controlled and regression of clinical signs was observed. Only epistaxis was sometimes present, but less severe than before treatment. After 6 months of treatment gemcitabine was discontinued by the refer veterinarian. Six weeks later the patient developed an abscess near left eye, facial deformity, and clear signs of pain/discomfort. Nasal radiographs showed invasion of right cavity and lysis of nasal bone. Dog was euthanized 8 months after starting treatment.

Conclusions

Combined use of gemcitabine and firocoxib showed good control of disease in this patient. Inhibition of COX-2 activity can play a role in nasal tumors treatment

SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION-3 (STAT3) EXPRESSION IN CANINE LYMPHOMA

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Introduction

Lymphoma is the most represented hematopoietic tumor in dogs and more commonly treated with chemotherapy. STAT3 is an oncogenic protein involved in the regulation of cellular mechanisms. STAT3 has been observed to be constitutively activated in many kinds of human malignancies including hematopoietic tumours, such as lymphoma, leukemia, and multiple myeloma. The aims of the present study were to evaluate the expression of STAT3 in spontaneous canine lymphoma.

Material and Methods

Twenty lymphomas of different histotype were included in the study. Formalin fixed tissue samples were processed for standard histopathological diagnosis and for immunohistochemical evaluation for CD3, CD79 alfa, STAT3 and STAT3-p-tyr705 (the tyrosine 705 phosphorylated form of STAT3). Statistical analysis was performed to evaluate significant relationships between STAT3 expression vs tumour grade and phenotype.

Results

The dogs included in the study were 11 males, 3 castrated males, 3 females and 3 spayed females; mean age was 9.26 + 2.84 years, ranging from 4.0 to 14.0 years. Eleven on 20 cases (55.0%) and 9/20 cases (45.0%) were classified as high grade and low grade lymphomas, respectively. B cell lymphomas represented 70.0% (14/20) of the cases included in the study, and the remaining 30.0% (6/20) were classified as T lymphoma. Thirteen on 20 cases (65.0%) were STAT3 positive, and 9/20 (45.0%) were STAT3-tyr-705 positive.

Conclusions

To our best knowledge this is the first preliminary study reporting STAT3 and STAT3-p-tyr705 expression in canine lymphoma. We observed that STAT3 expression was higher in high grade than in low grade lymphoma.

CLINICAL AND PATHOLOGICAL FINDINGS IN A RABBIT (ORYCTOLAGUS CUNICULUS) WITH A C-KIT POSITIVE GIST (GASTROINTESTINAL STROMAL TUMOUR)

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Introduction

A 9-year-old, male dwarf Angora rabbit (Oryctolagus cuniculus), weighting 1.1 kg, was presented to the referring veterinarian with a history of abdominal pain, anorexia and in generally poor condition. On physical examination a solid mass was detected in the abdominal cavity by palpation. No other physical abnormalities were apparent at that time.

Material and Methods

Laboratory investigations included complete blood cell counts and serum biochemical analysis. Histopathological and immunohistochemical evaluation were performed on surgically removed formalin fixed tissue samples.

Results

A complete blood cell count revealed a marked hypochromic macrocytic regenerative anemia, hypereosinophilia and lymphopenia. Serum biochemical analysis showed a marked hypoproteinemia with hypoalbuminemia and a moderately increased blood ureic nitrogen concentration. Histopathological evaluation of the intestinal lesion showed a proliferation of spindle cells with typical morphological features of myogenic gastrointestinal stromal tumour (GIST) which were positive for c-kit, smooth muscle actin, desmin and vimentin. Neoplastic cells were S-100 negative.

Conclusions

C-kit is the main marker considered to differentiate leiomyosarcoma from GIST. Leiomyosarcoma is c-kit negative. Based on WHO classification GIST can be divided into four morphologic patterns: storiform, myxoid, fascicular and epithelioid. Immunohistochemical studies categorized GIST as myogenic, neurogenic, with both myogenic and neural differentiation, and undifferentiated. Hypereosinophilia might be considered paraneoplastic as described rarely in other cases in humans and animals. To our best knowledge this is the first report describing this syndrome associated with myogenic GIST in a dwarf Angora rabbit.

CLINICAL-PATHOLOGICAL FINDINGS IN A PUPPY WITH PRIMARY CONGENITAL MESOBLASTIC NEPHROMA

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Introduction
A 104-day-old, male Pit Bull puppy was presented with a one week history of anorexia, abdominal pain and lethargy. At the clinical evaluation the puppy was hypothermic, at the abdominal palpation a large abdominal mass was revealed.

Material and Methods
Laboratory investigations, abdominal ultrasonograph, and cytological examination of the mass were performed at the first admission. The puppy died two days later and necropsy was performed. Histopathological and immunohistochemical evaluation of tissue formalin fixed tissue samples were performed.

Results
Laboratory investigations revealed marked azotaemia. Ultrasound examination of the abdominal cavity revealed a large left kidney appearing as a nearly isoechoic mass with the complete loss of corticomedullary definition. Cytological examination of the lesion revealed a pleomorphic spindle cell population of mesenchymal neoplasia. Histopathological diagnosis was suggestive of cellular mesoblastic nephroma. Neoplastic cells were vimentin positive, and cytokeratin, actin, desmin negative.

Conclusions
To our best knowledge congenital mesoblatic nephroma (MN) has been reported in only one case in a dog and similarly to the tumor described herein it was composed of mesenchymal derivatives with no nephroblastic derivatives (i.e., primitive glomeruli, nephrogenic tubules). MN is a rare tumor diagnosed in newborns and infants. MN is considered a benign tumor but, malignant behavior may also be expected. Because the rarity of reported cases of pediatric tumours in veterinary medicine, clinicians should be alerted to their occurrence. Although chemotherapy and radiation therapy could be considered, complete surgical excision is considered curative for most infant patients in human medicine.

CYTOGENETIC ANALYSES OF CANINE PROSTATE CANCER REVEAL POSSIBLE IMPORTANT PARAMETERS FOR THE DIAGNOSIS AND PROGNOSIS OF THE TUMOR DISEASE AND CONSERVED CHROMOSOMAL ABERRATIONS IN DOGS AND HUMANS

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Introduction

Besides man the dog is the only known mammalian species that spontaneously develops carcinomas of the prostate. Chromosome analyses of canine prostate carcinomas are rare. However, only just recently we have described a case of prostate carcinoma in a dog characterized by polysomy 13 as the sole cytogenetic abnormality. In this study we present two new cases of canine prostate cancer showing a clonal polysomy 13 along with complex karyotypic changes.

Material and Methods

Short term cell cultures were set up for both tumor samples The cultures were incubated in 5% CO2/air at 37°C for 5 days. The preparation of cell cultures for chromosome analysis followed routine methods. The cell suspensions were dropped onto ice-cold slides, which were then allowed to dry for 3 days before GTG-banding was carried out.

Results

Cell culture of both tumor samples resulted in well-growing cells with a moderate mitotic rate. Cytogenetic investigations of the first case revealed a hypodiploid karyotype (chromosome number between 67 and 72) and complex karyotypic changes. In the second case the chromosome number varied between 61 and 119 chromosomes and complex karyotypic changes were observed.

Conclusions

Numerical aberrations of canine chromosome 13 are observed in canine prostate cancer. Cytogenetic investigations of human prostate cancers have revealed the frequent occurrence of trisomies 7, 8 and 17. As human chromosome (HSA) 8q and the canine chromosome (CFA) 13 shares high homology, these results suggest that a conserved area on both chromosomes is involved in tumorigenesis in both species.

CANINE INVASIVE LOBULAR CARCINOMA OF THE MAMMARY GLAND: MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERIZATION OF THREE CASES.

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Introduction

Invasive lobular carcinoma (ILC) represents 15% of invasive human breast tumours. The histological pattern of ILC is characterized by a proliferation of non-cohesive cells, individually dispersed in a fibrous connective tissue. Invasive cells are epithelial even if they appear as discrete cells. This neoplasm is often associated with lobular carcinoma in-situ (LCIS) that is immunohistochemically Ck34βe12 positive and E-Cadherin negative. We describe the morphological and immunohistochemical findings of neoplastic canine mammary lesions comparable with human ILC.

Material and Methods

Three cases of canine mammary lesions, morphologically resembling ILC, were collected from the archive of the University of Pisa. Cases were morphologically described. Immunohistochemistry for Cytokeratins, Vimentin, Ck34βe12, E-Cadherin, Estrogen Receptor (ER), Progesterone Receptor (PR) and HER2 was also performed.

Results

The cases examined shared the histologic features of ILC: tumours were composed by a non-delimited, proliferation of discrete cells infiltrating fibrous connective tissue. Multifocally in situ carcinoma was present associated with invasive lesions. Cytology yielded low number of cells composed of fibroblasts and medium-sized round discrete cells. Invasive tumour cells and in situ lesions were Cytokeratins and Ck34βe12 positive. Cytokeratins successfully stained metastatic cells in lymphnodes, morphologically similar to sinuosoidal macrophages. Based on morphological and immunohistochemical characteristics, these tumours were classified as canine ILC with associated LCIS.

Conclusions

Since the behaviour of these tumours is aggressive and the histologic features resembling a discrete tumour may lead to misdiagnose a primary mammary carcinoma, it would be of interest to characterise those lesions and to take them into account in the differential diagnosis of mammary neoplasm.

CLINICAL/PATHOLOGICAL RESPONSE TO NEOADJUVANT TREATMENT OF CANINE MAMMARY TUMOURS WITH AGLEPRISTONE

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Introduction

A common language is necessary to report the results of cancer treatment in a consistent manner. The purpose of this work was to adapt guidelines used in human medicine to analyse the objective response of canine mammary tumours to Aglepristone.

Material and Methods

Forty-one entire bitches with primary mammary tumours were treated with 40 mg/Kg Aglepristone (31) or placebo (10). Tumour samples were taken before (day 1) and after (day 15) treatment. Reductions in tumour size of the primary tumour(s) measured with a calliper (days 1-15) were registered as clinical response (ClinR) (RECIST, Therasse et al 2000). Reductions in tumour cellularity in H&E-stained tissue sections (biopsies taken on days 1-15) were registered as pathological response (PathR) (Miller and Payne 2003).

Results

Partial ClinR (20% reduction from baseline) was observed in 29.0% and 10.0% of dogs in the treatment and control groups respectively. Stable/progressive diseases were observed in 64.5%/6.5% of dogs treated with aglepristone and 70%/20% of control animals, respectively. Complete ClinR was not observed. Partial ClinR was more frequent in benign and low grade malignant tumours. PathR was recorded in 10/28 treated and 1/9 control tumors studied. The decrease in cellularity was classified as grade 2 (<30% cell loss) in 7 cases and grade 3 (30-90% cell loss) in 3 cases. No relationship between ClinR and PAthR was observed.

Conclusions

Using human guidelines to analyze clinical and pathological responses in dogs with mammary tumours treated with Aglepristone was technically easy and showed partial responses in approximately one third of cases.

EGFR EXPRESSION IS PREDICTIVE OF A POOR PROGNOSIS IN FELINE CUTANEOUS SQUAMOUS CELL CARCINOMA

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Introduction

Treatment of feline cutaneous squamous cell carcinoma (FCSCC) is challenging due to its highly infiltrative and local destructive behavior. Dysregulations of epidermal growth factor receptor (EGFR) signaling have been found in many human cancers, including SCC. EGFR expression by neoplastic cells has been associated with a poor prognosis and makes SCC an attractive target for EGFR inhibitor therapy.

Material and Methods

The aim of this study was to characterize EGFR expression in FCSCC and assess its prognostic role. Nineteen formalin-fixed paraffin-embedded excisional biopsies of FCSCC were tested for EGFR expression using immunohistochemistry. A scoring system that took into consideration the staining intensity as well as the percentage of positive tumor cells was used. Relationships between EGFR expression and histopathological parameters (degree of differentiation, mitotic activity), disease free interval (DFI) and overall survival at 24 months were further investigated.

Results

Fourteen out of 19 tumors (74%) were positive for EGFR, with great variation in intensity and proportion of labeled cells. EGFR expression was not correlated with tumor differentiation or mitotic activity. Nine cats (47%) died of tumor-related causes (mean survival time, 9 months). EGFR score was higher in the cases with a negative outcome (6.22 vs 3.75, P=0.0352). Positivity for EGFR was significantly associated to decreased DFIs (P=0.0037) and survival times (P=0.0221).

Conclusions

Our data suggest that activation of the EGFR pathway is possibly involved in the progression of FCSCC. Additionally, EGFR expression carries a negative prognostic significance in these tumors and may provide a rationale for using EGFR inhibitors in association with conventional treatments.

WNT SIGNALING IN CANINE OSTEOSARCOMA: IF NUCLEAR BETA-CATENIN BARKS, DOES IT BITE?

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Introduction

Canine osteosarcoma (OS) is a devastating primary bone tumor, which is heterogeneous in nature and its pathogenesis is not completely understood. Wnt/beta-catenin signaling plays important roles in skeletal development and various oncogenic processes. Previous gene profiling studies on prognosis and metastasis of canine OS have shown enrichment for Wnt signaling genes. This study aimed to determine the reliability of nuclear beta-catenin localization as a hallmark for activation of this pathway and its prognostic value in primary tumors.

Material and Methods

Transcriptional activation of WNT/beta-catenin signaling was determined for 8 canine OS cell lines using a TCF-responsive luciferase reporter assay. The activity of the pathway was stimulated by Lithium chloride (LiCl), a GSK3β inhibitor. Localization of beta-catenin was observed using confocal fluorescence microscopy. The prognostic significance of beta-catenin expression and localization were determined by immunohistochemistry using a tissue array containing 110 tumors.

Results

Active WNT/beta-catenin signaling was observed upon LiCl stimulation in 7/8 cell lines, with 4 lines having an autocrine activity. Cytoplasmic beta-catenin was observed in all cell lines. In contrast, nuclear beta-catenin was detected only in 2 cell lines with autocrine activity, even upon LiCl stimulation. Immunopositivity for beta-catenin was detected in 43% of tumors, of which 54% had nuclear localization. Tumors having strong nuclear and cytoplasmic expression had significantly lower disease-free interval.

Conclusions

Presence of the nuclear beta-catenin is a reliable indicator of an active Wnt/beta-catenin in canine OS, but its absence does not indicate that the pathway is inactive. A subset of canine OS with nuclear beta-catenin has a poorer prognosis.

MASITINIB FOR MAINTENANCE CHEMOTHERAPY OF 2 DOGS WITH T-CELL MULTICENTRIC LYMPHOMA

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Introduction

Response to treatment of canine T-cell multicentric lymphoma is classicaly described as poor, especially for pleomorphic and lymphoblastic forms, with median survival times of less than 8 months in most studies focusing on the interest of « conventional » chemotherapy. In vitro studies have shown that canine T-cell might be sensitive to masitinib, owing to the inhibition of c-Kit/PDGFRa&b.

Material and Methods

Two Shih-tzu female dogs were presented for clinical staging and evaluation of therapeutic possibilities of multicentric lymphoma. Dogs were clinically staged 3-a and cytologic examination of enlarged lymph nodes was suggestive of clear cell lymphoma (CCL) and large granular lymphoma (LGL), respectively. Immunostaining was positive for CD-3 and negative for CD-79, indicating a T-Cell origin. Both dogs have been previously treated with glucocorticoids. The dog with CCL initially received vincristin (as part of a CHOP based protocol), but treatment was poorly tolerated and the owner refuses to perform the induction period. The dog with LGL received one initial administration of L-asparaginase. Both dogs received masitinib (12 and 12,2 mg/kg/day) after this initial induction period.

Results

Masitinib was well tolerated (clinically and according to haematology, serum biochemistry and urinanalysis). Progressive complete remission was reached in the dog with CCL, which remained present at the time of writing, 200 days after initial diagnosis. Rapide complete remission was reached in the dog with LGL, which remained present at the time of writing, 70 days after initial diagnosis.

Conclusions

Masitinib was well tolerated and could represent a therapeutic option in dogs with T-Cell multicentric lymphoma.

MULTICENTRE RETROSPECTIVE EVALUATION OF ORAL TUMORS IN 48 DOGS: 2006-2009.

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Introduction

Oral tumours (OT) account for 6% of canine cancer and are the fourth most common cancer overall.

Material and Methods

48 cases of canine oral tumours, presented to three different Veterinary Hospitals were reviewed.

Results

25 of the analysed tumors were malignant [13 malignant melanomas, 10 squamous cell carcinomas (SCC), 1 fibrosarcoma and 1 osteosarcoma] and 23 were benign (22 epulis type injuries, 1 papilloma). Dogs of mixed breed (n=11), animals over 10 years (n=23) and males (n=30) were most affected. Most tumors were located in the rostral mandibular area ($p<0,001$). The majority of animals had normal values of serum alkaline phosphatase (AP) (90%) and calcium (92,3%). Most tumors showed local staging (n=33) at the time of diagnosis hence surgical treatment was predominantly performed ($p<0.001$). Considering the most frequent histological types in this study; malignant melanoma affected predominantly mixed breeds (38,5%), with an average age of 12,8 years; SCC predominantly affected Border Collies (30%) with an average age of 8,5 years; whereas epulis were most common in Boxer dogs (27,3%) at an average age of 9,6 years. Clinical stage ($p<0,001$), age at the diagnosis ($p=0,041$) and the mitotic rate ($p<0,001$) varied significantly among the distinct neoplasms diagnosed.

Conclusions

Neither AP nor calcium is increased in oral tumours. Knowledge of parameters which differ significantly between tumour types, such as clinical stage, age at diagnosis and mitotic rate may assist a prompt and correct diagnosis.

EXPRESSION OF COX-2 IN REACTIVE AND NEOPLASTIC CANINE BONE TISSUE.

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Introduction

Accumulating evidence suggests, that cyclooxygenase-2 (COX-2) overexpression may be a causal factor for tumor growth and metastasis. Strong expression of COX-2 expression has been found in human osteosarcomas (92%).The aim of this study was to evaluate the expression of COX-2 in canine appendicular osteosarcoma (OS) and in reactive bone tissue.

Material and Methods

COX-2 expression was analyzed from 26 samples of appenticular osteosarcoma and 12 samples of reactive bone tissue by immunohistochemistry on paraffin wax-embedded tissue using the immunoperoxidase method. Histologic diagnosis of the bone pathologies was performed according to the WHO classification. The results were quantified according to previously published scoring systems and submitted to a statistical analysis.

Results

COX-2 overexpression was present in 18/26 cases of appendicular OS (69.2%), and in 6/12 cases (50%) of reactive bone tissue. No statististically significant difference in COX-2 expression was found between the two groups.

Conclusions

These preliminary results indicate an overexpression of COX-2 in osteosarcomas which suggests that this enzyme can play a possible role in the pathogenesis and growth of this tumor. Targeting COX-2 expression could be useful in the treatment of canine OS. Further studies are necessary.

FLOW CYTOMETRIC EVALUATION OF MINIMAL RESIDUAL DISEASE (MRD) IN CANINE LYMPHOMA TREATED WITH CHOP -BASED PROTOCOL: PRELIMINARY RESULTS.

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Introduction

Real-time PCR has been proposed for evaluating MRD in canine lymphoma. However it requires specific primers for each single case thus it is difficult to apply in a clinical practice. Few data are available on usefulness of FC in evaluation of MRD. This study reports preliminary evaluation of clinical usefulness of FC to assess MRD in canine multicentric high grade lymphoma (CMHGL) undergoing a 12-week, maintenance-free CHOP-based protocol.

Material and Methods

Untreated newly diagnosed CMHGL (B-cell) were enrolled. FC was performed at the time of initial staging (T0). Dogs in complete clinical remission (CR) were re-evaluated at week 14 with FC in order to assess MRD through the presence/absence of neoplastic lymphocytes in superficial lymph nodes, peripheral blood and bone marrow. Presence or absence of MRD was related to time to progression (TTP) and rate of relapse.

Results

10 cases of CMHGL (3 stage III and 7 stage V) were enrolled. MRD was absent in 8/10 cases: 6 cases had relapse (median TTP 189 days) and 2 cases are still in CR after 145 and 228 days. In 2/10 cases MRD was present and relapse was observed after 91 and 163 days respectively

Conclusions

Clinical CR was confirmed with FC in 8 out of 10 dogs. Even if 6 of these 8 dogs were negative for MRD, these cases experienced an early relapse. This could indicate a low sensitivity of FC in detecting MDR. However larger studies will help to define the role of FC to identify patients at risk of early relapse.

LOWER EXPRESSION OF CONNEXINS 26 AND 43 IN NON PIGMENTED BUCCAL MELANOMAS FROM DOGS.

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Introduction

Melanomas are quite frequent in pets, and the most common oral malignancy in dogs. In general, they present a poor prognosis, although a favorable clinical course and prolonged survival can be expected in most dogs with histologically well-differentiated melanocytic neoplasms (Esplin Vet Pathol 45:889-896, 2008). Non pigmented melanocytic neoplasms are less differentiated and considered more aggressive. Connexins form communicating channels in cell membranes, which can transfer molecules and ions smaller than 1 kD. Gap junction protein (connexin) expression and subcellular localization are reportedly altered in malignant neoplasms (Torres et al., Vet Pathol. 42(5):633-41, 2005 and Sanches et al Vet Pathol. 46(5):846-59, 2009).

Material and Methods

In this study, we aimed at establishing a comparison between pigmented and non (or less) pigmented melanomas of the oral cavity in dogs. Cell proliferation was quantified by counting PCNA positive nuclei. Connexins 26 and 43 expressions were studied by Western blot and immunohistochemistry.

Results

It has been found a statistically significant higher cell proliferation index in non pigmented oral melanomas when compared to pigmented melanomas. Cx 26 and Cx43 expressions were statistically lower in non pigmented melanomas

Conclusions

Therefore, our results agree with the current literature, which states that the most aggressive melanomas exhibit higher cell proliferation and lower expression of gap junction proteins, the connexins.

EPIDEMIOLOGICAL STUDY ON BREED AND AGE-RELATED INCIDENCE OF DIFFERENT TYPE OF CANCER IN DOGS LIVING IN NORTH-EASTERN ITALY

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Introduction

Few veterinary cancer registries and little up-to-date information on the incidence of different type of cancer in companion animals exist. In 2005, an incidence tumour registry of dog was established in Veneto Region. Incidence data of cancer and prospective breed-related risk factors in some dog breeds are here reported.

Material and Methods

Overall 3,417 samples were submitted and classified according with the WHO International histological classification of tumours of domestic animals. Through a telephone survey, the estimates and the characteristics of the canine population, with regards to sex, breed and the age stratification were obtained. Crude and specific incidence rates (IR) were calculated as annual rate per 100,000 animals.

Results

The overall IR was 288, and in purebred the IR for cancer was two folds higher than in crossbred. Boxer had a risk ratio (RR) for cancer and mast cell tumours, respectively 5 and 22 folds higher than crossbred. Labrador had a RR for cancer and mast cell tumours, respectively 1.5 and 6 folds higher than crossbred. English setter had a RR for cancer and mast cell tumours, respectively 1.4 and 10 folds higher than crossbred. German shepherd had a RR for mammary carcinoma and haemangiosarcoma, respectively 3.5 and 9 folds higher than crossbred. Lymphoma had an IR 3 folds higher in German shepherd than in crossbred. Age-specific IRs for cancer are also reported.

Conclusions

This epidemiological study provides insight into the individual risk factors of spontaneous tumours in dogs such as age and breed, which could affect susceptibility to cancer.

TREATMENT OF PRIMARY FRONTAL SINUS SQUAMOUS CELL CARCINOMA WITH PIROXICAM AND CARBOPLATIN IN TWO DOGS.

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Introduction

Canine primary frontal sinus squamous cell carcinoma (pFS-SCC) is infrequently reported. Mainly FS-SCC is an extension of nasal cavity SCC, with poor prognosis and short survival time despite surgical-, radiation-, and/or chemotherapy. However, favourable outcome has been described for treatment of canine oral SCC with piroxicam-carboplatin.

Material and Methods

Two dogs with facial deformity, limited to the frontal sinus region, and signs of diminished consciousness and headache, were histologically diagnosed with pFS-SCC. CT and MRI showed frontal sinus origin of the tumour, with invasion of the brain cavity and the orbit, but without nasal extension. No metastases could be detected. Treatment was started with piroxicam (0,3 mg/kg s.i.d. p.o), and carboplatin (300 mg/m² i.v., once in 3 weeks). After six 3-weekly treatments with carboplatin, the dosing interval was extended to 8-10 weeks.

Results

One dog showed CR; in the second dog all clinical symptoms disappeared, except minor facial deformity. Eight months after start of therapy, the owner of the dog with CR elected to stop carboplatin treatment after the seventh dose. Two months later this dog was euthanized because of a rapid, MRI-confirmed, recurrence of pFS-SCC. The dog with PR is still alive, 18 months after start of therapy, with excellent quality of life. In this dog carboplatin once in 10 weeks is required to stabilize residual disease.

Conclusions

Treatment outcome in this pilot study suggests that additional investigation of this therapy in a larger group of pFS-SCC patients is warranted. Low dose-intensity prolongation of carboplatin after six 3-weekly doses seems advisable.

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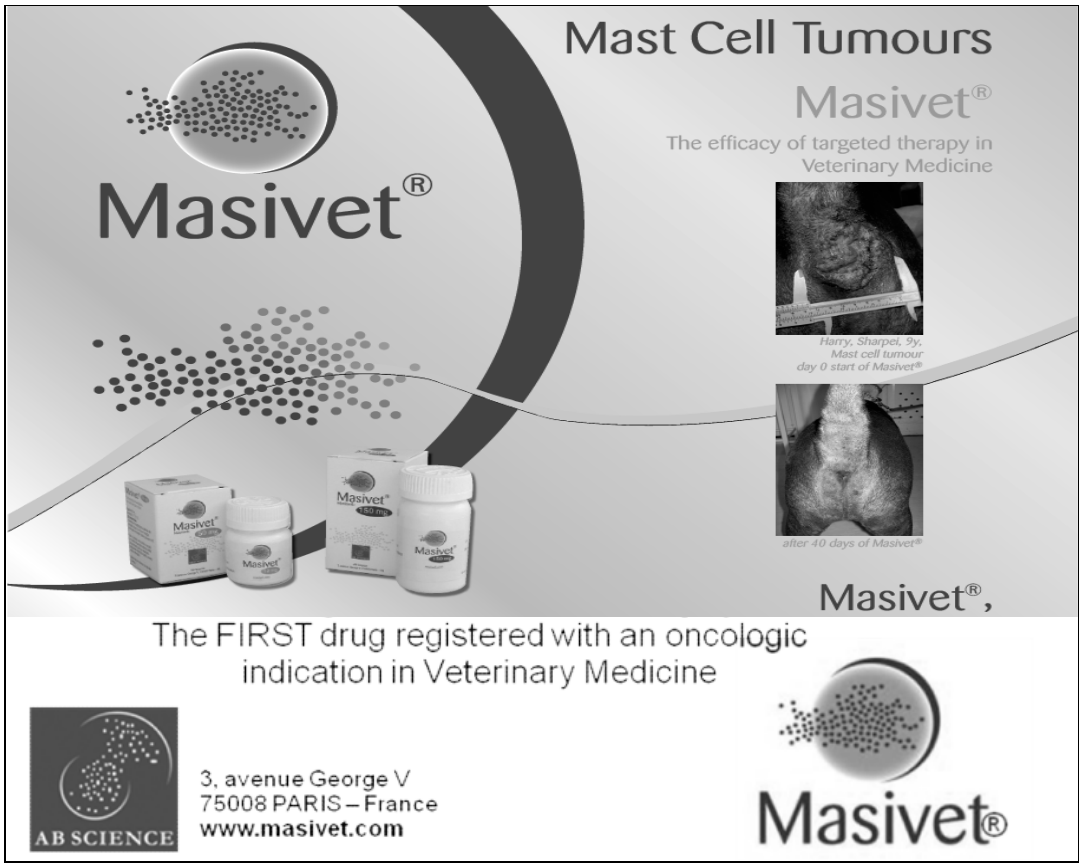

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