Prevalence of p53 dysregulations in feline oral squamous cell carcinoma and non-neoplastic oral mucosa

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Introduction

Feline oral squamous cell carcinoma (FOSCC) is the most common malignant oral tumor in cats. Mutations of the tumor-suppressor gene TP53 are frequent in human oral squamous cell carcinoma (HOSCC). TP53 mutations mainly involve the DNA-binding domain and lead to intranuclear accumulation of the abnormal p53 protein. The association between p53 dysregulations and tobacco smoke has been demonstrated in HOSCC, but only hypothesized in FOSCC. The immunohistochemical expression of p53 protein has been reported in 24-65% of FOSCC, but the presence of TP53 mutations has never been systematically evaluated. The aim of this retrospective study was to determine whether p53 immunohistochemistry accurately reflects the mutational status of the TP53 gene in FOSCC. Additionally, the prevalence of p53 dysregulation in FOSCC was compared with that of feline non-neoplastic oral mucosa, in order to investigate the relevance of these dysfunctions in cancer development.

Materials and Methods

All consecutive cases (2008-2018) of FOSCC were included, while 10 cases each of lingual eosinophilic granuloma and lymphoplasmacytic stomatitis were selected. Additionally, 10 post-mortem samples of histologically normal oral mucosa were prospectively collected from cats with at least 7 years and deceased for causes unrelated to oral pathology. All formalin-fixed and paraffin-embedded samples were screened for p53 immunohistochemical expression and TP53 mutations by Next Generation Sequencing (NGS) of exons 5-8, which encode the DNA-binding domain of the p53 protein. The associations between p53 dysregulations and exposure to environmental tobacco smoke, diagnostic category and tumor characteristic (location, histotype, degree of differentiation and mitotic count) were further assessed.

Results

p53 Immunohistochemistry

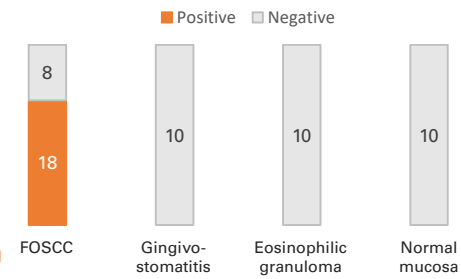
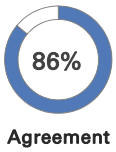


Figure 1



TP53 Mutation Analysis

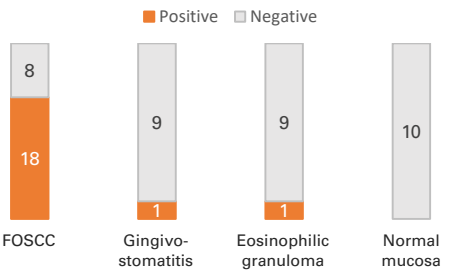


Figure 2

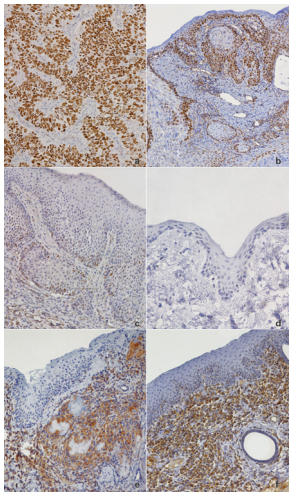


Figure 3

Cat, oral mucosa. Representative examples of p53 immunohistochemistry. (a-b) Squamous cell carcinoma. Intense nuclear labelling in a variable proportion of neoplastic cells. (c) Chronic gingivostomatitis. Weak staining in less than 20% of nuclei in the basal and suprabasal layers of the epithelium, which appears severely hyperplastic. (d) Normal oral mucosa. Lack of positive staining. (e) Eosinophilic granuloma and (f) chronic gingivostomatitis: moderate cytoplasmic staining of the inflammatory cells with no labelling of epithelial cells. Hematoxylin counterstain.

Eighteen FOSCC (69%) expressed p53 and 18 harbored TP53 mutations. The agreement between immunohistochemistry and mutation analysis in FOSCC was 77%. The overall agreement between immunohistochemistry and mutation analysis was 86%. None of non-neoplastic samples showed positive (>20% of labelled epithelial cells) p53 immunohistochemical staining, while one case each of eosinophilic granuloma and lymphoplasmacytic stomatitis harbored gene mutations (1 and 3 missense mutations respectively) (Figures 1, 2 and 3). In the 18 mutated FOSCC, 19 TP53 mutations were detected (16 missense mutations, 1 nonsense mutation and 2 in frame deletions); all the examined exons were involved (Figure 4). Both p53 immunohistochemical expression and TP53 mutations were significantly more frequent in FOSCC. p53 dysregulations were not associated with exposure to environmental tobacco smoke. There was no statistical relationship between p53 dysregulations and the other clinicopathological variables, except for a lower mitotic count in mutated cases (Tables 1 and 2).

Table 1
Relationship between p53 immunohistochemical expression and other clinicopathological variables in 56 histological samples of feline oral mucosa.

Variable	p53 negative	p53 positive	P
ETS (*n* = 50)	(4 missing)	(2 missing)	0.508
exposed	10	3	
not exposed	24	13	
Diagnosis (*n* = 56)			<0.001*
FOSCC	8	18	
chronic inflammatory lesions	20	0	
normal oral mucosa	10	0	
FOSCC location (*n* = 26)			0.657
dentate jaws	6	12	
non-dentate mucosa	1	1	
tongue	1	5	
FOSCC histotype (*n* = 26)			0.563
conventional	6	16	
non conventional	2	2	
Conventional FOSCC degree of differentiation (*n* = 22)			0.635
well differentiated	2	9	
moderately/poorly differentiated	4	7	
FOSCC MC (*n* = 26) *(median, range)*	16 (6-85)	11 (0-81)	0.388

ETS: environmental tobacco smoke
FOSCC: feline oral squamous cell carcinoma
MC: mitotic count

Table 2
Relationship between TP53 mutations and other clinicopathological variables in 56 histological samples of feline oral mucosa.

Variable	p53 negative	p53 positive	P
ETS (*n* = 50)	(3 missing)	(3 missing)	>0.999
exposed	9	4	
not exposed	24	13	
Diagnosis (*n* = 56)			<0.001*
FOSCC	8	18	
chronic inflammatory lesions	18	2	
normal oral mucosa	10	0	
FOSCC location (*n* = 26)			0.283
dentate jaws	4	14	
non-dentate mucosa	1	1	
tongue	3	3	
FOSCC histotype (*n* = 26)			0.563
conventional	6	16	
non conventional	2	2	
Conventional FOSCC degree of differentiation (*n* = 22)			0.635
well differentiated	2	9	
moderately/poorly differentiated	4	7	
FOSCC MC (*n* = 26) *(median, range)*	16.5 (10-85)	7.5 (0-79)	0.034*

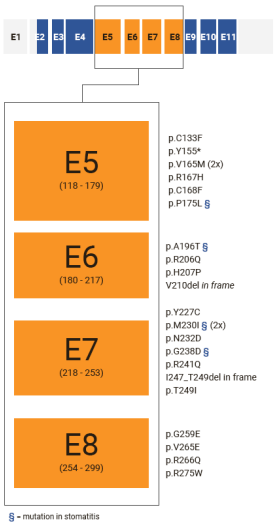


Figure 4

Localization of 23 TP53 mutations detected in samples of FOSCC and non neoplastic oral mucosa. Exons 5-8 were analyzed.

Conclusions

These results suggest an important role of p53 in feline oral tumorigenesis. Moreover, the immunohistochemical expression of p53 appears to reflect the presence of p53 mutations in most cases. It remains to be determined if the screening for p53 dysregulation, alone or with other markers, can eventually contribute to the early detection of this devastating disease.

References

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