High Prevalence of *Felis catus* Gammaherpesvirus 1 in Oronasal Tissues of Domestic Cats.

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Introduction

Viral infections account for over 15% of the human cancer burden.(1, 2) Demonstration of viral etiology usefully identifies the subset of tumors that may be preventable through vaccination.(3, 4) Epstein Barr virus (EBV) is an oncogenic member of the gammaherpesvirus family that commonly causes asymptomatic, lifelong infections in the human host.(5) Rarely, infected individuals develop EBV-associated nasopharyngeal carcinomas.(6) *Felis catus* gammaherpesvirus 1 (FcaGHV1) is a recently discovered virus in cats.(7, 8) Serology and blood PCR studies have suggested that that up to 30% of the domestic cat population, around 200 million cats, are infected with FcaGHV1, so the impact of this virus on feline health is of great interest.(9, 10) As a first step toward understanding the natural history of FcaGHV1, we have uncovered that oronasal tissues are a surprisingly common site of FcaGHV1 infection, and this data is foundational for our hypothesis that FcaGHV1 could cause a subset of feline oronasal carcinomas.

Specific Aims

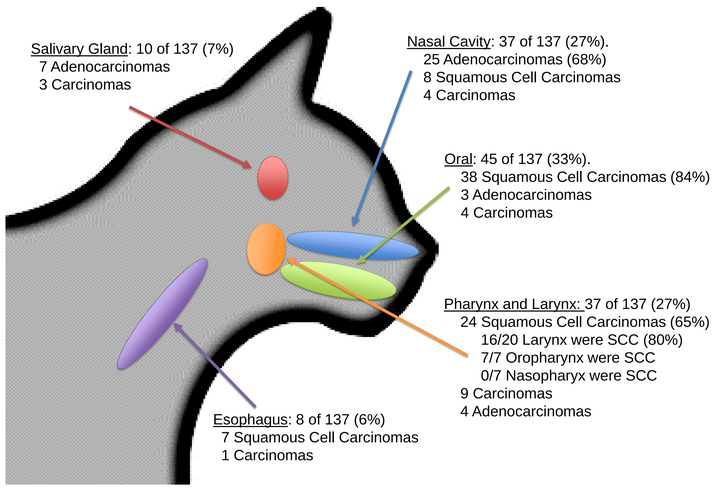
1. To establish the prevalence of FcaGHV1 in feline oronasal carcinomas vs. control tissues by PCR.
2. To explore whether FcaGHV1 is associated with specific histologically classified subsets of epithelial tumors.
3. Establish by *in situ* hybridization the cell specific distribution of FcaGHV1.

Cohort

- Samples were chosen from the UC Davis Anatomic Pathology Service tissue archives (2008-2016)
- 39 biopsy or necropsy samples were chosen based on tissue quality from a population of 137 oronasal epithelial tumors
- Tumor Classification: 18 Squamous Cell Carcinomas, 17 Adenocarcinomas, and 4 non-classified carcinomas
- Anatomic Distribution: 18 nasal/nasopharynx, 10 oral/oropharynx, 6 larynx, 3 esophagus, and 3 salivary.
- Non-tumor samples were taken from nasal tissues of cats without neoplasia; necropsied between 2009-2010.

Distribution of epithelial tumors in population cohort (n=137) (2000-2016)

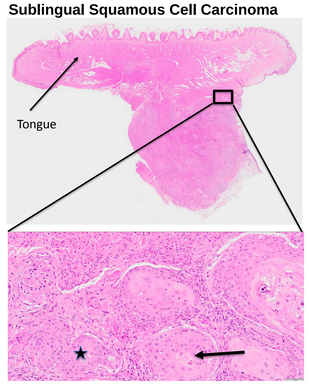
● 77 Squamous cell carcinoma (SCC) ● 40 Adenocarcinomas
● 20 Carcinomas (non-classified)



Feline Epithelial Tumors of Oral and Nasal Cavity

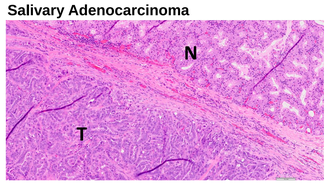
Oral and nasal carcinomas are common tumors in the cat, representing up to 10% and 8% of all tumors respectively. These tumors are distinguishable histologically, and subclassified by their putative cell of origin.

Sublingual Squamous Cell Carcinoma



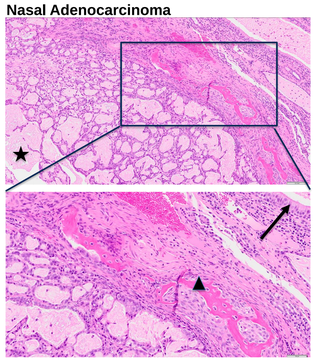
Oral **squamous cell carcinomas** are the fourth most common tumor in cats, and among characteristic histologic features are trabeculae and nests **(star)** of neoplastic, pleomorphic epithelial cells that breach the basement membrane and invade deeply into subjacent tissues. Characteristic keratinizing cells, like the surface epithelium from which it originates, are admixed with more basilar cells. Nests of neoplastic epithelial cells are often dysplastically differentiated from the basement membrane to a central area of keratinization **(arrow)**.

Salivary Adenocarcinoma



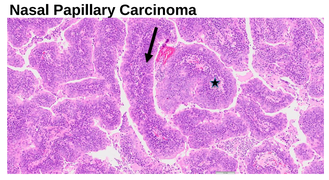
Adenocarcinoma cells are often highly anaplastic, but at least in some regions will reconstitute a glandular arrangement of poorly organized acini and ducts **(T)**. Secretions in these dead-end neoplastic glandular structures often leads to lakes of secretory products within neoplastic cysts **(star)**. Salivary and nasal glands have different microarchitecture and secretory products, so the appearance of these adenocarcinomas are highly variable. Differentiating **N**ormal from **T**umor tissue is dependent on understanding the cellular origin of the cancer cells.

Nasal Adenocarcinoma



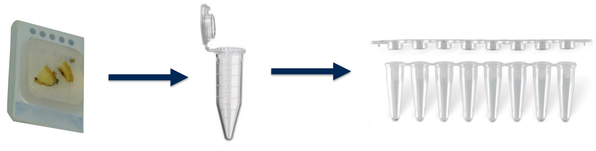
Nasal respiratory epithelium is composed of a pseudostratified ciliated columnar layer (arrow). These tumors are also capable of bony lysis (Triangle)

Nasal Papillary Carcinoma

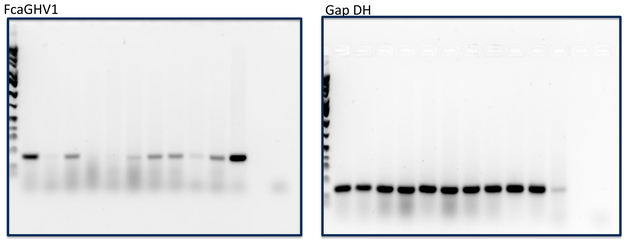


Papillomatous carcinomas are exophytic, with variably long and convoluted frond like extensions that protrude above the normal epithelial surface. Each frond is supported by a fibrovascular stroma **(star)** deep to the epithelial border. The neoplastic epithelium is multiple cells thick **(arrow)**, unlike the thin epithelium of normal nasal tissue

Materials and Methods



Paraffin embedded tissue was chosen based on tissue area, tumor:tissue ratio, and year tissue was collected. Paraffin was dissolved with xylene, and DNA then extracted from 40 micron scrolls of tissue using standard techniques. Primers were designed to amplify a 164 bp segment of the DNA polymerase gene of FcGHV 1 (NC_028099.1) Quality of DNA template was estimated by amplification of a segment of GAPDH. PCR amplified products were visualized using traditional agar gel electrophoresis. DNA sequencing of representative bands of the expected size confirmed that the correct viral segment was present and amplified. Negative controls, included in all PCR runs, lacked template DNA.



Both oronasal tumor tissue and non-tumor oronasal tissues (normal or inflamed) were tested by PCR for the presence of FcGHV 1. The data pictured above are our results on non-tumor tissues, but is similar to what we detected in tumor tissues. Amplified bands of the predicted size are present in most (8/10) samples. Viral nucleic acid was not detected in samples 3 and 4. This gel is representative of the data (in summary) that FcGHV 1 is present in both tumor and non-tumor samples. An amplified segment of GAPDH (present in all cells) serves to confirm that DNA was extracted and intact in all samples.

Table: FcaGHV prevalence by PCR amplification

	Tumor	Control	Total
Positive	35	8	43
Negative	4	2	6
Total	39	10	49

90% of the tumors, 80% of non-tumors, and 88% of all samples tested were positive for presence of viral amplicon by PCR.

	SCC	Adeno	Total
Positive	17	14	31
Negative	1	3	4
Total	18	17	35

94% of Squamous Cell Carcinomass tested positive for presence of viral amplicon by PCR
82% of Adenocarcinomas tested positive for presence of viral amplicon by PCR

Conclusion

- FcaGHV1 nucleic acid is commonly detected in oronasal tissue of domestic cats in California.
- Both normal cats and cats with carcinoma harbor FcaGHV1, therefore associations between FcaGHV1 and oronasal carcinoms cannot be determined by PCR alone.

Discussion

These results demonstrate that FcaGHV is more prevalent in oronasal tissues (90%) than blood (~20%) of cats in California.(8) We propose that this is a region of viral persistence, which leaves open the possibility that FcaGHV1 could contribute to oronasal cancers. By PCR analyses, there was no significant difference in viral detection between cats with or cats without oronasal carcinomas. PCR alone, therefore, cannot establish whether FcaGHV1 infection is oncogenic. Sorting out whether FcaGHV1 is an important etiological agent in feline carcinomas will require further studies designed to determine which specific subset(s) of cells are infected in normal cats vs. cats with carcinomas, and whether oncogenic genes of this gammaherpesvirus are expressed in tumors. In ongoing studies, we are testing cell specific infection by using in situ hybridization, which can determine whether FcaGHV1 nucleic acid is present within neoplastic cells. Given that the virus is so prevalent in oronasal tissue, this would be the minimum criteria for viral based oncogenesis. Future investigations comparing the viral transcriptome in tumor and non-tumor cells would help substantiate whether the virus is contributing to cell transformation.

References

1. Liao JB. Viruses and Human Cancer. *The Yale Journal of Biology and Medicine*. 2006;79(3-4):115-122.
2. Plummer M, de Martel C, Vignat J, Ferlay J, Bray F, Franceschi S. Global burden of cancers attributable to infections in 2012: a synthetic analysis. *Lancet Glob Health*. 2016;4(9)
3. Angioli R, Lopez S, Aloisi A, Terranova C, De Cicco C, Scaletta G, et al. Ten years of HPV vaccines: State of art and controversies. Critical Reviews in Oncology Hematology. 2016;102:65-72.
4. Jarrett O, Ganiere JP. Comparative studies of the efficacy of a recombinant feline leukaemia virus vaccine. Vet Rec. 1996;138(1):7-11.
5. Barton E, Mandal P, Speck SH. Pathogenesis and host control of gammaherpesviruses: lessons from the mouse. Annu Rev Immunol. 2011;29:351-97.
6. Khan G, Hashim MJ. Global burden of deaths from Epstein-Barr virus attributable malignancies 1990-2010. Infectious Agents and Cancer. 2014;9:38.
7. Troyer RM, Beatty JA, Stutzman-Rodriguez KR, Carver S, Lozano CC, Lee JS, et al. Novel Gammaherpesviruses in North American Domestic Cats, Bobcats, and Pumas: Identification, Prevalence, and Risk Factors. J Virol. 2014;88(8):3914-24.
8. Beatty JA, Troyer RM, Carver S, Barrs VR, Espinasse F, Conradi O, et al. Felis catus gammaherpesvirus 1; a widely endemic potential pathogen of domestic cats. Virology. 2014;460:100-7.
9. Stutzman-Rodriguez K, Rovnak J, VandeWoude S, Troyer RM. Domestic cats seropositive for Felis catus gammaherpesvirus 1 are often qPCR negative. Virology. 2016;498:23-30.
10. Peterson MN, Hartis B, Rodriguez S, Green M, Lepczyk CA. Opinions from the Front Lines of Cat Colony Management Conflict. Plos One. 2012;7(9).

Acknowledgments

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