

UC Davis

UC Davis Previously Published Works

Title

Use of nested polymerase chain reaction (PCR) for detection of retroviruses from formalin-fixed, paraffin-embedded uveal melanomas in cats

Permalink

<https://escholarship.org/uc/item/6zi8m893>

Journal

Veterinary Ophthalmology, 2(2)

ISSN

1463-5216

Authors

Stiles
Bienzle
Render
et al.

Publication Date

1999-06-01

DOI

10.1046/j.1463-5224.1999.00066.x

Peer reviewed

Use of nested polymerase chain reaction (PCR) for detection of retroviruses from formalin-fixed, paraffin-embedded uveal melanomas in cats

J. Stiles,*† D. Bienzle,‡ J. A. Render,§ N. C. Buyukmihci¶ and E. C. Johnson‡

*Department of Small Animal Medicine and ‡Department of Pathology, University of Georgia, College of Veterinary Medicine, Athens, Georgia 30602, USA, §Animal Health Diagnostic Laboratory and the Department of Pathology, Michigan State University, College of Veterinary Medicine, East Lansing MI 48824, USA, ¶Department of Surgical and Radiological Sciences, University of California, School of Veterinary Medicine, Davis, CA 95616, USA

Address communications to:

J. Stiles

†Present address: Santa Rosa Animal Eye Center, 3070 Cleveland Ave., CA 95403, Santa Rosa, USA.

Abstract

Thirty-six formalin-fixed, paraffin-embedded enucleated globes from cats with a diagnosis of diffuse anterior uveal melanoma were obtained. Sections of tumor were excised, deparaffinized, and subjected to nested polymerase chain reaction (PCR) to identify proviral DNA sequences from the feline leukemia virus (FeLV)–feline sarcoma virus (FeSV; 36 eyes), and the feline immunodeficiency virus (FIV; 18 eyes). All samples tested were negative for FIV DNA. Three samples were positive for FeLV–FeSV DNA. This is the first reported evidence of a possible link between naturally occurring feline anterior uveal melanoma and the presence of FeLV–FeSV DNA.

Key Words: feline leukemia virus, feline sarcoma virus, feline, polymerase chain reaction, uveal melanoma

INTRODUCTION

Uveal melanoma is a relatively common ocular disorder affecting cats. The tumors typically arise in the anterior stroma of the iris and are observed clinically as multifocal melanotic lesions on the anterior face of the iris. Eventually, the melanotic foci coalesce to cover the entire iridal surface, infiltrate the iridal stroma, and invade the ciliary body and sometimes the choroid. Secondary glaucoma and blindness are common sequelae, and life-threatening metastases may occur.^{1–16}

The feline sarcoma virus (FeSV) is a replication-defective, acutely transforming feline leukemia virus (FeLV) that has incorporated one of several cellular oncogenes.¹⁷ The two viruses have virtually identical electron microscopic appearance, share the same code proteins, and are classified into the same subgroups (A, B and C).^{18–20} The FeLV–FeSV complex of RNA viruses is known to cause a variety of diseases, including fibrosarcomas.¹⁸ Experimental studies have demonstrated that the Gardner strain of FeSV, when injected into the anterior chamber of kittens, produces invasive anterior uveal melanomas.^{21,22}

The feline immunodeficiency virus (FIV) is a retrovirus first described in 1987.²³ Although not directly linked to fibrosarcoma formation, immunodeficiency resulting from FIV infection may predispose cats to infection with other potentially transforming viruses or may result in decreased antitumor immune surveillance.²⁴ Thus, aggressive lymphomas have been noted at increased frequency in FIV-infected cats.²⁵

The study reported here was undertaken to determine whether FeLV–FeSV or FIV genes could be detected within formalin-fixed, paraffin-embedded sections of naturally occurring feline uveal melanomas.

MATERIALS AND METHODS

Thirty-six blocks of formalin-fixed, paraffin-embedded enucleated feline globes, in which diffuse anterior uveal melanoma had been diagnosed, were obtained. The age of the cats ranged from 1 year to 17 years, with an average age of 10 years. The eyes were from 22 male cats and 13 females. Breeds represented were: 30 domestic short or long hair, three Siamese, one Himalayan and one Persian. Two of the globes were from the same Himalayan cat. Information regarding the systemic health of the cats, including test results for FeLV or FIV, was not available.

Tissue preparation

To avoid cornea or other tissue in which viral DNA might be present,²⁶ sterile no. 11 scalpel blades were used to dissect a 3-mm cubic block of tissue from only the tumor. Samples were placed in sterile bullet tubes. Paraffin was removed from the sections with two xylene extractions and one wash in absolute ethanol. The dried tissue was resuspended in 50 mM Tris/1 mM EDTA/0.5% Tween-20 buffer, and incubated overnight at 37 °C with 200 µg/mL of proteinase K.

Samples were extracted twice with phenol:chloroform:isoamyl alcohol, and once with chloroform:isoamyl. DNA was precipitated with 3 M sodium acetate and absolute alcohol, and resuspended in water.

Polymerase chain reaction (PCR) amplification

Thirty-six uveal melanoma samples were subjected to nested polymerase chain reaction to identify high-molecular-weight FeLV–FeSV proviral DNA. Primers used for first-round PCR were: 5'-TTACTCAAGTATGTTCCCATG-3' and 5'-CTGGGGAGCCTGGAGACTGCT-3'.²⁷ These primers amplify a 166-bp segment encompassing nucleotides 67–232 of the FeLV long-terminal repeat (Genbank Accession nos M18247, M19392). PCR components consisted of 10 µL of template, 20 pmol of each primer, 2 units of *Taq* polymerase, buffer, and 200 µM of dNTPs in a volume of 50 µL. The final MgCl₂ concentration was 1.5 mM. The following cycling parameters were used: denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min, and extension at 72 °C for 2 min. For the second-round PCR, 5 µL of product from the initial round of PCR was used in an identical 50 µL reaction with the following primers: 5'-GGTTAAGCACCTGGGCCCTG-3' and 5'-GCAGCGGCCTTGAACTTCTG-3'. Cycling parameters were changed to denaturation at 94 °C for 1 min, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s. The resulting product was an 85-bp fragment encompassing nucleotides 126–210. The specificity of the nested fragment was verified by automated DNA sequencing of the product. The sequenced fragment had 96% homology with the expected product. As a positive control, DNA extracted from the persistently FeLV-infected cell line 3201/61E (provided by Dr James Mullins, AIDS Research and Reference Reagent Program) was included in each assay. Additionally, sections from a formalin-fixed, paraffin-embedded eye from a cat with ocular lymphosarcoma that had tested positive for FeLV was used as a positive control. Sections from this eye were consistently positive on PCR. Negative controls consisted of all reaction components except template DNA. The resultant amplicons were electrophoresed in 2% agarose gels, stained with ethidium bromide, and photographed. This PCR analysis did not differentiate between FeLV and FeSV proviral DNA.

Eighteen of the 36 uveal melanoma samples were additionally evaluated by semi-nested PCR amplification for FIV proviral DNA. First-round primers were NP 37 5'-CTACTGCTGCTGCAGCTGAA-3' and NP 38 5'-CACTGCATCCTAGCTGGTGC-3', which yielded a 485-bp product of the *gag* gene of FIV. Second-round primers were NP 38 and NP 39 5'-GGATGAAAGCT-TAAAGCAAC-3', which yielded a 159-bp product.²⁸ Other reaction components were identical to those for the FeLV PCR amplification. First-round cycling parameters were denatured at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, extension at 72 °C for 1 min, and a final extension period of 5 min at 72 °C. The second-round conditions were the same except that the annealing temperature was 55 °C. As a positive control 0.1 µg of plasmid DNA coding for the 34TF10 clone of FIV 29 (provided by Dr John Elder, AIDS Research and Reference Reagent Program) was used, and reaction components lacking DNA were included in each assay. Amplicons were electrophoresed in 2% agarose, stained with ethidium bromide and photographed.

RESULTS

All melanoma samples (18 eyes) evaluated for the presence of FIV DNA were negative. Three of the 36 samples evaluated for the presence of FeLV–FeSV DNA by nested PCR were positive (Fig. 1).

DISCUSSION

Experimental studies have clearly shown the association between FeSV and the development of anterior uveal melanoma in kittens.^{21,22} Intradermal injection of FeSV also produced melanomas of the skin in kittens.³⁰ The study reported here is the first evidence of a link between naturally occurring feline anterior uveal melanoma and the presence of FeLV–FeSV DNA.

FeLV/FeSV has been documented to play a role in the initiation of several types of neoplasms in cats. The virus has been most consistently associated with the development of lymphosarcoma,²⁷ although in the past 10 years a reduction



Figure 1. Ethidium bromide-stained 2% agarose gel, demonstrating positive PCR results for FeLV–FeSV DNA from feline anterior uveal melanomas. Lane 1, base pair marker; lane 2, negative control; lane 3, positive control from FeLV-infected cell line; lanes 4,5,7,9,11, negative samples; lanes 6,8,10, positive samples from feline uveal melanomas.

in the prevalence of viremia has been noted in cats with lymphoma.^{31,32} This probably relates to an overall reduction in the number of FeLV-positive cats. However, conventional testing for FeLV by ELISA or fluorescent antibody may lead to an apparent lower prevalence of FeLV positive cats than truly exists.³³ Tumor cells that are FeLV negative may be positive for feline oncornavirus cell membrane antigen (FOCMA), which proves a causal role for FeLV in the development of the tumor.³⁴ As additional support for this, one study using PCR detected proviral DNA in formalin-fixed, paraffin-embedded tumor tissue in seven of 11 FeLV-negative cats with lymphoma.²⁷ FeLV/FeSV genes were not detected in fibrosarcomas thought to arise from vaccination sites,³⁵ although the virus has been implicated in the natural occurrence of multicentric fibrosarcoma in cats.^{17,18}

Feline uveal melanomas arise as multifocal pigmented areas on the anterior surface of the iris, which suggests that the presence of a viral agent could incite formation of the tumor in multiple areas, including occasionally bilateral tumors. This is in contrast to uveal melanoma in the dog, which tends to form as a focal lesion that expands, rather than multifocal lesions that eventually coalesce.³⁶ Data were not available on the FeLV or FIV serologic test status of the cats in this study. Therefore, no correlation can be drawn between results of blood tests for viral agents, and the presence of FeLV–FeSV genes within the melanomas.

The presence of viral particles has previously been documented within human uveal melanomas, and in one feline uveal melanoma.³⁷ In that study, 57 ocular melanomas (53 human, three feline, one canine) were examined by transmission electron microscopy for the presence of viral particles. Five of the human melanomas had viral particles. One tumor had herpes-like particles, one had A-type oncornavirus particles, and three had particles consistent with togavirus. One feline tumor had togavirus particles as well. In the experimental studies in which kittens developed uveal melanomas following injection of Gardner strain FeSV, C-type virus particles were noted on electron microscopy.^{22,37} These viral particles were consistently seen budding from melanoma cell membranes. In these experimental studies with FeSV, the effect of the virus on uveal melanocytes was noted to include hypertrophy, hyperplasia and atypia, following which, spindle cell, mixed cell type and epithelioid melanomas developed. Metastases were also documented.

Tumor viruses may be latent within cells for extended periods of time. These cells can function relatively undisturbed and replicate without obvious effects, unlike cells infected by cytotoxic viruses. The mechanism by which FeLV may cause malignancy is by insertion of genetic material into the host cell DNA near a cellular oncogene, most commonly *myc*, resulting in activation and over-expression of that gene.³³ Unregulated division of that cell results, leading to a monoclonal malignancy. The virus may also incorporate the oncogene into its RNA sequence to form a recombinant virus, and insert this oncogene into every subsequently infected cell, thereby greatly enhancing

its transforming potential. In a study of feline lymphomas, transduction or insertion of the *myc* locus occurred in 32% of 119 tumors.³⁸ Accordingly, it may be possible that during infection with FeLV anterior uveal cells become latently infected, with viral persistence for long periods of time, perhaps years. At some point, cellular transformation of melanocytes into malignant melanoma cells may occur, resulting in the clinical appearance of ocular neoplasia.

Although the number of samples that were positive for FeLV–FeSV proviral DNA in this study was low, this may be due to the small sample size obtained from each tumor, and to DNA degradation with formalin exposure. Additionally, viral gene copy numbers in the tumor may also be low, as no samples were positive on the initial round of FeLV–FeSV PCR. Nested PCR dramatically increases the sensitivity of the test, thereby allowing for detection of very low levels of target DNA. Additionally, the sections of tumor in this study contained large amounts of melanin. This pigment remained suspended in the DNA samples even after several extraction steps and centrifugation. It is unknown whether melanin has an inhibitory effect on the sensitivity of PCR.

Based on the results of this study, it is possible that some feline uveal melanomas may be related in a causal fashion to the presence of FeLV–FeSV, although the significance in the overall disease entity is unknown. Further studies, including those with fresh tissues, are warranted.

REFERENCES

- 1 Blodi FC, Ramsey FK. Ocular tumors in domestic animals. *American Journal of Ophthalmology* 1967; **64**: 627–633.
- 2 Morgan G. Ocular tumors in animals. *Journal of Small Animal Practice* 1969; **10**: 563–570.
- 3 Engle GC, Brodey RS. A retrospective study of 395 feline neoplasms. *Journal of the American Animal Hospital Association* 1969; **5**: 21–31.
- 4 Bellhorn RW, Henkind P. Intraocular malignant melanoma in domestic cats. *Journal of Small Animal Practice* 1970; **10**: 631–637.
- 5 Cardy RH. Primary intraocular malignant melanoma in a Siamese cat. *Veterinary Pathology* 1977; **14**: 648–649.
- 6 Peiffer RL, Seymour WG, Williams LW. Malignant melanoma of the iris and ciliary body in a cat. *Modern Veterinary Practice* 1977; **58**: 854–856.
- 7 Souri E. Intraocular melanoma in a cat. *Feline Practice* 1978; **8**: 43–45.
- 8 Acland G. Intraocular tumors in dogs and cats. *Compendium of Continuing Education for the Small Animal Practitioner* 1979; **1**: 558–566.
- 9 Acland GM, McLean IW, Aguirre GD, Trucksa R. Diffuse iris melanoma in cats. *Journal of the American Veterinary Medical Association* 1980; **176**: 52–56.
- 10 Peiffer RL. The differential diagnosis of pigmented ocular lesions in the dog and cat. *California Veterinarian* 1981; **35**: 14–18.
- 11 Peiffer RL. Spontaneous ocular melanomas in the dog and cat: a comparative morphologic study. *Investigative Ophthalmology and Visual Science (Suppl.)* 1985; **26**: 21.
- 12 Dubielzig RR, Everitt J, Shaddock JA, Albert DM. Feline ocular melanoma and post traumatic sarcoma (Abstract). *17th Annual Meeting of the American College of Veterinary Ophthalmologists* 1986; **17**: 436–445.

- 13 Bertoy RW, Brightman AH, Regan K. Intraocular melanoma with multiple metastases in a cat. *Journal of the American Veterinary Medical Association* 1988; **192**: 87–89.
- 14 Patnaik AK, Mooney S. Feline melanoma: a comparative study of ocular, oral, and dermal neoplasms. *Veterinary Pathology* 1988; **25**: 105–112.
- 15 Dubielzig RR. Ocular neoplasia in small animals. *Veterinary Clinics of North America: Small Animal Practice* 1990; **20**: 837–848.
- 16 Duncan DE, Peiffer RL. Morphology and prognostic indicators of anterior uveal melanomas in cats. *Progress in Veterinary and Comparative Ophthalmology* 1991; **1**: 25–32.
- 17 Hardy WD. Feline oncoretroviruses. In: *The Retroviridae*, Vol. 2 (ed. Levy JA), Plenum Press, New York, 1993; 109–180.
- 18 Snyder SP, Theilen GH. Transmissible feline fibrosarcoma. *Nature* 1969; **221**: 1074–1075.
- 19 Snyder SP. Spontaneous feline fibrosarcomas: transmissibility and ultrastructure of associated virus-like particles. *Journal of the National Cancer Institute* 1971; **47**: 1079–1085.
- 20 Jarrett WF. Cat leukemia and its viruses. *Advances in Veterinary Science and Comparative Medicine* 1975; **19**: 165–193.
- 21 Shadduck JA, Albert DM, Niederkorn JY. Feline uveal melanomas induced with feline sarcoma virus: potential model of the human counterpart. *Journal of the National Cancer Institute* 1981; **67**: 619–627.
- 22 Albert DM, Shadduck JA, Craft JL, Niederkorn JY. Feline uveal melanoma model induced with feline sarcoma virus. *Investigative Ophthalmology and Visual Science* 1981; **20**: 606–624.
- 23 Pedersen NC, Ho EW, Brown ML, Yamamoto JK. Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. *Science* 1987; **235**: 790–793.
- 24 Terry A, Callanan JJ, Fulton R, Jarrett O, Neil JC. Molecular analysis of tumours from feline immunodeficiency virus (FIV)-infected cats: an indirect role for FIV? *International Journal of Cancer* 1995; **61**: 227–232.
- 25 Callanan JJ, Jones BA, Irvine J, Willett BJ, McCandlish IAP, Jarrett O. Histologic classification and immunophenotype of lymphosarcomas in cats with naturally and experimentally acquired feline immunodeficiency virus infections. *Veterinary Pathology* 1996; **33**: 264–272.
- 26 Herring IP, Troy GC, Pickett JP, Toth T, Champagne ES. Detection of feline leukemia virus in corneal tissues of infected cats by polymerase chain reaction and immunohistochemistry (Abstract). *29th Annual Meeting of the American College of Veterinary Ophthalmologists* 1998; **29**: 65.
- 27 Jackson ML, Haines DM, Meric SM, Mistra V. Feline leukemia virus detection by immunohistochemistry and polymerase chain reaction in formalin-fixed, paraffin-embedded tumor tissue from cats with lymphosarcoma. *Canadian Journal of Veterinary Research* 1993; **57**: 269–276.
- 28 Reubel GH, George JW, Higgins J, Pedersen NC. Effect of chronic feline immunodeficiency virus infection on experimental feline calicivirus-induced disease. *Veterinary Microbiology* 1994; **39**: 335–351.
- 29 Phillips TR, Talbott RL, Lamont C, Muir S, Lovelace K, Elder JH. Comparison of two host cell range variants of feline immunodeficiency virus. *Journal of Virology* 1990; **64**: 4605–4613.
- 30 McCullough B, Schaller J, Shadduck JA. Induction of malignant melanomas associated with fibrosarcoma in gnotobiotic cats inoculated with Gardner–Arnsstein feline fibrosarcoma virus. *Journal of the National Cancer Institute* 1972; **48**: 1893–1896.
- 31 Mauldin GE, Mooney SC, Meleo RE, Matus RE, Mauldin GN. Chemotherapy in 132 cats with lymphoma: 1988–94 (Abstract). *15th Annual Meeting of the Veterinary Cancer Society* 1995; **15**: 35–36.
- 32 Moore AS, Cotter SM, Frimberger AE, Wood CA, Rand WM, L'Heureux DA. A comparison of doxorubicin and COP for maintenance of remission in cats with lymphoma. *Journal of Veterinary Internal Medicine* 1996; **10**: 372–375.
- 33 Cotter SM. Feline viral neoplasia. In: *Infectious Diseases of the Dog and Cat*, 2nd edn (ed. Greene CE), W.B. Saunders, Philadelphia, 1998; 71–84.
- 34 Rohn JL, Linenberger ML, Hoover EA, Overbaugh J. Evolution of feline leukemia virus variant genomes with insertions, deletions, and defective envelope genes in infected cats with tumors. *Journal of Virology* 1994; **68**: 2458–2467.
- 35 Ellis JA, Jackson ML, Bartsch RC *et al.* Use of immunohistochemistry and polymerase chain reaction for detection of oncornaviruses in formalin-fixed, paraffin-embedded fibrosarcomas from cats. *Journal of the American Veterinary Medical Association* 1996; **209**: 767–771.
- 36 Collins BK, Moore CP. Diseases and surgery of the canine anterior uvea. In: *Veterinary Ophthalmology* 3rd edn (ed. Gelatt KN), Lippincott Williams & Wilkins, Baltimore, 1999; 755–795.
- 37 Albert DM. The association of viruses with uveal melanoma (Abstract). *77th Annual Meeting of the American Ophthalmology Society* 1979; **77**: 367–421.
- 38 Tsatsanis C, Fulton R, Hishigaki K *et al.* Genetic determinants of feline leukemia virus-induced lymphoid tumors: patterns of proviral insertion and gene rearrangement. *Journal of Virology* 1994; **68**: 8296–8303.