Feline uveal melanoma model induced with feline sarcoma virus

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This paper describes the first animal model of a virally induced uveal melanoma. Tumors developed following the injection of an RNA tumor virus, i.e., Gardner strain feline sarcoma virus, into the anterior chamber of newborn kittens. Histologically, the tumors were found to be iris and ciliary body melanomas, many of which showed invasion. The histology and ultrastructure of those tumors are described.

Key words: melanoma, feline sarcoma virus, model, ocular tumor

In the experiments presently presented, an oncogenic RNA virus, the feline sarcoma virus (FeSV), was successfully used to induce iris and ciliary body melanomas in cats. FeSV and the closely related feline leukemia virus (FeLV) are horizontally transmitted oncogenic RNA viruses.1-3 The feline leukemia-sarcoma virus (FeLV-FeSV) was first recovered from cases of experimental feline leukemia in 19644 and has subsequently been isolated from a number of cats with leukemias and sarcomas, particularly lymphosarcoma.5

At present, there are many unanswered questions concerning uveal melanomas in man for which it is hoped that an animal model, particularly one utilizing a naturally occurring oncogenic virus, might give insights. Areas of ignorance regarding human ocular melanomas include the etiology of these tumors. Here the possibility of a viral etiology has been suspected.6 The cell of origin, although widely suspected to be the "uveal nevus,"7 has not been conclusively demonstrated.8 The natural history of uveal melanomas has been speculated upon almost entirely on the basis of retrospective studies, and only recently have we begun prospective studies.9 The most effective mechanism of treatment of ocular melanomas is presently the center of considerable controversy.10 11 The development of a feline model for malignant melanoma and its subsequent study thus seems of value not only to gain further knowledge regarding the action of this naturally occurring oncogenic virus on the uveal cells of the cat but also to render insights into some aspects of uveal melanoma in man.

Materials and methods

Virus. Gardner strain FeSV was obtained commercially (Electronucleonics, Inc.) in a concentrated preparation containing approximately 10^11 to 10^12 viral particles/ml.

Cats. Random-bred domestic cats were obtained from local suppliers. They were acclimated to the laboratory environment for 3 to 6 weeks, during which time they were examined clinically, vaccinated against respiratory diseases and feline panleukopenia, and checked for the presence of feline leukemia antigen in circulating leukocytes by immunofluorescence. Cats were induced into estrus by light cycling, and the pregnant queens were housed in an isolation area until parturition. Injected kittens were reared in a separate room.

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Fig. 1. Flat, pigmented iris lesion (arrow) arising near site of injection of virus 2 weeks after injection.

Fig. 2. More extensive, raised iris tumor infiltrating iris 35 days after viral injection into anterior chamber (arrows).

Fig. 3. Subconjunctival and limbal presentation (arrow) of invasive melanoma found histopathologically to involve choroid, ciliary body, and iris, occurring 10 days after injection.

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**Induction of ocular melanomas.** One hundred and four eyes in 56 kittens ranging in age from 7 days to 15 days, were injected with the Gardner strain FeSV. The needle was inserted through the limbus, and a dose of 0.05 ml of virus was deposited at the inferior root of the iris.

**Clinical examination of animals.** The injected kittens were subsequently examined with a focused light and loupe and, when lesions were seen, examined with a slit-lamp biomicroscope. Clinical photographs were taken with a Kowa fundus camera at weekly intervals. Animals were sacrificed at periods ranging from 2 weeks to 235 days after injection, and complete autopsies were done. At the time of autopsy examination, gross photographs were taken of ocular and systemic lesions.

**Light microscopy.** Eyes were removed immediately after the time the animal was sacrificed or found dead and were fixed in acetic Zenker’s solution. Tissues were fixed in 10% buffered formalin and processed for light microscopic examination by standard techniques.

**Electron microscopic examination.** Electron microscopy was done on tissues and tumor freshly removed from the eye. The tissue was placed in 3% glutaraldehyde in 0.1M cacodylate buffer at pH 7.4. Specimens were divided while in glutaraldehyde into approximately 2 mm portions, washed in 0.1M cacodylate buffer, and postfixed in

Fig. 4A. Area of focal change in iris 4 weeks after injection, showing hypertrophy of iris melanocytes with atypical cells, (arrows) “streaming appearance”, and early disruption of iris pigment epithelium. (H&E; X640.)

2% osmium tetroxide. After this, en bloc uranyl acetate staining and processing were carried out on the Richert Electron microscopic tissue processor. Ultrathin sections were cut by standard techniques and examined with the JEOL JEM-7 electron microscope with an 80 kV accelerating voltage and a 50 nm objective aperture.

Results

Clinical observations. Of the 104 eyes injected (56 cats), tumors were clinically observed in 93 eyes. Tumors were usually clinically detectable by about 40 days after injection and always recognizable by 60 days. The
Fig. 4B. Another tumor at 16 weeks after injection, showing more advanced hypertrophy and atypia of iris melanocytes (arrows) and disruption of iris pigment epithelium. (H&E; ×500.)

The earliest lesion appeared to be a flat, discrete area of increased pigmentation of the iris. The majority of injected eyes (62 eyes, 36 cats)* showed progressive development of the initial lesion. This was seen as either a discrete mass enlarging within the iris and/or ciliary body (Fig. 1) or as a more diffuse tumor characterized by thickening of the iris. In most animals, both of these types of lesions continued to enlarge and eventually filled the anterior chamber (Fig. 2), and in instances in which the animals were permit-

*Fifty cats were injected bilaterally. When the clinical course of the tumors in each eye were different, the cat was counted once in each category.
Fig. 5. Early iris melanoma (M) 16 weeks after injection involving posterior iris and disrupting iris pigment epithelium (arrows), which is adherent to lens capsule (LC). (H&E; ×240.)

Histology and ultrastructure
Premalignant anterior uveal changes and melanoma. Sixty-three eyes from 36 animals were examined by light microscopy. In eyes removed 2 weeks after virus injection, hypertrophy and hyperplasia of the uveal melanomas were evident (Figs. 4A and 4B). In some eyes, this was diffuse, involving the entire iris and ciliary body, whereas in other specimens, areas of focal proliferation were seen. The involved melanocytes were frequently elongated and bizarre in appearance. In about 4 to 6 weeks, disruption of the iris pigment epithelium and migration of these cells into the involved iris stroma were observed as well.

In specimens removed within 12 weeks of viral injection, lesions interpreted as melanomas were observed. A total of 45 tumors showing some evidence of pigment formation and composed of spindle cells and/or epithelioid cells were observed involving the anterior uvea (Fig. 5). Diffuse lesions at this stage sometimes involved only a partial thickness of the iris. This appeared to progress to a full-thickness involvement after longer periods of time. Early tumors oc-
Fig. 6A. More extensive iris melanoma (M) in eye enucleated 7 weeks after injection. Tumor is adherent to lens (arrow shows lens capsule). (H&E; ×220.)

Fig. 6B. Site of perforation (arrows) of lens capsule by iris melanoma. Note curled lens capsule (LC) adjacent to perforation. (H&E; ×500.)
occurred at various sites on the iris, ranging from the pupillary border to the root of the iris. Some of these lesions encroached on the lens and even ruptured the lens capsule (Figs. 6A and 6B). These early lesions were composed primarily of spindle cells (Figs. 7A and 7B). With subsequent progression, invasion of the ciliary body from tumors arising primarily in the iris and invasion of the iris from the ciliary body tumors was observed. In larger tumors epithelioid cells became more prominent and not uncommonly predominant (Figs. 8A and 8B). With continued growth, invasion of the choroid and
Fig. 7B. Higher-power view of melanoma showing predominantly spindle-cell composition of tumor. (H&E; ×550.)

retinal detachment are seen (Figs. 9A and 9B). Chronic inflammatory cells were also a common finding within the tumors and in areas adjacent to them.

Changes in the ciliary epithelium, retina, and sclera. Disorganization and hyperplasia of the pigmented and nonpigmented ciliary epithelium were frequent findings (Fig. 10). In eight eyes, tumors were found arising from the ciliary epithelium (Figs. 11A and 11B).

In 31 eyes retinal folds (Fig. 12) were observed to be present. These folds were seen in eyes removed 2 weeks after virus injection and were constant in appearance throughout the age range up to 238 days after injection. The folds involved the full thickness of the retina and, depending on the plane of section, had either a wavelike or tubelike appearance. Photoreceptor cells, bipolar cells, and ganglion cells could be identified in the folds. The appearance of the underlying retinal pigment epithelium was variable, being absent in some instances, showing hyperplasia in others, and occasionally following the configuration of the folds.

In 12 eyes, fibrosarcomas (Fig. 13) were observed to arise from the limbus, apparently at the site of injection. These showed growth that generally paralleled that of the uveal melanomas. These tumors were composed of somewhat larger and narrower cells and were uniformly amelanotic. In eyes removed 20 weeks after injection, there sometimes was a merging of the two tumors.

Ultrastructural appearance of the induced feline melanomas. The electron microscopic appearance of the melanoma cells was basically similar to that described for human ocular melanomas. In the spindlelike melanomas, cells were seen showing the nuclear membrane infolding with a chromatin strip; spindle B-like cells were seen with filaments and rough endoplasmic reticulum. The epithelioid cells showed large, oval nuclei and well-formed nucleoli. Ribosomes, mitochon-
Fig. 8A. Mixed cell population in melanoma in eye enucleated 20 weeks after injection. S, Examples of spindle cells; E, epithelioid cells. (H&E; ×500.)

Fig. 8B. Another area of melanoma, showing predominantly epithelioid cells. (H&E; ×500.)
Fig. 9A. Invasion of choroid (C) and tapetum by melanoma in eye enucleated 22 weeks after injection. Retina (R) is detached. (H&E; ×80.)

Dria, basal bodies, centrioles, Golgi apparatus, and multivesicular vesicles were all observed. A significant difference from human melanoma cells, however, was the constant finding of virus particles budding from the cell membrane (Fig. 14). In cultured cells from the tumor, virus particles were also identified in the extracellular space (Fig. 15).

Late observations. In 14 of the 36 cats with progressing tumors, extraocular neoplasms have been identified in orbital muscles, skeletal muscle, pleura, and pericardium (Fig. 16). Intravascular spread was suggested by the presence of pulmonary vessels and by the presence of tumors and renal cortices. Electron microscopic examination of these
lesions should be useful in determining whether they are the result of viremia and local transformation of susceptible fibroblasts or are true metastases from the ocular tumors, which were responsible for or contributed to their development.

Discussion

The feline uveal melanoma model presently described employs the injection of a C-type oncornavirus or retrovirus (FeLV-FeSV) into the anterior chamber to induce iris and ciliary body melanomas in kittens. An
important preliminary observation for the present work was the finding by McCullough et al.\textsuperscript{19} that FeSV can induce malignant melanoma associated with fibrosarcoma in nonocular tissues of the cat. In additional preliminary work it was found that FeLV injected into the eyes of newborn kittens or into fetal kittens resulted in severe developmental abnormalities and the production of a retinal tumor resembling retinoblastoma.\textsuperscript{14} With these results in mind, we determined in additional preliminary studies the most effective strain of virus, the necessary age, and the route of administration for the production of melanomas of the iris and ciliary body.

In the experiments presently described, the Gardner strain FeSV was successfully used to induce ocular melanomas in cats. FeSV and FeLV are horizontally transmitted oncogenic RNA viruses.\textsuperscript{1-3} The FeLV-FeSV was first recovered from cases of experimental feline leukemia in 1964\textsuperscript{4} and has subsequently been isolated from a number of cats with leukemia and sarcomas, particularly lymphosarcomas.\textsuperscript{5} Attempts at purifying FeSV, separate from FeLV, have been inconclusive because most samples of FeSV appeared to be contaminated with "helper" FeLV.\textsuperscript{15-18} FeSV has been described as being virtually identical to FeLV by electron microscopic examination\textsuperscript{19, 20} and appears to share the same code proteins and therefore the same subgroups (i.e., A, B, and C) as FeLV.\textsuperscript{21}

Our present observations of FeSV in the induced tumors are consistent with the extensive previous ultrastructural studies. FeLV and FeSV have a slightly larger diameter than that of the avian and murine leukemia viruses\textsuperscript{22-26} but otherwise generally conform to the previous descriptions of C-type particles. FeLV and FeSV are circular or elliptical in cross-section, with a diameter of 90 to 110 nm, and are seen in two structural forms: an "immature" and a "mature" form.\textsuperscript{21} The im-
Fig. 11A. Tumor arising from nonpigmented ciliary epithelium in eye enucleated 16 weeks after viral injection. (H&E; ×200.)

Fig. 11B. Higher-power view of same area showing continuity of elongated tumor cells with transformed ciliary epithelium. (H&E; ×500.)
mature form has two concentric, centrally arranged, internal membranes with an electron-dense inner membrane and a less dense intermediate membrane. Occasionally, small spikes, which may play an important role in adsorption to host cells and infectivity, are found on the outer surface. The mature form has an outer limiting membrane with an elliptical, electron-dense nucleoid. Typically, virus particles are seen by electron microscopy, budding from the cell surface membranes; the cell membrane appears to contribute at least partially to the outer viral membrane. The chemistry of this virus has also been thoroughly studied with the structural viral proteins, the viral polypeptides, and the cytoplasmic RNA and polyribosomes in leukemic-infected cells, all characterized in some detail.

From a clinical standpoint, the FeLV-FeSV complex of viruses is extremely interesting in its ability to cause a variety of diseases, with nonregenerative anemia, panleukopenia-like syndrome, thymic atrophy, and leukemias and lymphomas being the most common. The increased susceptibility of viremic cats to unrelated disorders such as feline infectious peritonitis has led to the suggestion that FeLV-FeSV-infected cats may be generally immunosuppressed. It has been shown, however, that high titers of serum-neutralizing antibodies, which result in resistance to virus infection, can be found in these animals. Considerably more attention has been focused, however, on antibodies to feline oncornavirus-associated cell membrane antigen (FOCMA). This, as the name implies, is an antibody to the cell membrane of the virally induced tumor. Grant et al. have suggested that anti-FOCMA antibodies provide an antitumor immune surveillance function in vitro. This concept, however, has been questioned.

Recent studies have stressed that apparently healthy FeLV-FeSV-infected cats may excrete high titers of infectious virus in a
Fig. 13. Fibrosarcoma arising at site of limbal injection and invading cornea. (H&E; ×96.)

Fig. 14. Electron micrograph showing feline ocular melanoma cells removed directly from ocular tumor arising 20 weeks after viral injection. Virus particles (large arrow) are evident, as is a premelanosome (small arrow). (×50,000.)
Fig. 15. Feline melanoma cells maintained in culture for 14 days after removal from ocular tumor, 14 weeks after viral injection. Cells show virus particles budding from cell membrane and into the extracellular space (arrows). (X30,000.)

manner similar to animals with malignancy.\textsuperscript{2} The close environmental association of cats and man and the known potential infectivity of the FeLV-FeSV for human cells has raised obvious questions concerning the likelihood of horizontal transmission of this virus to humans and the possibility that FeLV-FeSV may play a part in human disease. No case of leukemia has ever been traced to FeLV-FeSV, nor has the incidence of leukemia increased in veterinarians working in contact with cats that have lymphosarcoma and leukemia.\textsuperscript{41} In addition, there has been a surprising failure to detect evidence of FeLV-FeSV infection in man on the basis of seroepidemiologic studies.\textsuperscript{42}

The role of FeLV and FeSV in the production of spontaneous feline ocular melanomas has not been studied. As in man, uveal melanomas in the cat appear to be relatively rare and yet are the most common primary intraocular neoplasm.\textsuperscript{43} The majority of spontaneous ocular melanomas in the cat occur in the iris and ciliary body.\textsuperscript{44} These tumors are described as typically showing rapid growth, appearing nodular, and giving rise to early metastasis.\textsuperscript{45, 46} At the light microscopic level the lesions presently induced closely resemble those described in the veterinary literature.\textsuperscript{45, 46} No previous ultrastructural studies of spontaneous ocular melanomas were found. In our own experience we have examined five feline uveal melanomas by electron microscopy, and their appearance was essentially similar to those presently seen, except for the fact that C-type virus particles were not present in the spontaneous lesions.

The induced lesions, which were studied by light and electron microscopy, also bore a close resemblance to previous descriptions of human uveal melanomas.\textsuperscript{47-50} A notable difference, however, is that in human melanomas the presence of C-type related virus particles is extremely rare.\textsuperscript{6}

Previous attempts to develop an experimental melanoma model with the papovavi-
Fig. 16. Invasion of extraocular muscle (MU) by melanoma growing into orbit 210 days after injection. (H&E; ×500.)

The feline model of uveal melanoma now described, we believe, can be used to determine aspects of the natural history and immunology of this neoplasm which may be applicable to the human counterpart. Also, this model should be of value in the study of diagnostic and therapeutic procedures which may prove applicable to human ocular melanomas.

REFERENCES

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