Electron Microscopic Study of Spontaneous Mammary Carcinomas in Cats and Dogs: Virus-like Particles in Cat Mammary Carcinomas

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SUMMARY

Electron microscopic study of 11 spontaneous cat mammary tumors revealed the presence of virus-like particles in 5 of the tumors examined. In three tumors, spherical particles with two concentric shells surrounding an electron-lucent center were found budding from, or free within, the cisternae of the endoplasmic reticulum. In one tumor, spherical particles with four concentric shells surrounding an electron-lucent center were observed budding from the cell membranes or free within the intercellular spaces. In the fifth tumor, both types of particles were present. One or two particles with large, centrally located nucleoids were found within the cisternae of the endoplasmic reticulum in three tumors examined.

Whether these particles are etiologically related to the tumors in which they were found or are merely passenger agents cannot be determined at the present time.

Eleven spontaneous dog mammary tumors were also studied; no virus-like particles could be found in any of these tumors.

INTRODUCTION

Spontaneous mammary carcinoma in high-cancer-strain female mice has been shown to be of viral etiology (3). These tumors contain characteristic virus particles, which can be consistently detected on electron microscopic examination (1, 6). However, with very few exceptions, virus particles could not be found in spontaneous mammary carcinomas developing in other species, including humans. Therefore, it was of interest to examine spontaneous mammary carcinomas that developed in cats and dogs.

MATERIALS AND METHODS

Animals. Spontaneous mammary tumors from 11 cats and 11 dogs were examined. Eight of the 11 cat tumors and all 11 dog tumors were obtained from the Animal Medical Center, New York, N. Y., through the kind cooperation of Dr. Peter C. Brodey. Three cat tumors were obtained from Dr. Robert S. Brodey, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pa.

Eleven spontaneous dog mammary tumors were also studied; no virus-like particles could be found in any of these tumors.

RESULTS

Mammary Tumors in Cats. Examination of 11 cat mammary tumors revealed the presence of virus-like particles in 5 out of 8 tumors (Table 1). The particles were spherical with electron-lucent centers and several concentric shells. They varied slightly in morphology and in their location relative to the cell.

In 3 of the 5 tumors, particles with 2 concentric shells were found (Figs. 2 to 4, arrows). In only a few isolated particles, a 3rd intermediate shell was observed (Figs. 5 and 6). The inner shell (Fig. 5, d) in all of these particles appeared more electron dense than the outer shell (Fig. 5, o). When an intermediate shell was observed (Fig. 5, i), it appeared to be of moderate electron density and in close proximity to the inner shell. The average diameter of the particles was about 90 m. They were located within the cisternae of the endoplasmic reticulum and in the perinuclear cisternae of epithelial cells. Occasionally, particles appeared to bud from the membranes of the endoplasmic reticulum into the interior of the cisternae.

Cat Mammary Tumors. Of the 11 spontaneous cat mammary tumors studied, there were 8 adenocarcinomas, 1 lobular carcinoma, 1 colloid carcinoma, and 1 undifferentiated carcinoma. The ages of the cats ranged from 8 to 17 years. The breeds and other data are indicated in Table 1.

Eleven spontaneous dog mammary tumors were also studied; no virus-like particles could be found in any of these tumors.
Table 1

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>Age (yr)</th>
<th>Breed</th>
<th>Histology</th>
<th>Electron microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(d)</td>
<td>Domestic</td>
<td>Adenocarcinoma</td>
<td>+ 2</td>
<td>N</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>Domestic</td>
<td>Adenocarcinoma</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>Persian</td>
<td>Adenocarcinoma</td>
<td>+ 4</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>Siamese</td>
<td>Adenocarcinoma</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>Siamese</td>
<td>Adenocarcinoma</td>
<td>0</td>
</tr>
<tr>
<td>6(e)</td>
<td>12</td>
<td>Domestic</td>
<td>Undifferentiated carcinoma</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>8-9</td>
<td>Domestic</td>
<td>Lobular carcinoma</td>
<td>+ 2</td>
</tr>
<tr>
<td>8</td>
<td>11</td>
<td>Calico</td>
<td>Adenocarcinoma</td>
<td>+ 2</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>Domestic</td>
<td>Adenocarcinoma</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>13</td>
<td>Domestic</td>
<td>Adenocarcinoma</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>12</td>
<td>Domestic</td>
<td>Colloid carcinoma</td>
<td>+ 2</td>
</tr>
</tbody>
</table>

\(a\), virus-like particles present; 0, no virus-like particles observed.

\(b\) 2, virus-like particles with 2 concentric shells surrounding an electron-lucent center; 4, virus-like particles with 4 concentric shells (the outermost 2 consisting of a unit membrane) surrounding an electron-lucent center.

\(c\) N, virus-like particles with an outer shell and an electron-dense, centrally located nucleoid.

\(d\) Found in street; age undetermined.

\(e\) No information was available on the breed.

A low-magnification electron micrograph of a group of epithelial cells is shown in Fig. 1. A higher magnification of the outlined area in Fig. 1 (Fig. 1a) demonstrates a single particle (arrow) budding from the membrane of the endoplasmic reticulum. Single particles scattered throughout the cell (Fig. 2) or groups of up to approximately 5 to 10 particles (Fig. 4) were most often observed.

In the 4th tumor, particles with 4 concentric shells were observed. These particles (Fig. 7) had an inner, dense shell (d), an intermediate shell of less electron density (i), and an outer unit membrane made up of 2 shells (u). They occasionally appeared to bud from the cell membrane of epithelial cells and were also found free within the extracellular spaces. The average diameter of these particles is 98 mμ. Fig. 8 is a low-magnification electron micrograph of groups of epithelial cells. An enlargement of the outlined area in Fig. 8 (Fig. 8a) illustrates a particle (arrow) budding from the cell membrane.

The 5th tumor contained both types of particles described above; those with 2 shells (or more rarely 3 shells) found in the endoplasmic reticulum cisternae, as well as particles with 4 shells located intercellularly.

In 3 tumors examined, 1 or 2 particles containing nucleoids were observed. These particles (Figs. 9 and 10) were spherical with an outer shell (o) and a dense, rather large, centrally located nucleoid (n). They were located within the cisternae of the endoplasmic reticulum. The particle in Fig. 9 appears almost hexagonal in shape.

**Mammary Tumors in Dogs.** Spontaneous mammary tumors from 11 dogs were examined for the presence of virus-like particles. In spite of a careful search, particles were not found in any of these tumors.

**DISCUSSION**

Spontaneous mammary carcinomas in female mice of high-cancer strains such as C3H, RIIII, or A contain type B virus particles, which can be readily identified in the electron microscope. However, results of similar studies performed thus far on spontaneous mammary tumors occurring in several other species have been essentially negative. Although type C particles were observed in transplanted rat tumors derived from spontaneous (17), chemically induced (5), or X-ray-induced (9) mammary carcinomas, no virus particles were detected in any of the primary tumors. Recently, virus-like particles resembling intracytoplasmic A and type C particles were described by Chopra and Mason (4) in a mammary carcinoma that developed spontaneously in a rhesus female monkey.

With only a few exceptions, electron microscopic examination of human breast cancer failed to reveal the presence of virus-like particles. In an extensive and thorough study, Haguenau (15) did not observe virus-like particles in 91 human breast cancer biopsies. Results of studies carried out by other investigators [reviewed by Gross (13)], including a more recent study by Sykes et al. (25), were also negative. However, Dmochowski et al. (8) observed virus-like structures resembling type B and C particles in several human mammary carcinomas.

In a preliminary study (14), we reported the presence of virus-like particles in several spontaneous cat mammary carcinomas. In our present study, virus-like particles were observed in 5 out of 11 spontaneous cat mammary tumors. In 3 of these tumors, particles with 2 concentric shells were...
Type C particles are generally associated with leukemias in extracellular spaces. The structure and location of these particles are similar to those of immature C particles. In some respects from mouse mammary tumor virus particles, while the type A particles observed in mouse mammary tumors is more sharply defined and denser than the intermediate shell in cat mammary tumors. The type A particles comprised a unit membrane. However, the intermediate shell in mouse mammary tumors have 4 shells, and the outer 2 shells are located, and surrounded by a shell. These particles resemble "intracisternal A" (24) particles, which were previously observed in malignant (See Refs. 1, 2, 6–9, 11, 12, 20, 22, 30, and 33 in Ref. 10) as well as in some normal cells (10). They also bear a similarity to immature guinea pig leukemia virus particles (11, 21, 22). In another tumor, particles with 4 concentric shells were observed in extracellular spaces. The structure and location of these particles are similar to those of immature C particles. In 1 tumor, both types of particles were observed. Only 1 or 2 particles with large centrally located nucleoids similar to mature C particles appeared in each of 3 tumors examined. Type C particles are generally associated with leukemias in mice (2, 7), chickens (16), and cats (18–20, 23).

The particles present in the cat mammary tumors differ in some respects from mouse mammary tumor virus particles. The immature particles found in both cat and mouse mammary tumors have 4 shells, and the outer 2 shells comprise a unit membrane. However, the intermediate shell in mouse mammary tumors is more sharply defined and denser than the intermediate shell in cat mammary tumors. The type A particles found in cat mammary tumors are intracisternal, while the type A particles observed in mouse mammary tumors are intracytoplasmic. The few mature particles observed in cat mammary tumors contain large, centrally located nucleoids, whereas the nucleoids of mature mouse mammary tumor virus particles are smaller, eccentrically located, and surrounded by a shell.

The significance of particles found in the cat mammary tumors cannot be fully evaluated at this time. It is possible that the particles have no causal relationship to the tumors in which they were found. It is well known that certain spontaneous and transplanted tumors in mice may carry virus particles as latent passengers. This was first observed by Graffi et al. (12), who found that the mouse leukemia virus is commonly carried as a passenger in transplanted mouse tumor cells. Particles found in cat mammary tumors may therefore represent a passenger virus. However, it is also possible that the particles observed in cat mammary tumors are etiologically related to cat mammary carcinoma. Additional studies are needed to determine the significance of these particles.

REFERENCES

Fig. 1. Electron micrograph of a group of cells from a spontaneous cat mammary tumor. X 16,000. Fig. 1a, higher magnification of the outlined area in Fig. 1 showing a virus-like particle (arrow) budding from a membrane of the endoplasmic reticulum. X 87,000.

Fig. 2. Part of a cat mammary tumor showing several virus-like particles (arrows) with 2 concentric shells within cisternae of the endoplasmic reticulum. X 35,200.

Figs. 3 and 4. Virus-like particles (arrows) with 2 concentric shells within the cisternae of the endoplasmic reticulum of cat mammary tumor cells. Fig. 3, × 55,000; Fig. 4, × 68,000.

Figs. 5 and 6. Virus-like particles (arrows) in cisternae of the endoplasmic reticulum; one particle (Fig. 5, right) appears to be budding from the membrane of the cisterna. These particles have a dense inner shell (d), an outer shell (o), and an intermediate shell (i). Fig. 5, × 116,000; Fig. 6, × 87,000.

Fig. 7. A virus-like particle in an intercellular space. The particle contains a dense inner shell (d), an outer unit membrane (u), and an intermediate shell (i). × 106,000.

Fig. 8. A group of cells from a spontaneous cat mammary tumor. × 20,000. Fig. 8a, enlargement of the outlined area showing a virus-like particle (arrow) budding from the cell membrane. × 87,000.

Figs. 9 and 10. Virus-like particles present within the cisternae of the endoplasmic reticulum. Each particle has an outer shell (o) and an electron-dense centrally located nucleoid (n). Fig. 9, × 116,000; Fig. 10, × 80,000.
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