Preliminary Electron Microscope Study on Virus-like Particles in a Spontaneous Mammary Tumor of Collared Lemming

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SUMMARY

The present electron microscopic study of a spontaneous mammary tumor (diagnosed histologically as adenocarcinoma) of collared lemming was undertaken in search for the possible presence of particles resembling oncogenic RNA virus particles. The study has demonstrated the presence of a large number of characteristic virus-like particles. The virus-like particles were similar to the hamster type H virus particles in morphology and intracisternal cytoplasmic location, previously observed in some mouse mammary tumors and human breast cancer. These particles were found budding from and free within the cisternae of the rough endoplasmic reticulum. They measure 1450 Å average outer diameter with small nucleoids of 700 Å diameter. Normal mammary gland tissues of collared lemmings were also examined in the electron microscope. However, no virus-like particles could be found in any of these tissues. Whether these virus-like particles are etiologically related to the mammary tumors of collared lemmings or are merely passenger agents remains to be determined.

INTRODUCTION

Since spontaneous mammary tumors in mice have been shown to be of viral etiology (5), the search for virus particles associated with mammary tumors in human beings and also in animals of various species has been of great interest. Thin section electron microscopic examination has revealed the presence of characteristic oncogenic RNA virus particles in the mouse mammary tumors (3, 17). In recent years, particles resembling murine oncogenic RNA viruses have been reported in the human mammary cancers (9, 21, 22, 27, 39) as well as in mammary tumor of a rhesus monkey (7, 10) and in the mammary tumors of rats (8, 11, 12, 23, 33) and of cats (26). As far as is known, no virus-like particles have been detected in spontaneous mammary tumors in animals of other species. It was, therefore, of interest to search for virus-like particles by electron microscopy in a spontaneous mammary adenocarcinoma of collared lemming.

MATERIALS AND METHODS

Tumor Tissue. At the Institute of Arctic Biology, University of Alaska, an adult female collared lemming (Dicrostonyx groenlandicus) developed a spontaneous mammary tumor. The tumor-bearing lemming and 2 normal lemmings were killed with pentobarbital sodium and necropsied immediately. After samples of the mammary tumor and 2 normal mammary gland tissues were fixed in buffered neutral 10% formalin, they were sent to the Department of Virology at The University of Texas System Cancer Center M. D. Anderson Hospital and Tumor Institute, Houston, Tex.

Electron Microscopy. The tissues fixed in 10% neutral formalin were cut into smaller pieces, fixed in 3% glutaraldehyde, postfixed in 2% osmium tetroxide in Millonig's buffer, dehydrated in a series of graded ethanol solutions, and embedded in Epon-Araldite. Thick sections were cut on a Porter-Blum MT-2 Sorvall ultramicrotome, and following examination of Paragon multiple-stained thick sections under the light microscope, adjacent thin sections were cut, double stained with uranyl acetate and lead citrate, and examined in a Siemens-Elmiskop 1-A electron microscope at 80 kV at magnifications varying from 2,000 to 15,000.

RESULTS

Mammary Tumor. As Van Pelt and Dieterich (47) reported, the spontaneous mammary tumor of collared lemming was found to be histologically an adenocarcinoma of the mammary gland by light microscopy. Electron microscopy of thin sections of the lemming mammary tumor has revealed the presence of a number of characteristic virus-like particles within the cisternae of the rough endoplasmic reticulum (Figs. 1 to 5 and 7) and occasionally in the perinculear cisternae of epithelial cells (Fig. 6). The structure of these virus-like particles appeared to be similar to the type H virus particles in hamsters described first by Bernhard and Tournier (4). The particles appear round or slightly oval and uniform in size with an average diameter of...
1450 Å and are limited by an outer double-layered membrane about 150 Å thick, observable at good resolution (Fig. 4). Each of these particles had a round, small, centrally located nucleoid measuring an average of 700 Å in diameter (Fig. 4). The inner part of the nucleoid appeared to exhibit lesser electron density comparable to the cytoplasmic matrix (Figs. 4, 6, and 7). The electron-lucent zone between the outer envelope and nucleoid was split off by characteristic thin radial spokes (Figs. 4 to 6).

The virus-like particles were released by the budding process from the membrane of the rough endoplasmic reticulum (Figs. 2 and 3). Occasionally, 2 budding particles possessing a shared outer envelope were seen within the cisternae of the rough endoplasmic reticulum (Fig. 7). Particles resembling virus were never found within the extracellular space or inside the nucleus.

Normal Mammary Gland Tissues. Two mammillary gland tissues from 2 normal collared lemmings were examined for the presence of virus-like particles. In spite of a careful prolonged search, no virus-like particles were found in any of the tissues examined.

DISCUSSION

Following Bittner's discovery (5) of an extrachromosomal factor in the milk of mice, now known as type B virus (14), and the original observation of characteristic virus-like particles in thin sections of mouse mammary tumors (17), details of their structure (1, 2) and mode of their development (19, 20) have been reported. Particles resembling type C virus (14) have been reported in transplanted rat mammary tumors derived from spontaneous (8, 11, 33), chemically induced (12), and X-ray-induced (23) mammary tumors of rats. Recently, 2 types of particles resembling intracytoplasmic type A and type C virus have been found in a spontaneous mammary tumor of a female rhesus monkey (7, 10). More recently, the presence of virus-like particles has been reported in 5 of 11 spontaneous cat mammary tumors examined (26). The significance of particles found in the mammary tumors has so far not been established.

During recent years, many investigators have examined human breast cancer biopsy specimens by means of electron microscopy in search for the presence of virus-like particles. Negative findings regarding the presence of particles resembling virus in human mammary cancer have been reported by some workers (32, 42). Virus-like particles resembling the known murine oncogenic RNA viruses have been detected in several human mammary cancers (9, 21, 22, 39). In addition, small virus-like particles also have been observed in a number of human mammary tumor biopsy specimens (27).

In the present study of a spontaneous mammary adenocarcinoma of collared lemming, a large number of virus-like particles have been demonstrated within the cisternae of the rough endoplasmic reticulum. The morphological characteristics of these particles are similar to type H virus particles in hamster tumors (4) except for their size. The type H-virus-like particles in hamster tumors measure 800 to 1000 Å, while those in the tumor of collared lemming measure 1450 Å. Type H virus particles were first demonstrated in either normal or virus-transformed BHK 21/13 cells (4). Subsequent studies have revealed the presence of type H virus particles in several clones of BHK 21 cells (6, 16, 29, 35, 38, 40, 44, 45), in cells of lines derived from virus-induced hamster tumors (6, 14, 29, 30), in virus-transformed hamster cell lines (6, 13, 29, 35, 37, 46), in spontaneous hamster tumors (24, 25, 34, 41, 43), and in virus- or chemically induced hamster tumors (28, 36, 41). The presence of type H virus particles has been reported in mouse mammary tumors (18), in Soehner-Dmochowski virus-induced tumors of rats grown in tissue culture, and in bone tumors of hamsters induced by this virus, both in the original tumors and in the tumors grown in tissue culture (18). The presence of type H or "type R" virus-like particles has also been reported in human breast cancer biopsy specimens (39). Although the virus-like particles present in the lemming mammary tumor differ in respect to size from type H virus particles, the somewhat larger size of particles may be due to differences in fixation.

It is impossible to elucidate the part played by the virus-like particles in the development of a lemming mammary tumor in which they were found on the basis of an electron microscope study. Viruses may be found in tumor tissues as passenger agents and may have no relationship to the etiology of the tumor (31). The part played by virus-like particles found in mammary tumor of collared lemming will have to be further investigated with respect to a possible etiological significance.

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REFERENCES

S. Hiraki, R. W. Van Pelt, and L. Dmochowski

Fig. 1. Low-magnification electron micrograph of a group of cells from a spontaneous mammary tumor of collared lemming. × 8,000.

Fig. 2. Higher magnification of the outlined area in Fig. 1, showing 2 virus-like particles budding from a membrane of the rough endoplasmic reticulum. × 82,500.

Fig. 3. A virus-like particle in process of budding from a membrane of the rough endoplasmic reticulum. × 96,000.

Fig. 4. Two virus-like particles present within the cisterna of the rough endoplasmic reticulum. Each particle has an outer envelope, an electron-dense nucleoid, and a characteristic radial structure between the nucleoid and the outer envelope. × 96,000.

Fig. 5. Three virus-like particles in the cisternae of the rough endoplasmic reticulum. × 50,000.

Fig. 6. A virus-like particle in the perinuclear cisterna. × 50,000.

Fig. 7. Two budding virus-like particles with a shared outer envelope within the cisterna of the rough endoplasmic reticulum. × 97,500.
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