

ELECTRON MICROSCOPIC OBSERVATIONS ON STRUCTURES RESEMBLING MYXOVIRUS IN HUMAN SARCOMAS

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Human tumors of mesenchymal origin contain cytoplasmic structures resembling ribonucleoprotein strands of paramyxoviruses. Similar structures have previously been reported in collagen diseases. The nature and function of these structures remain unresolved.

THE COMPARISON OF EXPERIMENTALLY INDUCED tumors of animals and naturally occurring tumors of man lacks valid criteria. One discrepancy concerns the viral etiology of animal and human malignant neoplasms. In certain animal tumors, such as murine leukemia, sarcoma, and mammary carcinoma, RNA-containing type C and type B virus particles occur in large numbers. Bioassays have irrefutably proved that these virus particles are the causative agents of these tumors.^{7,21,27,32} In some other types of experimental tumors, no mature virions appear but there is oncogenic viral DNA incorporated into the genome of the host cell.⁹ Not only DNA- but also RNA-containing viruses may exist in the neoplastic cells in the form of subviral structures.¹⁶ Cell-fusion, heterokaryon formation, and "rescue" by a helper virus may be needed for the maturation of the subviral structures into complete virions.^{16,28,29}

Most human tumors when examined in the electron microscope or tested in bioassays appear to be devoid of mature virions. Extensive search is required to find a few elusive virus-like particles in human tumors. Herpes, adeno- and reoviruses, as well as elementary bodies of mycoplasma, occasionally occur in human tumors probably as passengers and not

as etiologically important agents.^{1,8,20} Occasionally, type C and related virus-like particles were found in neoplastic tissues deriving from human leukemia, lymphoma, and sarcoma.^{8,35} The number of these particles rapidly diminishes in cells cultured *in vitro*; thus, these virus-like particles have never been identified as the human counterparts of oncogenic type C viruses of animals. On the contrary, culturing *in vitro*, particularly of lymphoid cells deriving from human lymphoma and from other lymphoproliferative entities, results in the rapid increase in the amount of a herpes-type (Epstein-Barr) virus.²²

Very little attention has been paid in the past to the existence of subviral structures in tumor cells of man. Preliminary findings concerning such structures, however, have recently been reported by Stewart³⁶ who observed the occurrence of filamentous structures and budding type C-like virus particles in tissue cultures of human liposarcoma and Hodgkin's disease. In the latter case, these structures were identified later as ribonucleoprotein strands of simian paramyxovirus 5.³⁷ Lymphoid cells grown in culture from patients with infectious mononucleosis⁴ or from idiopathic thrombocytopenic purpura³² occasionally contain lattice-like arrangements of short strands which, in cross section, appear as aggregates of small virus-like particles. The viral nature of these latter structures have been questioned; the structures were presented also as cellular elements.^{2,3}

Thus, merely morphological studies failed to determine in most instances whether the filamentous structures represented viral nucleoprotein strands or cellular material. If the structures are of viral derivation, it remains

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unresolved whether these structures are components of a passenger virus or of a virus etiologically related to the induction of mesenchymal tumors of man. Thus, the need for a complex study has become evident. Such a study should deal with the occurrence and morphological features of these hypothetically subviral structures, and it should encompass attempts at isolation and identification of these structures as well as bioassays to establish whether the structures possess any oncogenic potency or not. The present paper is a preliminary report on morphological observations concerning structures that may be of viral derivation, as found in biopsies and tissue cultures of human tumors of mesenchymal origin.

MATERIAL AND METHODS

Specimens of tumors: Twelve tumor biopsies (3 rhabdomyosarcomas, 3 liposarcomas, 2

chondrosarcomas, 1 osteosarcoma, 1 fibrosarcoma, 1 neurofibrosarcoma, and 1 Kaposi's sarcoma) and 12 primary tissue cultures of tumors (8 rhabdomyosarcomas, 2 chondrosarcomas, 1 fibrosarcoma, and 1 osteosarcoma) were examined electron microscopically.

Electron microscopy: Finely minced freshly biopsied tumor tissue or cultured cells dispersed by trypsinization or scraping were placed immediately in glutaraldehyde, post-fixed in osmic acid, dehydrated in graded series of ethanol, embedded in Epon Araldite, cut with an LKB ultratome, stained with uranyl acetate followed by lead citrate, and examined in an RCA 3G electron microscope.

Tissue cultures: Finely minced fresh tumor biopsy material was placed in glass T flasks under perforated cellophane membrane and incubated in Ham's F10 medium containing 20% heat inactivated fetal calf serum, penicillin G 200u/ml, streptomycin 100 μ g/ml, and kanamycin 115 μ g/ml. Cultured cells were

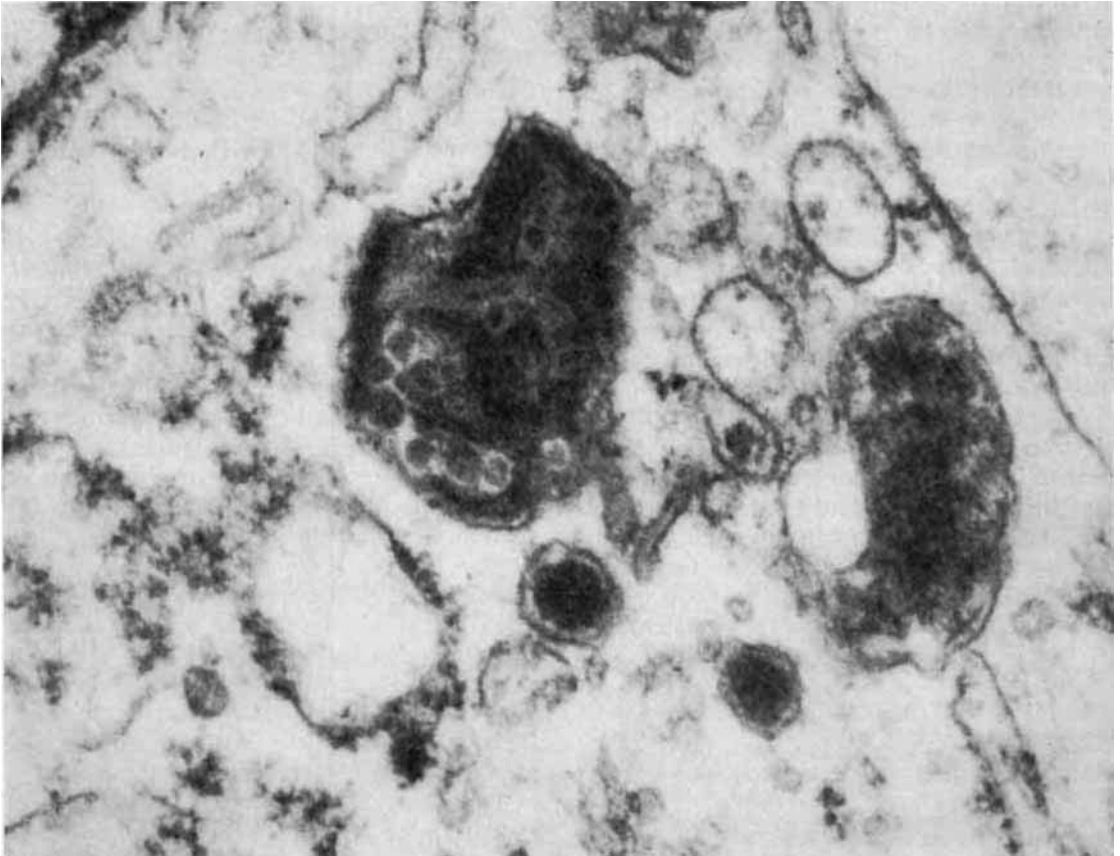


FIG. 1. Mature virus-like particles in primary tissue culture of rhabdomyosarcoma from the breast of a 42-year-old woman. The virus-like particles have double membranes, electron-dense core and diameters of 120 μ m (Electron microscopy, $\times 85,000$).

transferred to new cultures or harvested for morphological studies by trypsinization or by scraping.

RESULTS

Occurrence of apparently subviral structures: Of 12 biopsied tumors, all the 3 rhabdomyosarcomas and 3 liposarcomas, 1 osteosarcoma, 1 fibrosarcoma, and 1 Kaposi's sarcoma contained filamentous and tubular cytoplasmic structures. The 2 chondrosarcomas and the neurofibrosarcoma, however, in the biopsy material examined up to this time, have failed to reveal such structures. In 4 of 8 primary tissue cultures of rhabdomyosarcoma, similar structures were found. The same findings were obtained in the tissue cultures of 1

osteosarcoma, 1 fibrosarcoma, and 1 chondrosarcoma.

The discovery of the structures required extensive search; several sections of each specimen and several hundreds of fields were examined. The distribution of the structures was found to be quite irregular; in some sections, it was easy to find the structures, whereas other sections of the same specimen were devoid of them. Small clusters of mature virions were seen only exceptionally in the specimens examined (Fig. 1); these findings will be reported elsewhere after further studies.

Normal kidney, skin, and leukocytes were examined for the presence of similar structures. Extensive search involving a total of 50 tissue specimens yielded negative results.

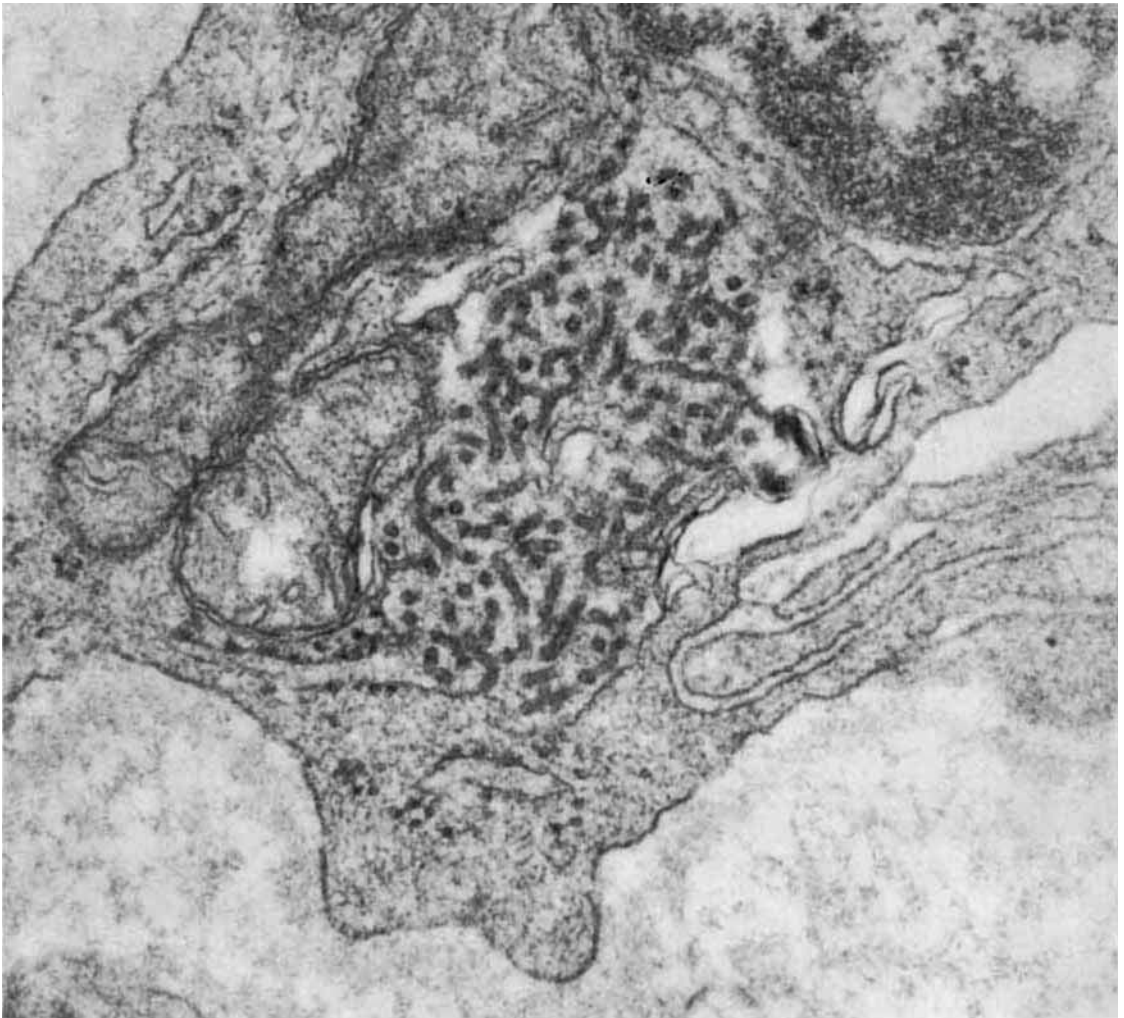


FIG. 2. Cytoplasmic network of tubular structures in the biopsy material from a patient with rhabdomyosarcoma (Electron microscopy, $\times 90,000$).

Morphological description of the structures: The filamentous and tubular structures are located in the cytoplasm in aggregates of varying sizes. The aggregates may be membrane-bound. In the case of the Kaposi's sarcoma, the structures were found between the nuclear membranes. The filaments measure 200–220 Å in diameter and up to 1000 Å in length. In cross sections, the curved filaments appear as both electron-dense or lucent round bodies (Figs. 2–4).

DISCUSSION

Further studies are needed to determine the nature and significance of these structures. It is uncertain whether the structures as found in different tumors are of the same type. While some of these structures closely resemble unenveloped ribonucleoprotein strands of paramyxoviruses,¹⁹ other slightly different, i.e., smaller, structures, particularly those appear-

ing in cultured lymphoblasts,^{4,32} are thought to represent cellular material.^{2,3,6} During the replication of parainfluenza virus type 2 in HeLa cells, cytoplasmic areas containing a dense network of filamentous structures without mature virions were seen.¹⁹ It was postulated that the viral capsid is acquired when the viral nucleoprotein strands are assembled beneath the cell membrane and the virus particles pass through the cell membrane by a budding process.¹⁹ It is compatible with this view that the filamentous structures described in this report occur at cytoplasmic sites where replication of viral nucleoprotein takes place.

Myxoviruses and type C oncoRNA viruses display morphological resemblances, thus some of the structures described herein may represent ribonucleoprotein strands of a human oncogenic virus of the type C or related class. Type C viruses have recently been incriminated in the causation of human

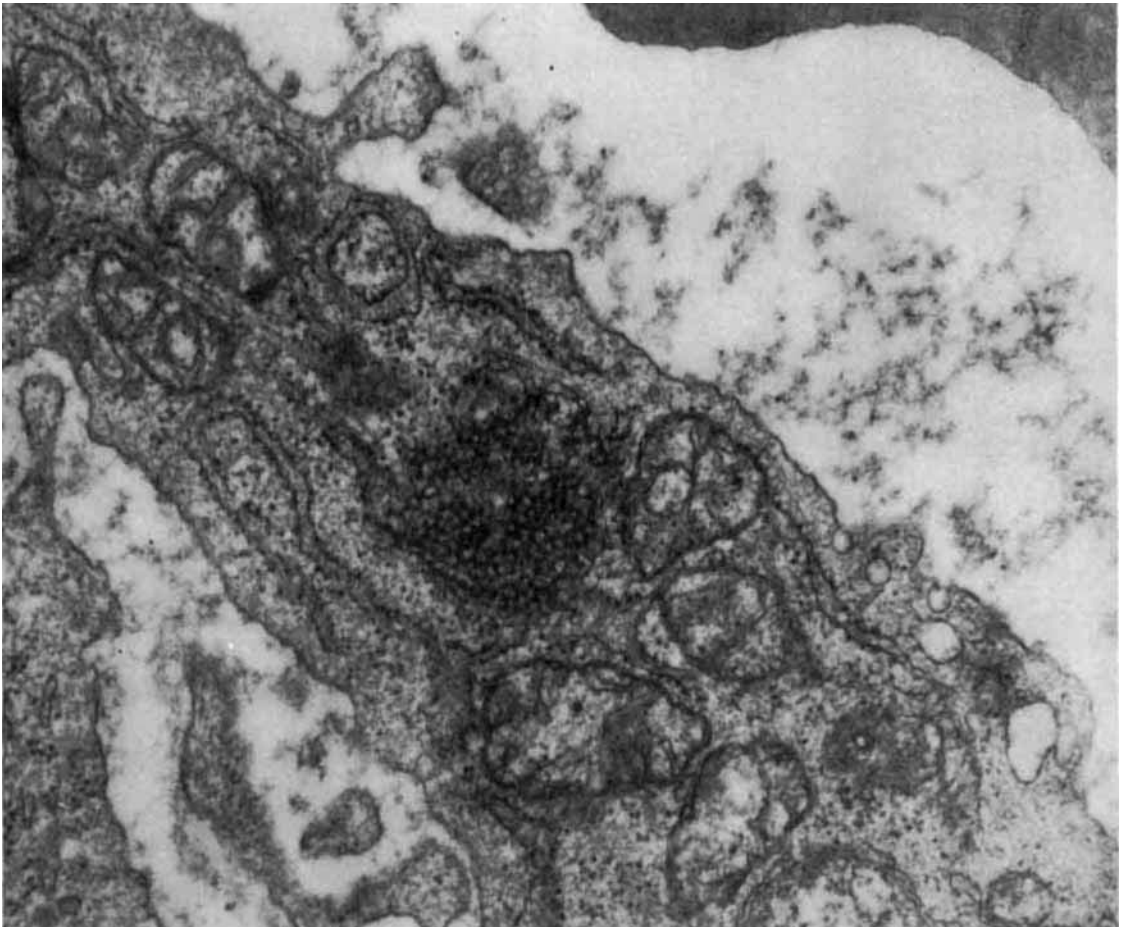


FIG. 3. Interwoven tubular structures in the cytoplasm of a cell grown in primary culture from a biopsied fibrosarcoma (Electron microscopy, $\times 47,500$).

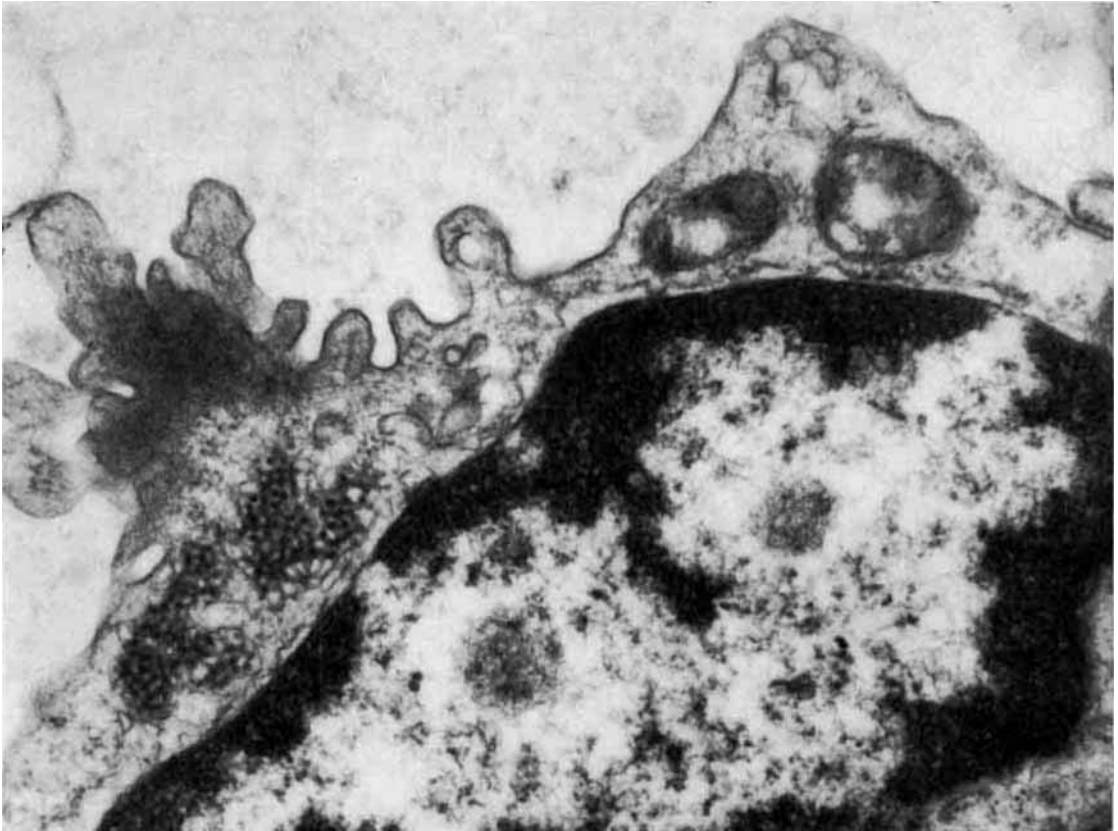


FIG. 4. Cytoplasmic tubular structures appearing in biopsied sample of Kaposi's sarcoma (Electron microscopy, $\times 47,500$).

sarcomas.^{11,23} In tumors caused by Rous sarcoma virus in primates, crystalline and filamentous cytoplasmic structures somewhat similar to those reported in this paper have been found.²⁴

The occurrence of quite similar structures in collagen diseases of man, particularly, as first shown by us, in systemic lupus erythematosus,^{13,17,18,33} polymyositis,^{5,26} Sjögren's syndrome,³⁰ and discoid lupus,¹⁴ indicates that these structures are not confined solely to tumors. The ribonucleoprotein composition of the filamentous strands found in lupus was shown by digestion with RNAase.¹² Isolation of viral agents failed from biopsy materials containing the filamentous structures.¹⁰ Thus, it was also proposed that similar strands represented cellular and not viral elements.^{2,3,17,25} Even if these structures are of viral derivation, as we have proposed,^{13,31,34} it remains to be determined whether they play the role of a passenger or of an initiator in collagen diseases and in mesenchymal tumors. Members of the orthomyxo- and paramyxovirus groups

may elicit or aggravate "autoimmune" reactions by endowing the host cells, in which viral components replicate with "neoantigenic" features; thus, subviral structures may be of great etiological significance in collagen diseases.³⁴ Paramyxovirus and coronavirus-like structures have recently been found associated with viral hepatitis,^{15,38,39} but it remains to be determined whether these structures play the etiologic role or act only as passengers. Autoimmune sequelae of viral hepatitis are well known; the harboring of subviral structures within parenchymal liver cells could explain such "autoimmune" reactions.

The structures described in this paper may represent unenveloped ribonucleoprotein strands of an ubiquitous virus which is widely spread in human populations and seldom reaches full maturity. It is evident that further morphological observations, in addition to virologic, serologic, and biologic studies, are needed in order to elucidate the significance of these filamentous structures in mesenchymal tumors of man.

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