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Abstract

Adenovirus 12-induced tumor has been so far considered to be an undifferentiated sarcoma, but in the present study it has been possible to obtain such electronmicroscopic findings that substantiate well the theory of the neuro-ectodermal supporting cell origin as suggested by the observation at optical level. In other words, a specific clinging picture of cellular membranes and the presence of desmosomes have been demonstrated. In addition, though only in rare instances, the presence of virus-like particles have been verified, and some comments have been made about the relation between tumor and the appearance of virus as well as about carcinogenic mother cell.

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ELECTRON MICROSCOPIC STUDIES ON THE TUMOR INDUCED BY ADENOVIRUS TYPE 12

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Adenovirus type 12, as has been demonstrated by TRENETIN, YABE *et al.*[1], exhibits carcinogenic activity in animals and the malignant tumors are developed at the site of inoculation. As for the pathological pictures of the tumor induced by this adenovirus, several pathologists have so far interpreted it to be an undifferentiated mesenchymal neoplasm or sarcoma but precise nature of this tumor remains unclarified. On the other hand, OGAWA *et al.*[2], in their morphological investigations on this tumor, have obtained an evidence suggestive of an epithelial character even in those cells that appear to be sarcomatous on various findings, and through the sequential observations of neoplastic tissue changes suspected it to be of neuro-ectodermal supporting cell in origin. For the purpose to find out the cellular origin, morphological characteristics of the tumor cell were studied electronmicroscopically with reference to its histological pictures.

MATERIALS AND METHODS

To newborn hamsters less than 24 hours old 0.1 ml of adenovirus type 12 ($10^{2.5}$ $TCID_{50}$/0.1 ml) maintained in HeLa cell cultures was inoculated intraperitoneally, and after the latent period of about 20 days, 11 tumors developed in these animals were taken out. One to three specimens were prepared from each of the eleven tumors, and they were either fixed first with buffered 5% glutaraldehyde (pH 7.2) and then with Millonig fixative (1% osmic acid), or directly fixed with the latter fixative without prefixation. Next, they were dehydrated in graded concentration of ethanol and embedded in Epon 812 and methacrylate resin. These were sliced into ultrathin sections with an JUM-5A ultratome and stained with uranyl acetate, and the observations were done in the JEM-7 electronmicroscope. While the respective groups of tumor specimens were stained with hematoxylin-eosin (HE), some of them were stained by silver impregnation or by Mallory-Azan for the histological examinations.

OBSERVATIONS AND RESULTS

At the level of light microscope the tumor cells present spindle shape ar-

ranged in a sarcomatous, irregular array and occasionally showing a strong polymorphism. Among the 11 tumors larger than bean size none of them has revealed any specific arrangement of the cells. Stroma being scarce, no specific fibroplasia can be recognized.

Electronmicroscopically, it is usually common among these tumor cells that the structures of nucleus and cytoplasm are simple, scarcely presenting specific differentiation morphology (Fig. 1). Most of the cells have somewhat ovoid nucleus whose nuclear envelope is smooth, sometimes showing a marked indentation, but the karyoplasm diffusely appears fine granular, and occasionally chromosomal nodules are formed in a close contact with the nuclear envelope. In the karyoplasm there are observed one to three nucleolus of irregular shape and high electrondensity, but the nucleolonemma is indistinct. In rare instances there can be seen in the karyoplasm, vesicle-like or granular small masses without a limiting membrane, which seem to be of the intracytoplasmic origin. However, neither virus particles nor matrix area can be observed.

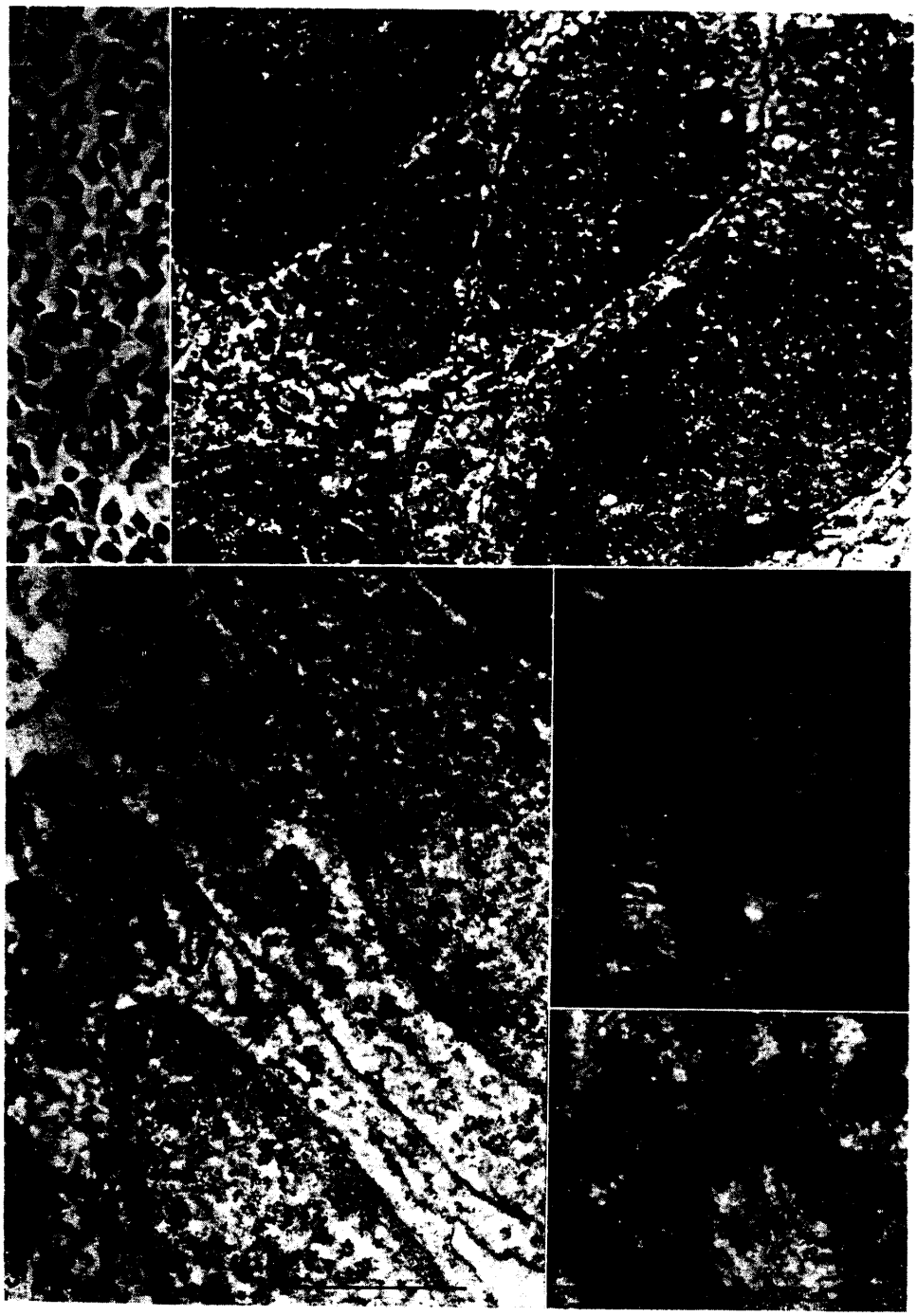
The number of intracytoplasmic organellae is small and the structure is simple, having diffuse but dense ribosome granules, and the density of the cytoplasmic matrix is generally high. There can often be encountered Golgi complex whose membrane is well developed and appears lamellar (Fig. 5). Around this area numerous small vesicles are scattered, but no cyst formation can be detected. Mitochondria are found discretely and most of them appear oval on the cross-section, and some among them are found to contain mitochondrial granules (Figs. 2, 3, 4a). Although the number of endoplasmic reticulum is small, they generally assume the structure of vacuolar, smooth-surfaced vesicles. However, there are sometimes tubular, rough surfaced elements of endoplasmic reticulum scattered discretely, and these can also be observed locally arranged in a lamellar array. In rare instances, there is seen a central body. In addition, there appears an intracytoplasmic component with pseudo-myelin structure, but no phagosome can be recognized. It is also characteristic that only a very few of lysosomes and lipid granules can be detected. The cell membrane is generally in a smooth contact with one another, and very occasionally there can be seen the formation of desmosomes a little distant from the cystic area formed in the intercellular space

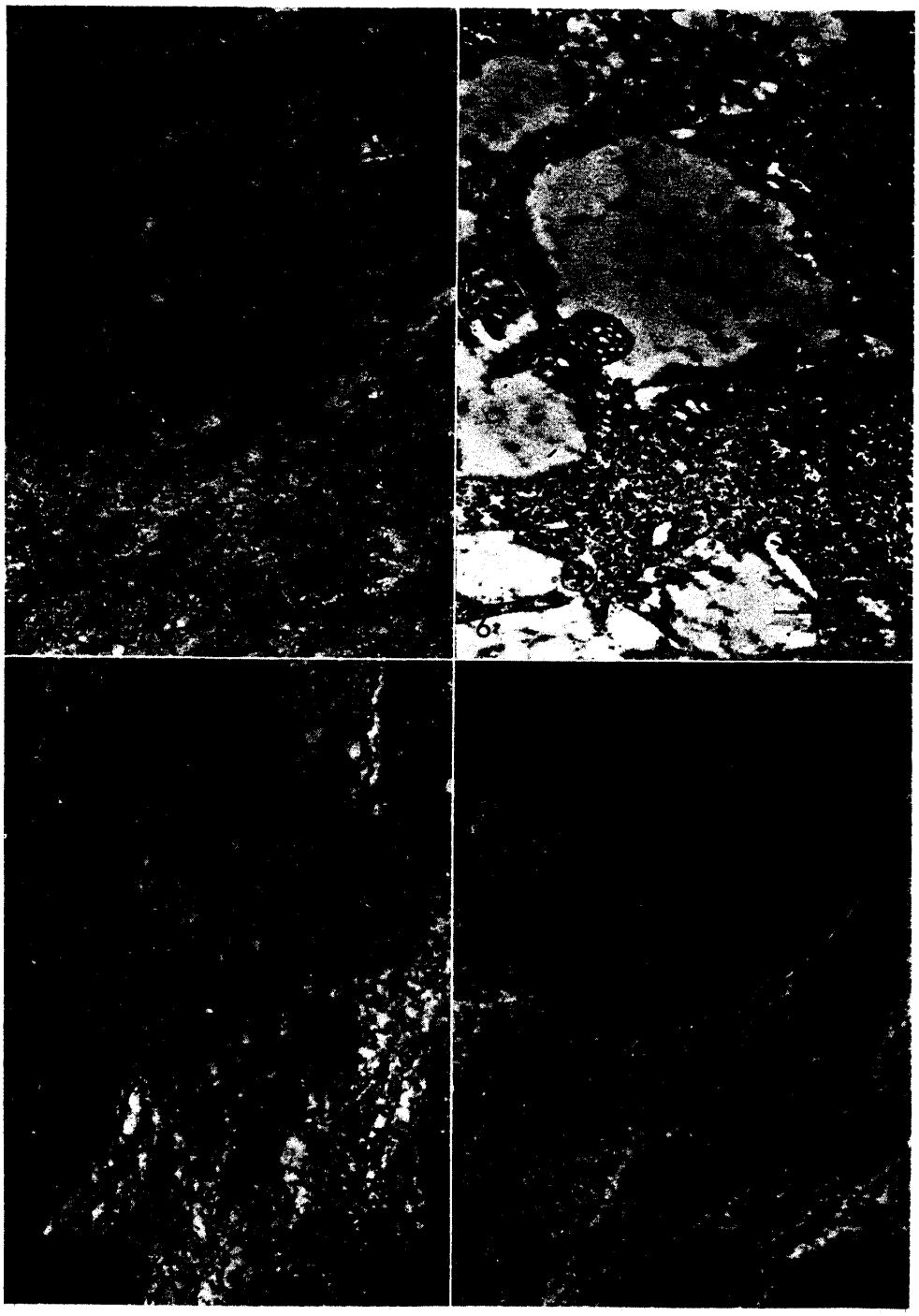
Fig. 1 A light micrograph of adenovirus 12-induced tumor demonstrating spnidle shaped cells irregularly arranged in sarcoma-like pattern. ×600

Fig. 2 The general appearance of the tumor, showing no specific morphology of the nucleus and the cytoplasm. Adenovirus 12-induced tumor. ×10000

Fig. 3 The cell membranes are in a smooth and close contact with one another, and occasionally there can be observed the formation of desmosome (arrow). Adenovirus 12-induced tumor. ×26000

Fig. 4a and 4b Various figures of desmosomal connection (arrows) appear between the contacting surfaces of the two tumor cells. Adenovirus 12-induced tumor. ×49000





(Figs. 3, 4a, 4b). What is most characteristic is interdigitation or clinging figure of adjacent cells in some of the specimens, and in extreme cases there are formed cyst-like fields of varying size in between the cells as if they have projected tentacles forming small networks (Figs. 6, 7). In addition, there can be seen distinct microvilli clinging tightly to each other with their brush-border projections. However, tumor cells are generally in a very close contact with each other, leaving a little intercellular space. As for stroma there can be observed diffusely homogenous substance in which there are fibrillar formations running through it wavily (Fig. 10). In some portions there can be observed collagen fibers, but no distinct picture of the basal membrane.

In the nucleus viral matrix and virus particles can not at all be seen. However, in some of the cells, though very rare, there have been found oval, free virus-like particles of about 70×90 mμ in diameter, high electron density, linked to each other like beads, and located within the cell body but close to the cell membrane (Figs. 8, 9). Internal structures of these particles are indistinct, and they seem to have no association with the membraneous structure of the cell.

DISCUSSION

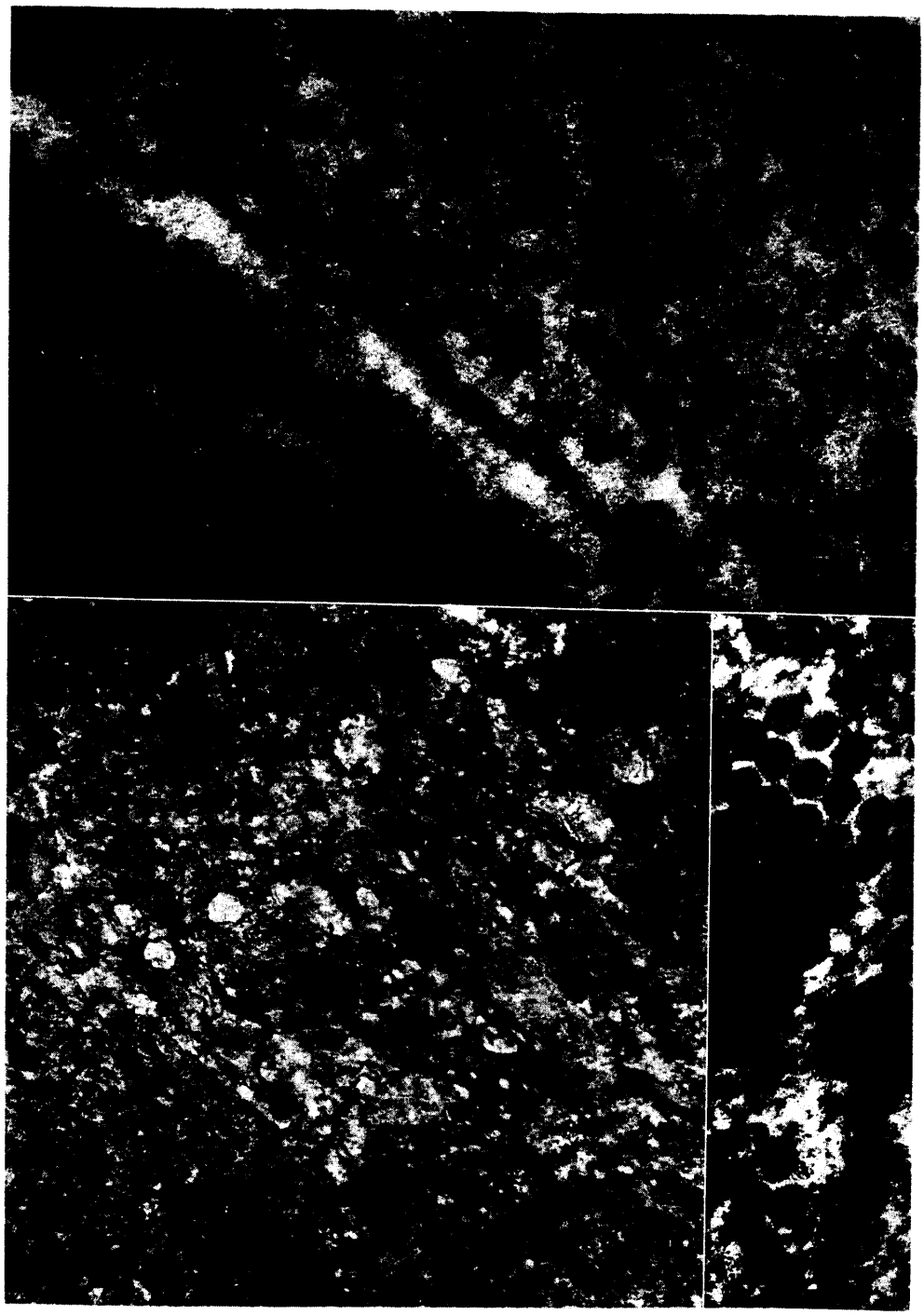
At the optical level the adenovirus 12-induced tumor has so far been considered as an undifferentiated sarcoma, and in somewhat deeper studies, HUEBNER (BERMAN)[3] has also suspected it to be mesothelial and endothelial in origin By other investigators it is suspected as lymphosarcoma, reticulosarcoma, etc.[4,5,6], but its true origin remains obscure. According to OGAWA *et al.*[2] the cells of this tumor sometimes are arranged themselves in a specific string-like formation, and especially in those minute ones spindle-shaped cells are arranged perpendicular to the stroma in a palisade manner, and in some cases these cells surround the fine stroma with or without blood capillaries making a pseudo-roset formation. Furthermore, on the basis of epithelioid character and strong polymorphism of cell patterns observed more easily in the early neoplastic nodules and on the fact that the small primary tumor nodules are formed uniformely on

Fig. 5 Showing Golgi complex, and also ribosome granules distributed diffusely and densely. Adenovirus 12-induced tumor. ×22000

Fig. 6 There can be seen markedly elongated cytoplasmic protrusions which are clinging to those of other cells, forming networks, and there are distinct cyst-like spaces among the cells. Adenovirus 12-induced tumor. ×16000

Fig. 7 There can be observed the characteristic interdigitation or clinging figure of adjacent cells, and forming cyst-like fields in between the cells. Adenovirus 12-induced tumor ×12000

Fig. 8 There can be found a free virus-like particles with high electron density (arrow), and located within the cell body but close to the cell membrane. Adenovirus 12-induced tumor. ×10000



fine peripheral nerve fibers in thc stretch-specimens of mesentery as well as in the serial tissue sections, it has been concluded that this tumor is of neuro-ectodermal supporting cell origin, derived either from immature capsule cells or Schwann cells. Available main histological diagnoses may be listed as shown in Table 1, but there are as yet no electronmicroscopic observations made on this tumor. For references, some examples other than adenovirus type 12 and in the case of animals other than hamster are described.

Table 1. Histopathological Diagnosis of Adenovirus-induced Tumor

Year	Investigator	Type of adenovirus	Animal used	Diagnosis
1962	TRENTIN, YABE *et al.*[1] (four pathologists)	12	H	Undifferentiated sarcoma
1962	YABE *et al.*[13]	12	H	Undifferentiated sarcoma
1962	HUEBNER *et al.*[3]	12 and 18	H	Primitive undifferentiated mesenchymal neoplasms with epithelioid characteristics
1964	YABE *et al.*[14]	12	Mouse	Undifferentiated malignant tumor
1964	RABSON *et al.*[15]	12	Mastomys and mouse	Undifferentiated malignant tumor
1964	GIRARDI *et al.*[16]	7, 12 and 18	H	Undifferentiated sarcoma
1964	PEREIRA *et al.*[4] (BEATTI)	12	H	One of a lymphosarcoma in some places suggestive of a reticulosarcoma
1965	OGAWA *et al.*[2]	12	H	Malignant tumor of immature neuro-ectodermal supporting cell in origin
1965	LARSON *et al.*[5]	7	H	Malignant lymphoma, lymphosarcoma or undifferentiated sarcoma
1965	PEREIRA *et al.*[6] (BEATTI)	31	H	Similar to that of tumors produced in hamsters by adenovirus type 12
1965	SARMA *et al.*[19]	Avian adenovirus (CELO)	H	Well-differentiated fibrosarcoma

H = Hamster

From the multiplicity of the nature of malignant tumor cells, the tumor cells generally lose their morphological characteristics of the original mother cell due to anaplasia, on the other hand some cells exaggerate abnormally a part of the characteristics. Hence it is natural to expect that even these malignant tumor

Fig. 9 Higher magnification of the area in Fig. 8. Virus-like particles having an outer membrane are 70×90 mμ in average diameter and linked to each other like beads. Adenovirus 12-induced tumor. ×80000

Fig. 10 The stroma is composed of diffusely homogenous substance in which there are wavily flowing streams of fibrillar formations running through it, but no distinct picture of the basal membrane. Adenovirus 12-induced tumor. ×23000

Fig. 11 Adenovirus type 12 as observed within the nucleus of the culture HeLa cell. ×90000 (By courtesy of Dr. J. TAWARA)

Bar indicates 1μ

cells appear to have lost their intrinsic characteristics. On the other hand, they would have preserved their morphological or functional characteristics in some way. Similarly, the same can be said of the findings at the ultramicroscopic level. Generally, in undifferentiated cells the development of intracellular organellae is relatively poor, but in those more differentiated cells as a rule specialized, complex structures are observed. In the case of the adenovirus 12-induced tumor in which the development of intracytoplasmic organellae is poor but with extremely abundant free ribosomes, the cell division takes place vigorously, proving evidently that it is of undifferentiated cells. Now, from the facts that these tumor cells show no distinct pinocytotic vesicles nor basement membrane structure, that they show poorly developed vesicles which lack specific arrangement, that they possess no lysosomes, and that they reveal neither phagocytotic activity nor fibroplasia, the various diagnoses mentioned in the foregoing such as fibrosarcoma and angiogenic sarcoma can be ruled out. The finding that has the most important bearing is the formation of desmosomes, though rare, and this with exception of showing the similar structure in the non-epithelial tissue such as cardiac muscle of turtle[20], will amply support the evidence of the epithelial origin. Furthermore, the clinging structure of cellular projections coupled with the histological picture of this tumor suggests a complex structure as observable in the neurogenic cell. Malignant neurilemmoma is a tumor that presents a complex histologic picture, and according to OOTA[7] and TAKAHAMA[8] in the electronmicroscopic finding of human cases, it reveals a specific structure in that cytoplasmic protrusions cling to those of adjacent cells entwining their cellular membranes and another specific picture of the desmosome formation, while the cytoplasm does hardly show any specific structure except occasional formation of basal membrane but without any differentiation processes like myelin. The fine structures of the adenovirus 12-induced tumor practically coincide wtih these electronmicroscopic findings of human nerve-supporting cell tumor.

From these as well as from the histologic characteristics previously pointed out by OGAWA *et al.*[2] it seems reasonable to assume that the original mother cell of this tumor is the undifferentiated nerve-supporting cell which is one of the glial cell series.

In spite of the fact that this tumor has been formed by the inoculation of adenovirus type 12, all the attempts at isolation of virus from tumors by means of tissue culture method have failed completely (Table 2). Consequently, it is believed that infectious virus particles are absent in this tumor. However, HUEBNER *et al.*[9] claim that this tumor contains viral complement-fixing antigen in high concentration. On one hand, SMITH and MELNICK[10] have found electronmicroscopically adenovirus-like particles in the filtered fluid of adenovirus 12-

Table 2. Isolation or Detection of Virus from Adenevirus-induced Tumor

Year	Investigator	Type of adenovirus	Method	Result
1962	HUEBNER *et al.*[3]	12 and 18	T. C.	(−)
1963	TRENTIN, YABE *et al.*[17]	12	T. C. and E. M.	(−)
1963	HUEBNER *et al.*[9]	12 and 18	T. C.	(−)
1963	YABE[18]	12	T. C.	(−)
1964	RABSON *et al.*[15]	12	T. C.	(−)
1964	SMITH *et al.*[10]	12	E. M.	(+) ? Adenovirus-like particle
1965	LARSON *et al.*[5]	7	T. C.	(−)
1965	SARMA *et al.*[19]	Avian adenovirus (CELO)		(−)

T. C. = Tissue culture
E. M. = Electronmicroscope

induced tumor by negative staining method, and on the other, in their density-gradient fractionation study they have pointed out that these particles from size, subunits, morphology and density are found to resemble incomplete adenovirus. Thus they conclude that the presence of such incomplete viral units helps to explain why attempts to isolate infectious virus from adenovirus 12-induced tumor have failed despite the serological evidence that viral elements are present. Nonetheless, up to the present there are no reports on the presence of virus with thin section specimens of the tumor.

Adenovirus is said to be about 75 mμ in diameter. The virus-like particles detected in the present experiment exist only within the tumor cell. Of course, the possibility of contamination and the presence of passenger virus cannot be overlooked, but taking into considerations the reports by DALES[11] and TAWARA[12] about the proliferation pattern of HeLa cells in which virus has been propagated, it is fairly safe to assume this particles to be adenovirus from its size and morphology.

Now, the following suggestions and problems may be pointed out: 1) Whether or not the induction of adenovirus type 12 is always limited to specific target cells and it takes the same manner of carcinogenesis or not; 2) On the basis of polymorphous nature of histologic picture whether several kinds of cells may be mixed in the tumor or from the multiplicity of histologic pictures whether the tumor is composed of the cells of a single strain derived from a single mother cell possessing polymorphous capacity; and 3) Even though technically it is difficult to study the initial step of carcinogenesis by electron microscope, further studies are necessary on the behaviors of virus in the course of tumor development.

CONCLUSIONS

Adenovirus 12-induced tumor has been so far considered to be an undifferentiated sarcoma, but in the present study it has been possible to obtain such electronmicroscopic findings that substantiate well the theory of the neuro-ectodermal supporting cell origin as suggested by the observation at optical level. In other words, a specific clinging picture of cellular membranes and the presence of desmosomes have been demonstrated. In addition, though only in rare instances, the presence of virus-like particles have been verified, and some comments have been made about the relation between tumor and the appearance of virus as well as about carcinogenic mother cell.

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