Ultrastructure of Sarcoma 180

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SUMMARY

Sarcoma 180 has been studied with the electron microscope. Desmosomes have been demonstrated at the intercellular junctions in the tumor cells. The desmosome consists of a plate-like cytoplasmic condensation on which bundles of tonofilaments end. The intercellular space at the desmosome level may show a dense, fibrillar material. The presence of desmosomes and tonofilaments demonstrates the epithelial nature of this tumor and thus its identification as a carcinoma. The abundance of peri- and interchromatin granules, the nucleolar persistence during mitosis, and the presence of dense bodies either close to mitotic chromosomes or in the cytoplasm during nuclear reconstruction, as well as other features of the tumor cells, are briefly described.

INTRODUCTION

Sarcoma 180, also known as Crocker's tumor, originated in the mouse as a spontaneous tumor of epithelial lineage, localized in the axillary region. This tumor was discovered in 1914 by Dr. W. H. Woglom at the Crocker Laboratory in the United States and was maintained by successive transplants (28, 29) after which it was accepted that its behavior was that of a sarcoma. The electron microscope has provided great aid in the knowledge of many tumors, whether human, animal, or experimental, but does not allow the discrimination between normal and neoplastic cells, whether human, animal, or experimental, but does not allow the discrimination between normal and neoplastic cells, although it permits the study of additional cytological details (2, 3, 5, 12, 13, 15, 16, 21, 23, 24, 30). In this field we must remember the important papers and studies on this subject performed by Dalton et al., Dmochowski et al., Moore et al., and Seman et al. (7–10, 19, 20, 25, 26). These authors, in addition to studying several transplantable tumors from mouse and human mammary neoplasm by means of electron microscopy, corroborated the finding of viral particles. Among these tumors are found those of the breast, the extrachromosomal heredity and relation with virus which were interpreted by Bittner in 1936 (4, 7–10, 19, 20, 25, 26). In a previous paper on the light microscopic features of Sarcoma 180 (34), the author showed that the morphological and biological characteristics of this tumor were epithelial. This conclusion was based on the kind of metastasis produced and especially on the distribution of the reticulin fibers stained with argentiechniques. Furthermore, in 1961 Lustig (17) showed that embryonal tissue inhibits sarcomatous proliferation and induces a differentiation of glandular type. The purpose of this paper is to prove the epithelial nature of this tumor, by the ultrastructural study of the tumor cells and the demonstration of the existence in these cells of the characteristic intercellular junctions that exist in several types of epithelial cells and that have received according to their location and special features the names of zonula occludens, zonula adherens, desmosomes, and terminal bars (1, 6, 11, 14).

MATERIALS AND METHODS

The observations were carried out on Sarcoma 180, transplanted s.c. in C3H mice after about 15 days of tumor development. Small pieces of the tumor was removed from mice under ether anesthesia and cut in smaller fragments (about 1 mm in diameter) on a glass plate with 4 or 5 drops of fixative. These fragments of tissue were fixed in 2.5% gluteraldehyde in 0.1 M phosphate buffer (pH 6.9) for 2 hr in the refrigerator. After 24 hr in washing buffer the pieces were postfixed in 1% osmium tetroxide in phosphate buffer for 1 hr. They were dehydrated in gradually increasing ethanol and embedded in Maraglas. Ultrathin sections of 750 to 1000 A thick were cut in a Porter-Blum ultramicrotome and stained with a saturated solution of uranyl acetate in methanol and then with lead citrate according to the method of Reynolds (22). Sections of Maraglas embedded tissues, cut 0.95 μm thick, were stained with Unna's blue for observations with light microscopy and to observe the cellular areas separated by connective stromal tracts. Observations and micrographs were taken at several magnifications with a Zeiss EM9 electron microscope.

RESULTS

The ultrastructure of tumor tissue was studied in interphase cells and during mitotic divisions. The intercellular junctions between the plasmatic membranes of adjacent cells were the prime target of this study.

Interphase Cells. They form the largest portion of the tissue. Necrotic areas, which are larger with the age of the tumor, were found among these cells. The cells acquire polyhedral shape and a very large nucleus which is bound by a characteristic nuclear envelope with pores. This envelope contains invaginations that penetrate deeply in the nuclear space and carry a cytoplasmic matrix in their inner part, as well as various organoids and inclusions. These invagina-
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Mitotic Cells. We have seen not only normal mitotic aspects in the tumor cells, but also abnormal mitotic features, which will be described in detail. During prophase the chromosomes became conspicuous as lumps of condensed chromatin still in relation with the nuclear membrane. The nuclear envelope is gradually fragmented and disappears while the centriolar movements begin. The nucleolus loses its characteristic morphology and becomes more homogeneous, with a granular aspect, and it is fragmented. Remarkably, the nucleolus persists during metaphase and anaphase as clumps in close relationship to some parts of the chromosomes (Fig. 4). In metaphase, as in normal cells, the chromosomes are oriented in an equatorial plate, many of the chromosomes show nucleolar material near their ends and the spindle microtubules are evident. Occasionally, multipolar mitosis with irregular orientation of the microtubules is observed. In some cases the lack of centriolar migration with the 4 centrioles in one pole of the spindle and the chromosome mass in the other was observed. With regard to the centriolar structure in no case was an alteration of its morphology observed. In anaphase the migration of the chromosomes toward the spindle poles begins and in telophase the nuclear envelope is reconstituted, while the chromosomes become uncondensed. Cyto-
kinesis takes place simultaneously as a symmetrical narrowing of the plasmatic membrane and the remaining cytoplasm which becomes an intercellular bridge between the 2 daughter cells. The convergence of the spindle microtubules was found in this place. Finally the 2 cells are completely divided. During anaphase a remarkable structure was found. It consists of an accumulation of small dense bodies, spherical or rod-like, which are localized near the chromosomes (Fig. 6). Later, when the nuclear envelope is reconstituted at telophase, it appears as a dense mass with zones of low electron density and is located in the cytoplasm adjacent to the nucleus (Fig. 7).

Intercellular Junctions. When the tumor cytoarchitecture is analyzed with light microscopy, it is observed that the cells are arranged in cords and nests. When the intercellular regions already described with the light microscope are observed with the electron microscope, close contacts between cells were seen. Thus, it was concluded that the presence of these characteristic specializations of the cell periphery allows the identification of Sarcoma 180 as a carcinoma. In fact, the plasma membrane is thickened in many points of contact and the adjacent cytoplasm becomes more dense (Fig. 1). These dense regions occur at variable intervals in the extended area of close contact of the plasma membranes. Fine and abundant tonofilaments that fall perpendicularly to the desmosome plates are observed. Thin filaments running in the cytoplasmic matrix were also seen (Figs. 2 and 3). Each desmosome is plate shaped, with a major longitudinal axis, and in the intercellular space, a fibrillar material of moderate density is found. The regions of the plasma membranes interspersed among the desmosomes are closely joined to each other.

DISCUSSION

The most important observation made in this study of Sarcoma 180 is the finding of desmosomes located at multiple points of contact among tumor cells. These cells are intimately joined to each other and the intercellular substance is practically absent. The finding of desmosomes and tonofilaments in these cells is considered definite proof of the epithelial nature of these cells. Thus, this tumor is identified as a carcinoma, although the immaturity of the cells has been the source of the misinterpretation of this tumor as a sarcoma when observed with light microscopy. The desmosomes found in this tumor show the general features of these regions already described in the literature. Each cell has several desmosomes, each one appears as a condensed plate at each side of the intercellular contact region, and bundles of tonofilaments end on some of the plates. However, due to the immaturity of the cells, some features of typical desmosomes are occasionally absent. The quantity of tonofilaments ending on the desmosomal plates varies heavily and occasionally the plates lack tonofilaments. These special features of the desmosomes in these tumor cells may be attributed to an incomplete differentiation of the cells (18). The degree of condensation of the desmosomal plates also varies markedly.

The importance of the identification of the histogenesis of...
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this tumor is obvious. As this tumor is widely used as an experimental material in pharmacology, pathology, and other areas, workers should be aware of the epithelial nature of this tumor. The remaining ultrastructural features of these tumor cells have been described in other tumors. The abundance of perichromatin granules (which have been described also in normal tissues of rodents) (3, 31) and the experimental material in pharmacology, pathology, and other areas, workers should be aware of the epithelial nature of this tumor is obvious. As this tumor is widely used as an experimental material in pharmacology, pathology, and other areas, workers should be aware of the epithelial nature of this tumor. The remaining ultrastructural features of these tumor cells have been described in other tumors. The abundance of perichromatin granules (which have been described also in normal tissues of rodents) (3, 31) and the high frequency of abnormal mitosis should also be noted. Finally, the submicroscopic study of this tumor has not revealed any viral particle.

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REFERENCES

Fig. 1. Low-power electron micrograph showing desmosomes located at several places in cell surface (pointers). In one nucleus an apparent inclusion is formed by a transverse section of a nuclear envelope invagination (/). In Figs. 1 to 9, the tissue was fixed in glutaraldehyde and postfixed in osmium tetroxide. All sections were stained with uranyl acetate and lead citrate.

Fig. 2. Tonofilaments ending on the desmosome plates (pointer). N, nucleus. $\times$ 57,000.

Fig. 3. The cytoplasm of the tumor cells shows abundant tonofilaments. $\times$ 47,500.
Fig. 4. Nucleus of a cell at mitotic prophase. Perichromatin granules and a large nucleolus (n) joined by finely granular and evenly arranged material are shown. × 28,000.

Fig. 5. High-magnification electron micrograph of a nucleus showing the perichromatin granules (pointer) surrounded by a clear space. The chromatin is highly condensed. × 52,250.

Fig. 6. Groups of dense granules (pointer) close to the metaphase chromosomes. A bundle of microtubules (M) ends on a nearby centromere. × 57,000.

Fig. 7. Group of dense granules similar to those of Fig. 6 but in the cytoplasm of a cell at telephase. Desmosomes (d) are observed at the cell surface. × 19,250.
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