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Ultrastructure of the Lewis Lung Carcinoma

T. SATO, K. TAKUSAGAWA, N. ASOO, F. ARIJI, K. SHIDA, N. KUMANO and K. KONNO

Department of Internal Medicine, The Research Institute for Tuberculosis and Cancer, Tohoku University, Sendai, Japan

Abstract—*Electron microscopic observations were made on the Lewis lung carcinoma, which has frequently been used in various experiments. Although the tumor cells had desmosomes, interdigitations, microvilli and basement membranes which were of epithelial nature, they did not show either squamous or adenomatous differentiation. On the basis of microscopic and ultrastructural findings, this tumor falls into the category of large cell carcinomas. At the periphery of the tumor, sinusoidal clefts between the strands of tumor cells contained many red blood cells without fibrin. While perfect blood vessels were observed towards the center of the tumor, large necrotic areas were observed. Dilated large vessels possessed some endothelial fenestrations. Light endothelial cells were also observed, which were penetrated by red blood cells. Interstitial components were very scant and collagen fibres were frequently observed to be dissolved.*

INTRODUCTION

THE LEWIS lung carcinoma (3LL) originated in a spontaneous tumor of a C57 black mouse [1] and has been maintained by successive transplantation. This tumor has been described as an anaplastic epidermoid carcinoma with a marked hemorrhagic tendency [1].

Since the 3LL produces multiple lung metastasis regularly, spontaneously and consistently [2], and is extremely refractory to most chemotherapeutic agents [3], this tumor has been widely used as an experimental material in the various fields. However, we have not been aware of reports concerning its fine structures.

This study is to examine both ultrastructural features of the 3LL and the correlation between tumor cells and stroma because of spontaneous metastasis to the lungs.

The fact that this tumor readily metastasizes to the lung might be explained from the following findings at the peripheral zone of tumor mass: (1) irregular shapes of tumor cells; (2) a few microvilli, many cytoplasmic processes, prominent ectoplasm and poorly developed cytoplasmic organelles; (3) scant intercellular junctions; (4) wide intercellar spaces and sparse intercellular components; and (5) imperfect blood vessels.

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MATERIALS AND METHODS

The Lewis lung carcinoma has been maintained by subcutaneous implantation into C57B1/6 male mice in our laboratory. The tumor was excised and the minced gruel was inoculated into subcutaneous tissue on the back of a normal mouse. The samples were removed, together with skin and subcutaneous tissue, at 7, 11, 18 and 23 days after implantation. They were divided into two groups. One of them was sliced about 1 mm thick. The slices were fixed in 2.5% Millonig-buffered glutaraldehyde (pH 7.4) for 2 hr, postfixed in 1% Millonig-buffered OsO_4 (pH 7.4) for 2 hr, then dehydrated in an ethanol series and embedded in Epon 812. The embedded slices were adequately cut in pieces from the periphery to the center of the tumor mass. Semi-thin sections of the pieces were stained with toluidine blue for orientation, and appropriate regions were selected for ultra-thin sectioning. The ultra-thin sections were stained with uranyl acetate and lead citrate, and examined with a Hitachi HU-11B electron microscope operated at 75 kV.

The other was embedded in paraffin and stained with hematoxylin and eosin.

RESULTS

Tumor cell

The tumor cells appeared polyhedral to spindle shaped (Figs 1 and 2). The nuclei were

very irregular and possessed deep invaginations and intranuclear pseudoinclusions which contain some cytoplasm. Multinucleated large tumor cells were often observed (Fig. 3). Clumping of chromatin was observed peripherally about the nuclear membrane and interchromatin granules were plentiful. The nucleoli were large and prominent, and consisted predominently of a granular component. Oval and round mitochondria had irregular cristae and were often swollen. Rough endoplasmic reticulum was of moderate amounts and flattened cisternae contained a material of moderate electron density; Golgi complexes were moderately developed. Lysosomes were scattered and frequently exhibited myelin-like structures (Fig. 4). Microtubules were often observed and free ribosomes were abundant.

Many microfilaments about 100 Å thick were often observed in the central region of tumor cells with an eccentric nucleus (Fig. 5). However, bundles of tonofilaments and keratohyalin granules were not found. On the other hand, no tumor cells had either mucus granules or extra- and intracellular lumens. No viral particles were found, either.

Correlation between tumor cells and stroma

Tumor cells were freely scattered at the periphery of the tumor and were pleomorphic (Fig. 2). These cells possessed prominent ectoplasm, many cytoplasmic processes and a small number of short and blunt microvilli on the cell surfaces (Fig. 2). Intercellular spaces were wide and collagen fibres were sparse (Fig. 2). Towards the central region of the tumor, plasma membranes of tumor cells came nearer to each other, and the interdigitations and short desmosomes between the adjacent cells increased (Fig. 6). One or two layers of basement membrane-like materials were often observed between tumor cells (Fig. 4). Although necrotic areas enlarged as the tumors increased in size and age, inflammatory cells were rarely observed. A few degenerating tumor cells were often observed distinctly among the viable cells (Fig. 7). Interstitial cells were very scanty and collagen fibres were frequently observed to be dissolved (Fig. 8). Elastic fibres were not found.

Blood vessels in the tumor were unexpectedly scanty and consisted of endothelial cells, with or without a pericyte, and a layer of basement membrane. Pinocytotic vesicles were few in general, and intraluminal cytoplasmic processes were often long and tortuous. Extravascular spaces were narrow and contained a few collagen fibres. Occasionally, tumor cells were seen close to a basement membrane of an endothelial cell. The endothelial fenestrations were often observed in dilated large vessels (Fig. 9). Platelets without fibrins were sometimes observed in a group in the lumen. Red blood cells penetrated degenerating endothelial cells with light cytoplasm and came out into extravascular space (Fig. 10). At the margins of the tumor, many red blood cells were observed in sinusoidal clefts between the strands of tumor cells (Fig. 11). Fibrin was rarely observed. Blood vessels close to necrotic areas were not always degenerate. Lymphatic vessels were not found.

DISCUSSION

Tumor cell

The Lewis lung carcinoma is commonly used in a variety of experiments. It is known that the 3LL originated in a spontaneous pulmonary carcinoma of a mouse, and its histology was anaplastic epidermoid carcinoma with marked hemorrhage [1]. But Salsbury *et al.* [4] described the histology of the 3LL as being rather more similar to that of a sarcoma with blood vessels lying in close relation to tumor cells than that of a carcinoma with blood vessels and tumor cells separated by a fibrous tissue stroma. However, the tumor cells ultrastructurally exhibited microvilli, interdigitations and desmosomes, and were often accompanied with a basement membrane. These findings led us to consider that this tumor was of epithelial origin [5, 6].

Microscopically, the tumor cells did not show stratification, definite keratin formation or intercellular bridges. It has been reported previously that ultrastructurally, epidermoid carcinoma cells possess well-developed desmosomes and complex interdigitations between cells, many tonofibrils in the cytoplasm and relatively poorly developed cytoplasmic organelles [6, 7]. Adenocarcinoma cells show glandular differentiation and well-developed organelles [6, 7]. The examined tumor should be diagnosed as large cell carcinoma because of the absence of characteristic microscopic and ultrastructural features of epidermoid carcinoma or adenocarcinoma. Since transplantable tumors usually lose their resemblance to the original pattern and change to undifferentiated tumor with successive transplantations [8], it is probable that this tumor has changed from epidermoid carcinoma to undifferentiated carcinoma.

A mass of randomly oriented, nonspecific microfilaments measuring about 100 Å in diameter were observed in the central region of

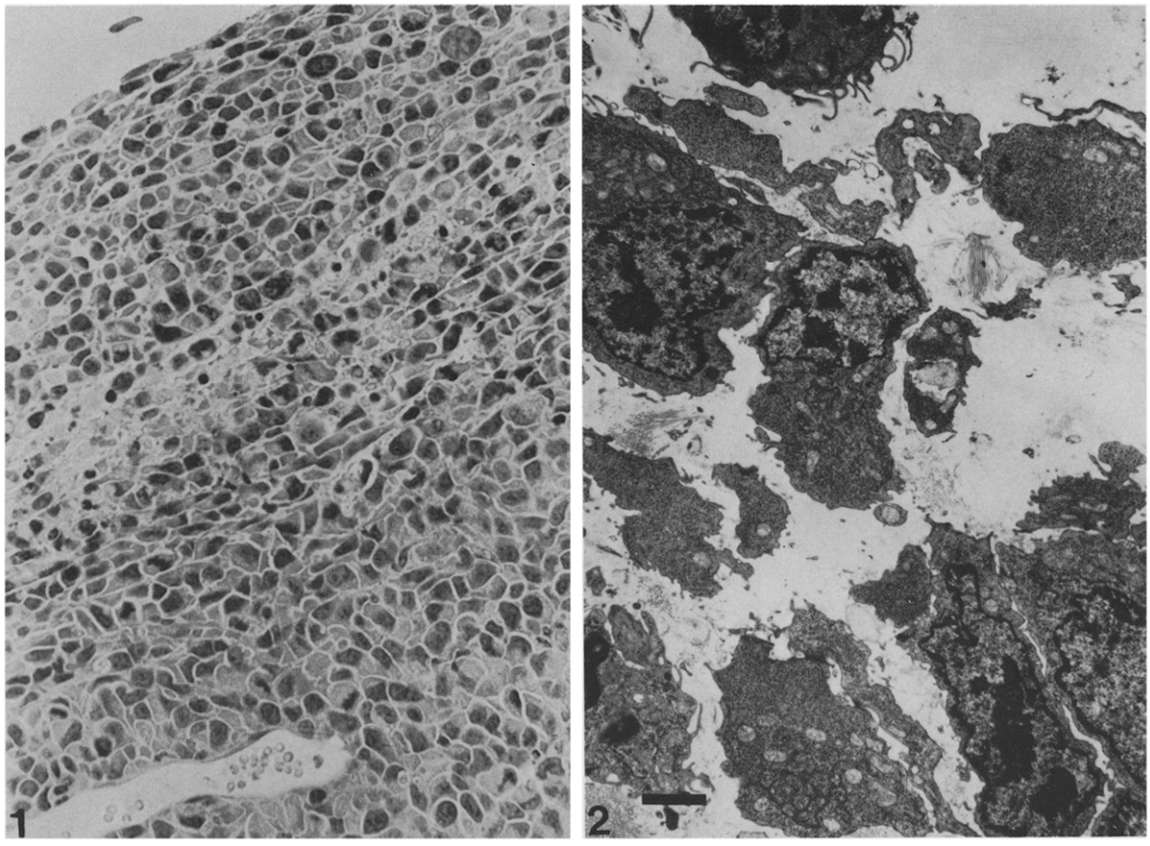

Fig. 1. *The sinusoidal clefts with many red blood cells between the strands of tumor cells are seen at the margin of the tumor. The tumor cells shift from fusiform to polyhedral and become larger toward the center of the tumor. Large tumor cells are seen scattered. Hematoxylin and eosin.* ×300.

Fig. 2. *Tumor cells at the periphery of the tumor. The cells are irregular and have many cytoplasmic processes, a few microvilli and prominent ectoplasm. Intercellular spaces are wide and collagen fibers are sparse. Bar* = 2 μm.

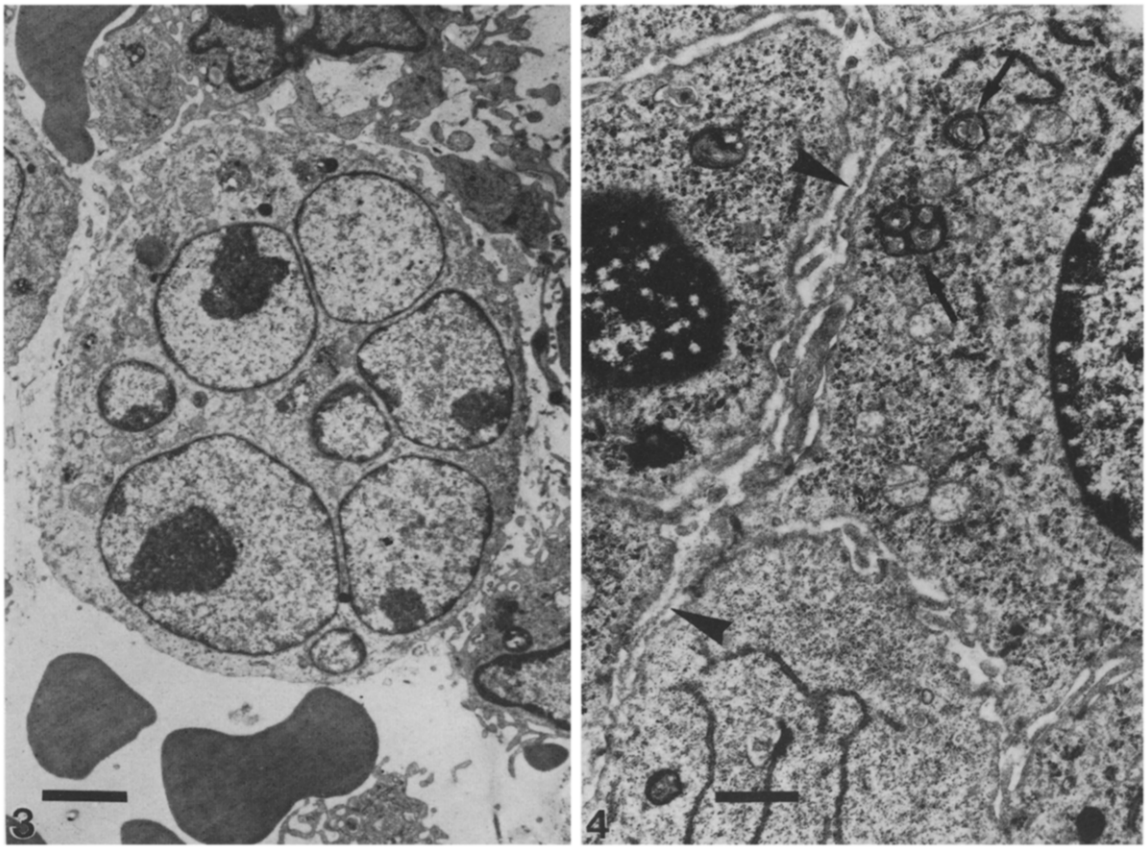

Fig. 3. A multinucleated large tumor cell. Bar = 2 μm.

Fig. 4. Tumor cells are packed. Lysosomes are scattered and frequently exhibit myelin-like structures (arrows). A layer of basement membrane-like material (arrowheads) is observed between tumor cells. Bar = 1 μm.

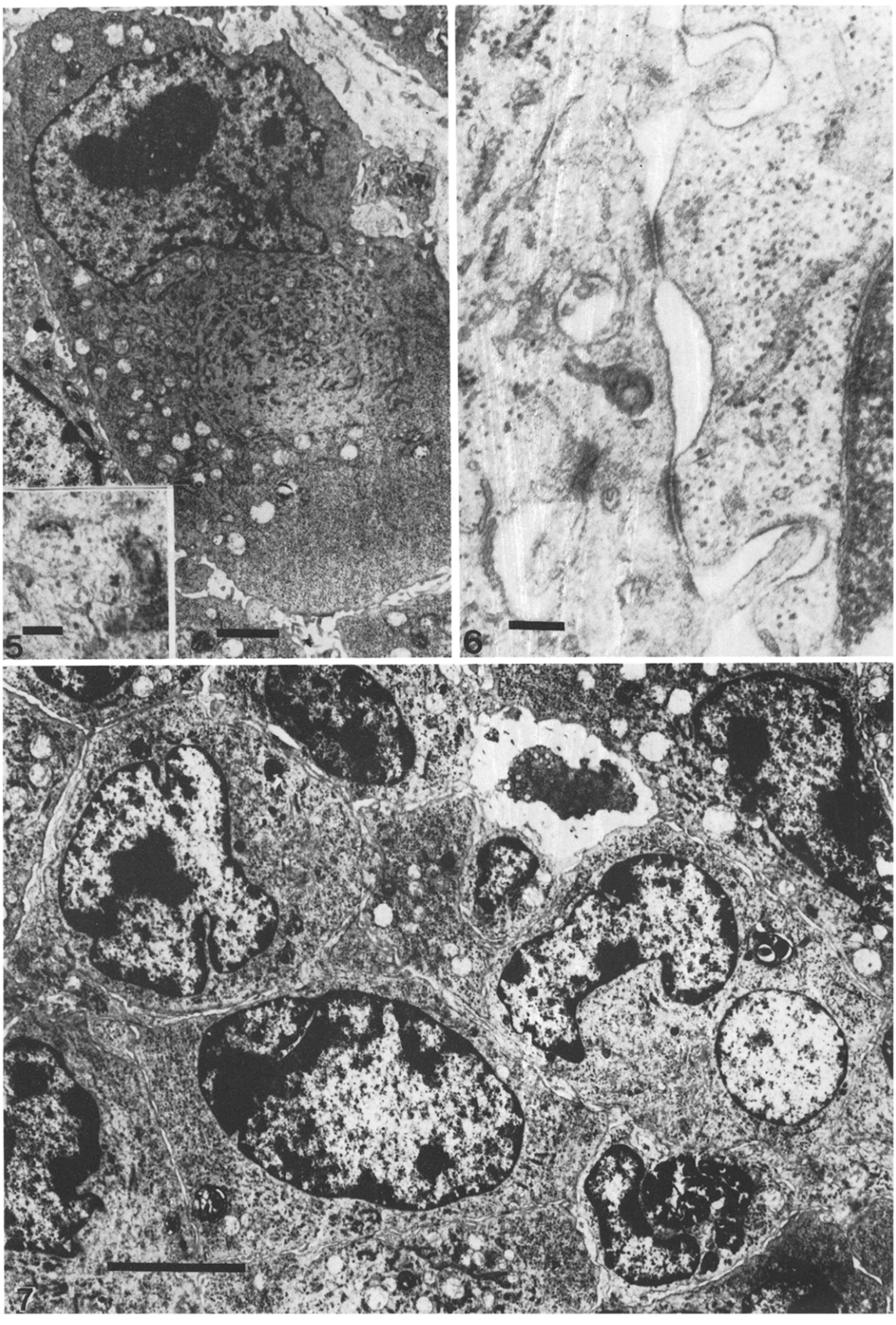

Fig. 5. *A clear zone with microfilaments is seen in the central region of a tumor cell with an eccentric nucleus. Bar* = 2 μm. *Inset: microfilaments in the clear zone. Bar* = 0.2 μm.

Fig. 6. *Interdigitations and short desmosomes between the adjacent cells. Bar* = 0.2 μm.

Fig. 7. *A few degenerating tumor cells are seen among the viable cells without inflamatory cells. Bar* = 5 μm.

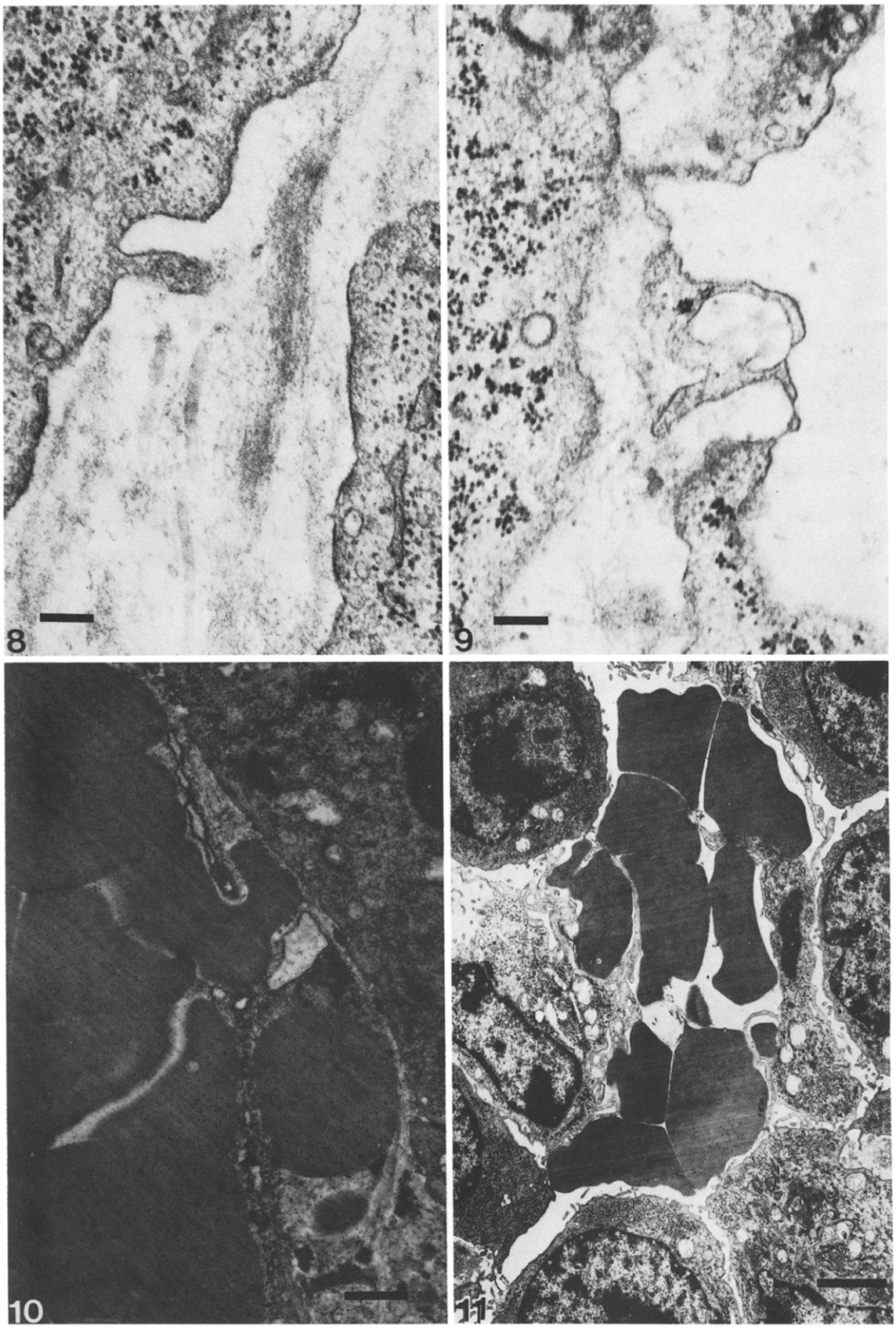

Fig. 8. *Stromal collagen fibres are degenerated and amorphous substances are seen in the intercellular space. Bar* = 0.2 μm.

Fig. 9. *The endothelial fenestrations of a large vessel. Bar* = 0.2 μm.

Fig. 10. *A red blood cell in the vascular lumen is just penetrating through the endothelial cell by forming a protrusion. Another red blood cell is present in an extravascular space. Bar* = 1 μm.

Fig. 11. *A passage between the strands of tumor cells containing red blood cells. Bar* = 2 μm.

a tumor cell with an eccentric nucleus. Similar aggregates have been observed in several other different types of tumors [9], and it has been considered that they are indicative of squamoid metaplasia [10] or have no particular significance [11].

Tumor stroma

James and Salsbury [12] observed the blood vessels of the 3LL by light microscopy and reported that the 3LL possessed poorly formed sinusoidal vascular channels, in which an endothelial lining was absent and blood cells were in direct contact with tumor cells at the margins of the tumor. Moreover, they thought that red blood cells were streaming between strands of tumor cells and blood flow rate was very much lower than in the discrete vascular channels [13]. Warren and Shubic [14] observed ultrastructurally many channels containing red blood cells in the melanoma transplants which possessed no endothelial lining, and considered that the clefts between the strands of tumor cells were sinusoidal in nature and were fed and drained by capillaries in melanoma transplants [14]. Although the sinusoidal channels were observed ultrastructurally in the examined tumor as above-mentioned clefts, it was uncertain whether red blood cells were simply caused by hemorrhage or by streaming. However, the latter possibility might be present because fibrin was not seen in this channel.

Sometimes, endothelial fenestrations were observed in the larger vascular channels. These have been reported in many tumors, although the original tissue does not normally have them [15]. It was considered that the propensity for bleeding in tumors was caused by endothelial fenestrations [16].

Blood vessels towards the center of the tumor with some areas of necrosis have generally intact endothelial cells, in contrast to some peripheral vessels, as described by Salsbury *et al.* [4]. Areas of hemorrhage were often observed in the neighborhood of necrotic zones. The endothelial cells with numerous dilated endoplasmic reticula were penetrated by red blood cells.

The fact that this tumor was inclined to metastasize to the lung might depend on both the tumor cells themselves and the correlation between them and tumor stroma [17–19]. The tumor cells showed irregular shapes and possessed a few microvilli, many cytoplasmic processes and prominent ectoplasm, and did not contain such abundant cytoplasmic organelles at the peripheral zone of the tumor. In addition, intercellular junctions observed were very few. Interstitial components were very scanty and intercellular spaces were very wide. These findings suggest that tumor cells are readily able to release from tumor mass. Moreover, it might favor dissemination of tumor cells that tumor blood vessels at the periphery of the tumor were structurally imperfect vascular architecture [20, 21].

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