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Light and Electron Microscopic Study of Malignant Melanoma Cells, Isolated from the Peripheral Blood

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Licht- und elektronenmikroskopische Untersuchungen maligner Melanom-Zellen, isoliert aus dem peripheren Blut

Zusammenfassung. Zellen maligner Melanome, die aus dem strömenden Blute isoliert wurden, konnten durch Melaninnachweis und elektronenmikroskopische Besonderheiten charakterisiert werden. Alle zeigten mehr oder weniger starke degenerative Veränderungen.

Summary. Cells of malignant melanoma isolated from the blood were studied by light and electron microscopy. Most of the cells were identifiable beyond any doubt by the presence of melanin and by their characteristic structure. All of the tumour cells isolated from the blood showed signs of damage of variable degree.

Introduction

The aim of the present study was to find out how often tumour cells were detectable in the peripheral blood of patients with malignant melanoma; furthermore, to study changes occurring in these cells under light and electron microscopy. No electron microscopic evidence has been reported thus far for cells of malignant melanoma isolated from the blood.

Material and Methods

Fifty patients with malignant melanoma were studied for tumour cells in their peripheral blood. In 20 patients samples were taken after preoperative irradiation with an average dose of 4000 r prior and 30 min after surgical removal of the tumour.

Ten ml heparinized blood from the cubital vein were used. The tumour cells were isolated by Seal's silicone-flotation method on slides, in 10 cases by Malmgren's hemolysis method on a millipore filter of five micron pore, and stained with Papanicolaou's stain.

The tumour cells isolated by Seal's procedure were studied electron-microscopically in eight cases. In two patients the primary growth was examined in ultrathin sections. The specimens were fixed with Palade's 2 per cent osmium tetroxyde, embedded in Araldite, contrasted during embedding with uranyl acetate, subsequently by Karnovsky "A", and photographed with JEM 6/C electron microscope.

Results

In 31 out of 50 patients tumour cells were detectable in the peripheral blood. The isolated cells were readily identified by means of light microscopy. The melanin pigment of these cells showed a dust-like or granular dispersion through out the cytoplasm; there were coarse-grained, darkbrown pigment granules covering the nuclear structure (Fig. 1). Approximately 20 per cent of the cells

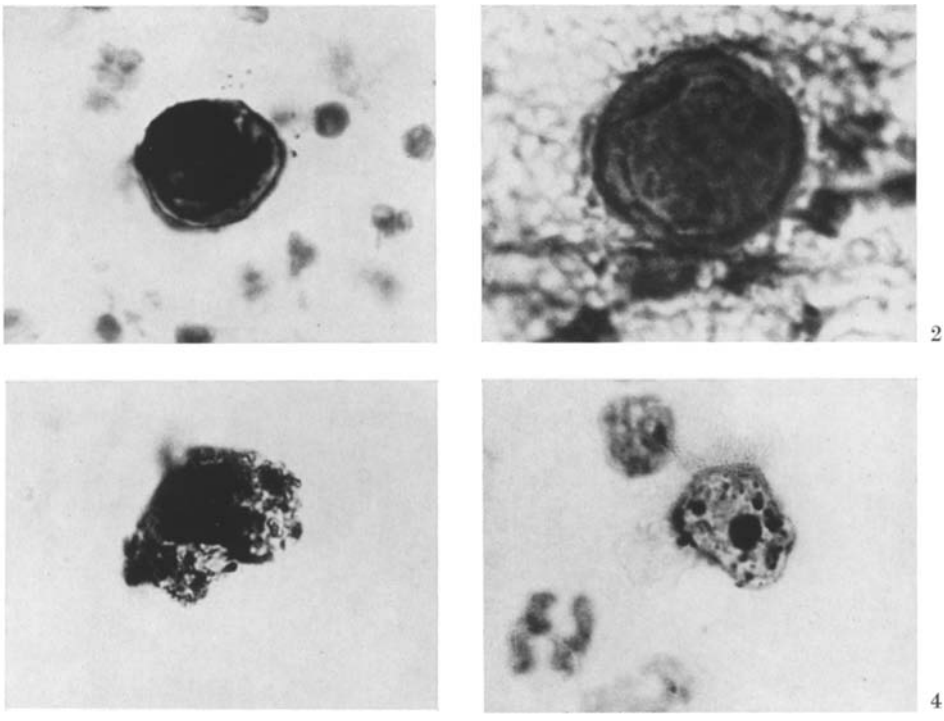

Fig. 1. Pigmented tumour cells isolated from the blood of a patient with malignant melanoma. Streptolysin-O; filter; Papanicolaou's staining; × 530

Fig. 2. Amelanotic tumour cells isolated from the blood of a patient with malignant melanoma. Streptolysin-O; filter; Papaniclaou's staining; × 530

Figs. 3 and 4. A damaged tumour cell and a naked nucleus isolated from the blood of a patient with malignant melanoma. Streptolysin-O; filter; Papanicolaou's staining; × 530

were amelanotic. Even in the absence of melanin they were readily distinguished from other cells by their size as well as by their coarse chromatin pattern and their prominent nucleoli in their large excentric nuclei (Fig. 2). Beside intact tumour cells, numerous damaged cells, cell fragments and naked nuclei were seen (Figs. 3 and 4).

In one patient tumour cells were demonstrated only after treatment, none before; in six of the patients the number of malignant cells increased in blood samples drawn after manipulation. In 13 patients therapeutic measures did not seem to affect the number of detectable tumour cells.

The cells of the primary tumour showed the typical ultrastructural features of cells of malignant melanoma. The cytoplasm contained numerous ribosomes and mitochondria. The Golgi-zone was well developed. There were numerous granules of melanin inside the melanosomes of unequal size, their average diameter being 300 mμ (Fig. 5). Components of melanosomes were highly varied in shape; some had rod-shaped or granular patterns of melanin of different density, or the melanin had a lamellar arrangement with cross-striations of distinct periodicity (Fig. 6).

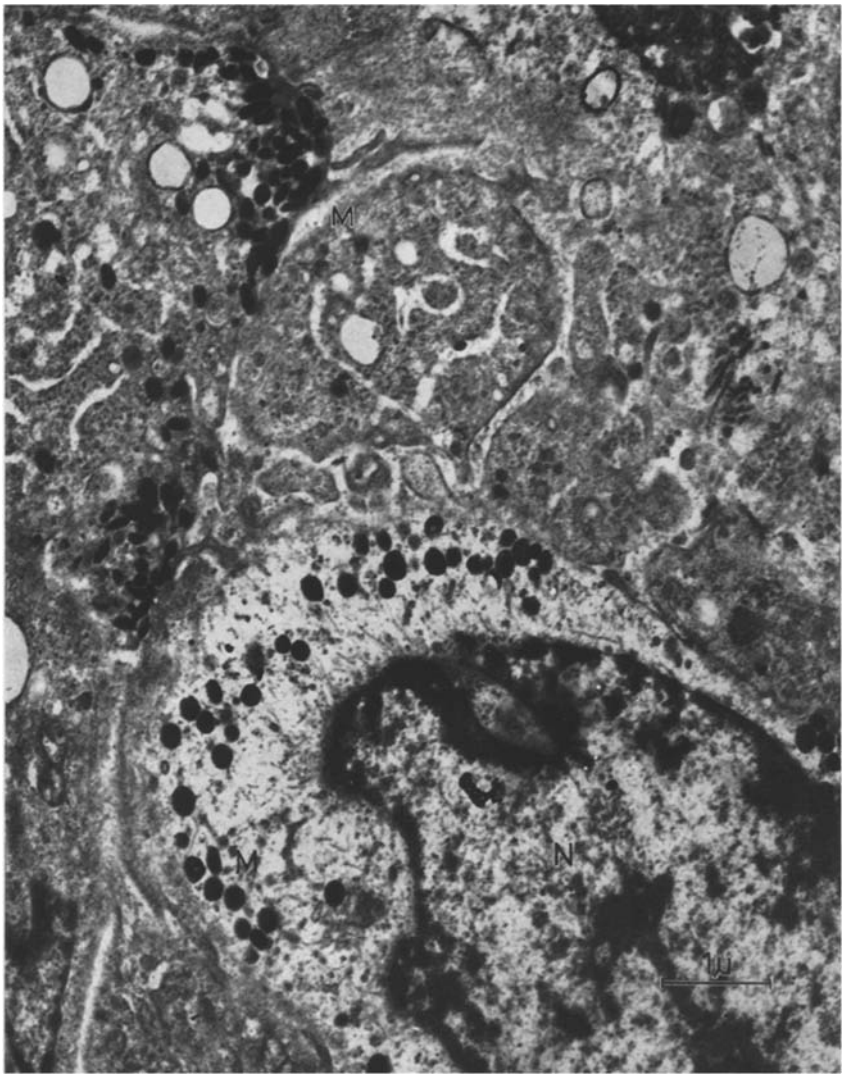

Fig. 5. Malignant melanoma cell with irregular nucleus in the primary tumour (*N*). The cytoplasm contains numerous mitochondria, ribosomes, lipid granules and melanosomes (*M*) of variable size. Electron micrograph: 3,900 × 4.5

The cells isolated from the blood showed different degrees of cellular damage. The nuclei reflected the process of pyknotic disintegration through all its stages. The cytoplasm became poor in organells, though mitochondria were generally still recognizable. In most cells melanosomes were still present but they were poorer in melanin although they preserved their granular or lamellar structure (Fig. 7). Some of the isolated cells contained no pigment, but their strongly enlarged, irregular nuclei with cytoplasmic indentations left no doubt about their neoplastic character (Fig. 8).

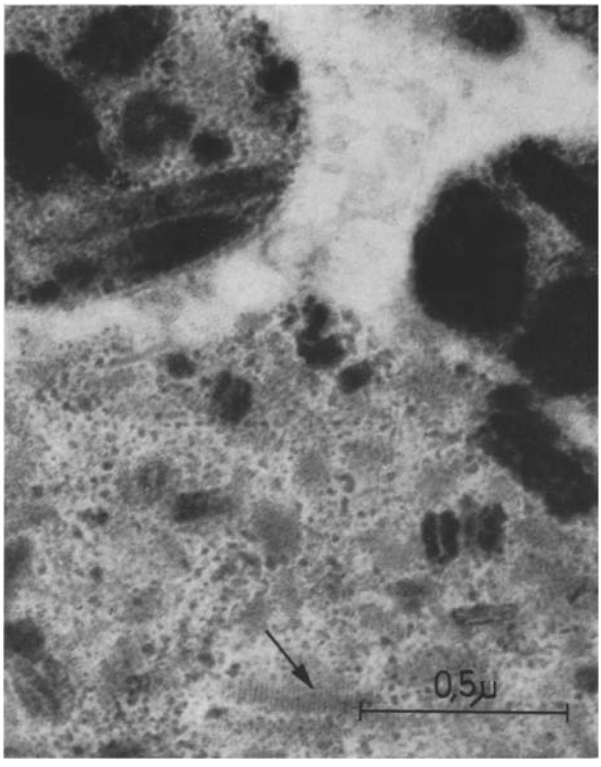

Fig. 6. The pigment granules in the melanosomes are arranged as rod-shaped, lamellar pattern in which cross striations with distinct periodicity are recognizable. Electron micrograph: 25,500 × 2.5

Discussion

Most cells from malignant melanoma contain melanin and are thus readily distinguished under the light microscope from any other cellular element of the blood. The tumour cells isolated from the blood are identifiable beyond any doubt on the ground of their fine structure so characteristic of malignant melanoma. Study of tumour cells circulating in the blood is thus particularly rewarding in this type of tumour.

In 31 out of 50 of our patients with malignant melanoma, i.e. in nearly two-thirds of our material, we were able to identify tumour cells in the peripheral blood. Other investigators report figures between 30 and 70 per cent. Though the range of reported positive findings could hardly be greater, malignant melanoma differs from other types of malignant tumours (squamous cell or glandular carcinoma) by its tendency for increased numbers of circulating tumour cells in the peripheral blood (Cole, 1961; Döbrössy, 1966; Moore, 1965; Romsdahl, 1962). The hazards of surgery in promoting cellular dissemination from the primary tumour into the blood-stream, thus in favouring spread of tumour, seem from our own observations to be possible too (Cole, 1961).

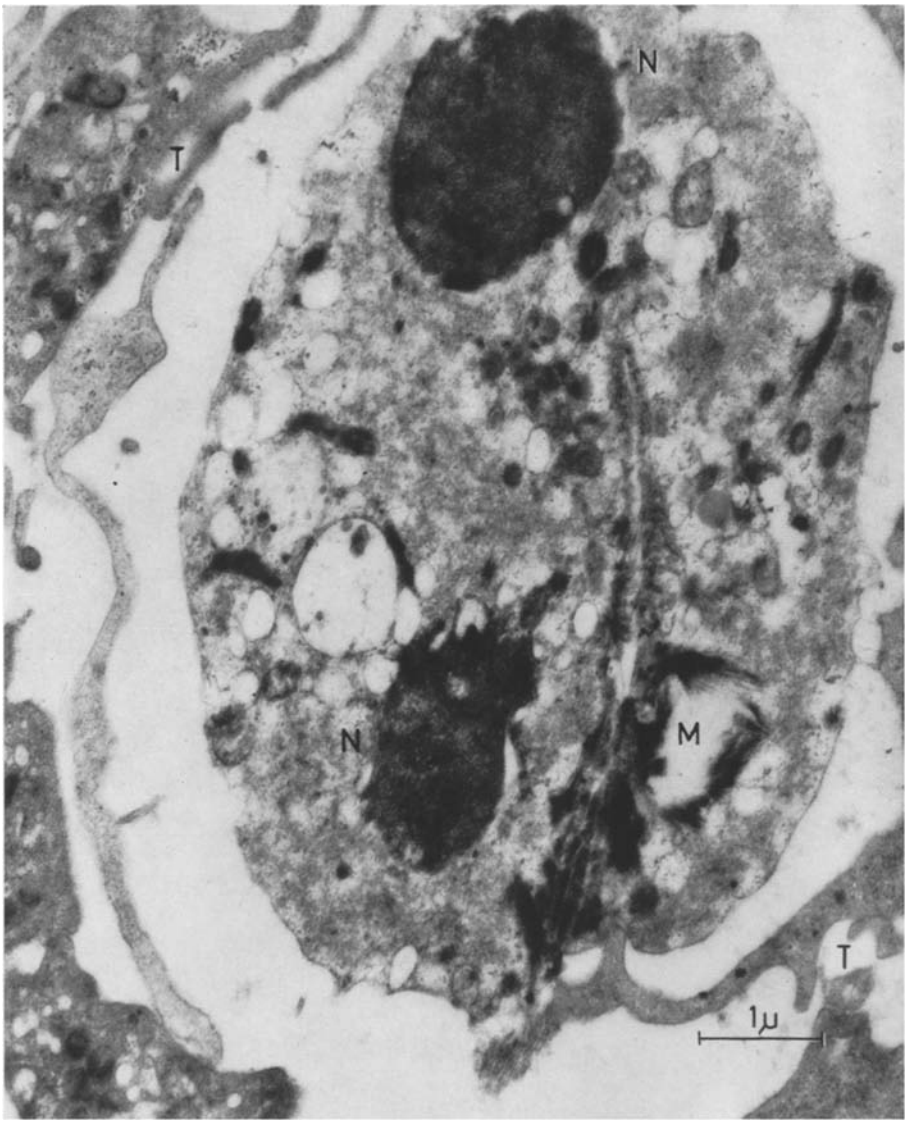

Fig. 7. Malignant melanoma cell isolated from the blood. The cytoplasm shows nuclear fragments (*N*) resulting from pyknotic disintegrations and numerous vesicles. A lamellar pattern of melanosomes (*M*) is also recognizable. Cellular degeneration is extensive. There are several platelets (*T*) in the field. Electron micrograph: 7,900 × 2.5

Extensive ultrastructural changes were seen in the cells isolated from the peripheral blood. From our other studies (SUGÁR, in prep.) these changes proved not to be artefacts produced by manipulations in the procedure of isolation. Disintegration of the malignant cells proceeds *in vivo*, beginning possibly even earlier than cellular entrance into the blood-stream. Damage of the cells obviously

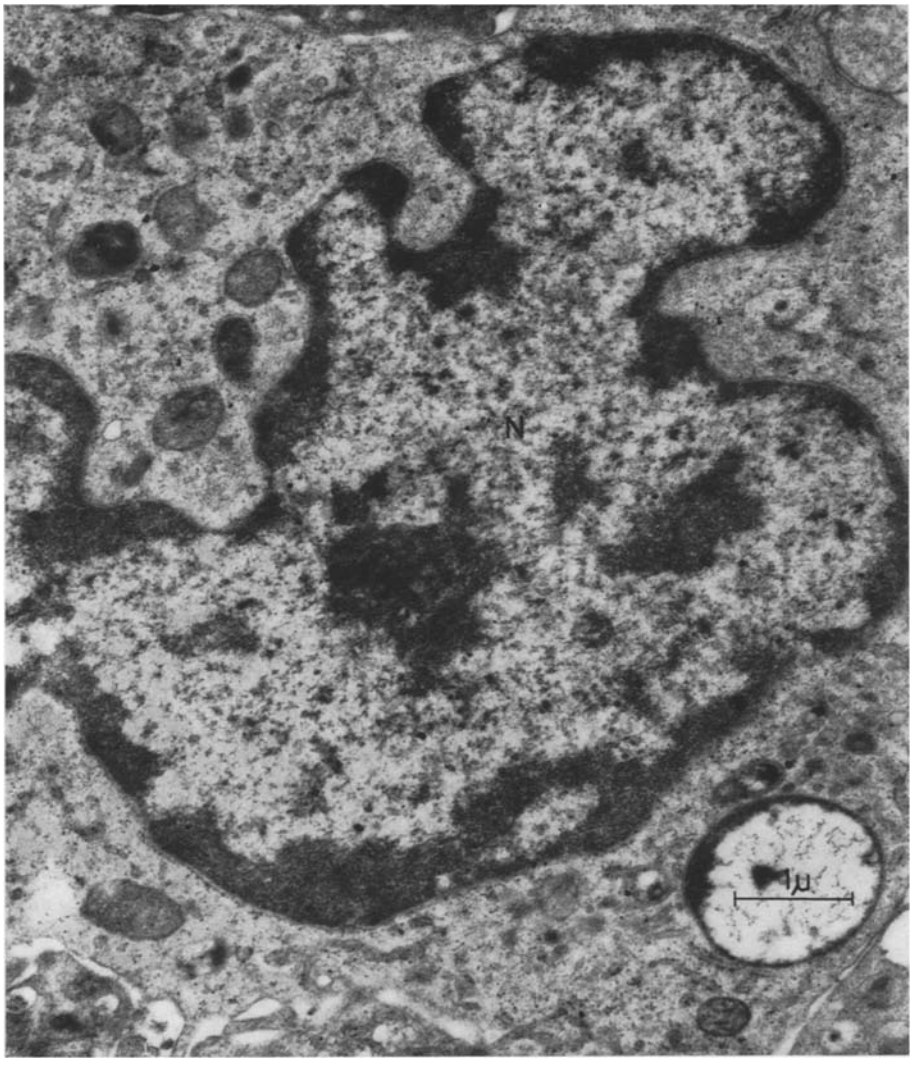

Fig. 8. Amelanotic tumour cell isolated from the blood. Enlarged, irregular nucleus (*N*) with cytoplasmic indentations. Electron micrograph: 7,500 × 2.5

interferes with their viability and growing potential. This is probably the reason why all malignant cells circulating in the blood-stream fail to give rise to haematogenous metastases.

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