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STRAINS OF HUMAN MELANOMA TRANSPLANTED INTO NUDE MICE AND RATS

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Various tumors have now been transplanted into nude mice and rats directly from man or after passage through tissue culture. Serial passage of these tumors in nude animals in some cases leads to the formation of tumor strains with stable characteristics. The obtaining of various transplantable strains of human tumors and, in particular, sets of strains of tumors identical in histogenetic features, is important for experimental cancer research.

This paper describes a series of strains of human melanoma in the collection of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR.

EXPERIMENTAL METHOD

Nude mice based on line BALB/c, 6-8 weeks old, and nude rats 4-6 weeks old, of our own breeding, were used. Human tumors obtained from operation material were transplanted subcutaneously into mice in the form of fragments. In one case human melanoma cell line MeWo was used for transplantation [4]. In this case 10^7 cells in 0.5 ml were injected subcutaneously into mice. A suspension containing 150 mg of human tumors, previously subjected to serial passage in mice, in 0.5 ml was injected subcutaneously into nude rats. In every case, both in nude mice and in nude rats after the second passage of the tumor, the suspension was transplanted serially and subcutaneously. The species to which tumors growing in nude animals belonged was determined by electrophoresis of lactate dehydrogenase in agar gel. Histological characteristics of the tumors were studied by the use of sections stained with hematoxylin and eosin, with picrofuchsin, and by the PAS reaction.

For electron-microscopic investigation the tumor tissue was fixed in a 2.5% solution of glutaraldehyde, postfixed in OsO_4 solution, dehydrated in alcohols of increasing concentration, and embedded in Epon 812 resin mixture. Semithin and ultrathin sections were cut on LKB-111 ultramicrotomes and the ultrathin sections were examined in the JEOL-100C electron microscope. Semithin sections were stained with toluidine blue, ultrathin with an aqueous solution of uranyl acetate and lead citrate.

EXPERIMENTAL RESULTS

Four strains of human melanoma transplantable into nude mice and rats were obtained; three strains (Mel-2, Mel-3, and Mel-5) were obtained from operation material.

Strain Mel-2, transplanted into nude mice and rats with an interval of 27-30 days, has undergone 53 passages. The tumor which was the source for this strain corresponded in structure to the typical picture of an alveolar-solid epithelial-like melanoma, producing pigment (Fig. 1a). Strain Mel-2 had an alveolar-lobular structure and consisted of large cells with

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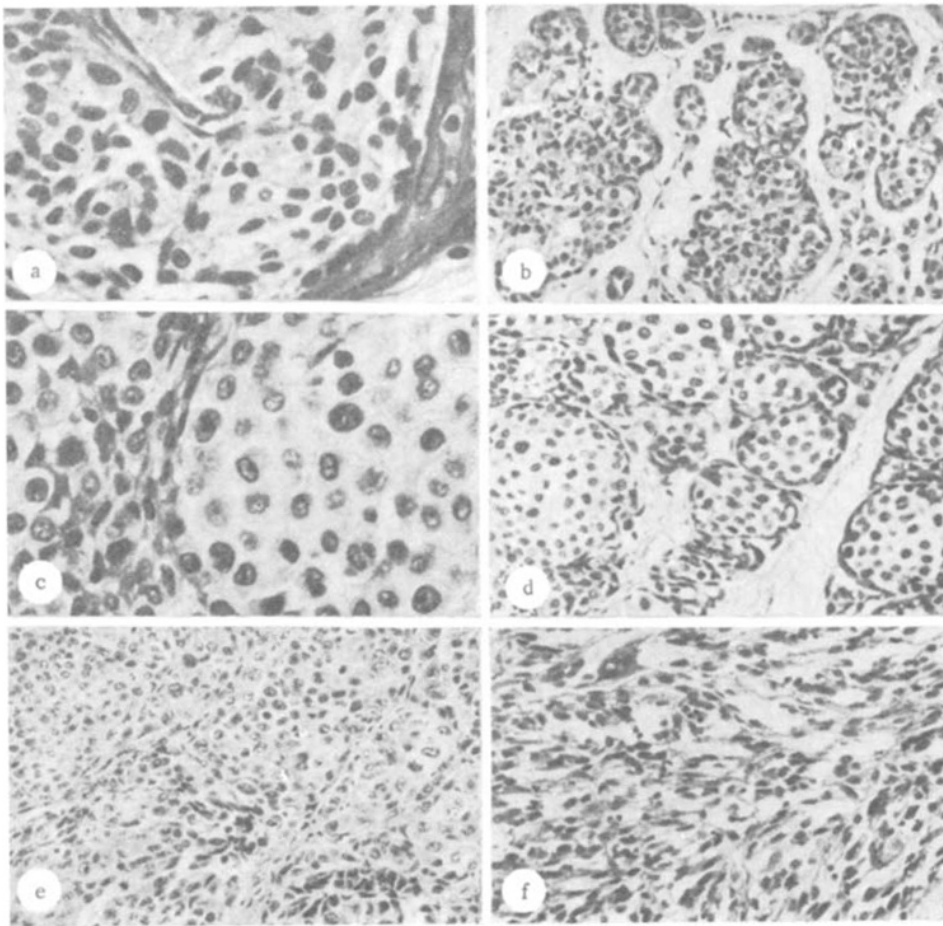


Fig. 1. Morphological picture of melanoma. a) Original tumor for strain Mel-2 (melanoma of alveolar-solid structure); b) strain of melanoma Mel-2, third generation (melanoma of alveolar-solid structure); c) original tumor for Mel-2 (melanoma of alveolar-solid structure); d) strain of melanoma Mel-3, 29th generation (melanoma of alveolar-solid structure); hematoxylin-eosin, 160 \times ; e) strain of melanoma Mel-5, second generation (melanoma of alveolar-solid structure); f) strain of melanoma Mel-1, 52nd generation (sarcoma-like melanoma). Staining: a, c-f) with hematoxylin and eosin; b) with picrofuchsin. Magnification: a, c) 400 \times , b, d-f) 160 \times .

pale cytoplasm, with no signs of melanin production when examined at the light-optical level (Fig. 1b). The original material for strain Mel-3 was a tumor with alveolar-solid structure, producing pigment (Fig. 1c). Strain Mel-3, transplanted after 22-24 days, in both early (6th-15th) and late (41st-56th) generations, is a melanoma consisting of pale polygonal cells, forming alveolar-lobular structures, separated by thin bands of connective tissue. No melanin production could be detected at the light-optical level (Fig. 1d). The source for strain Mel-5 was a tumor containing a large quantity of pigment. However, strain Mel-3, transplanted into nude animals after 16-18 days, contained no pigment in the very first passages when examined at the light-optical level. The tumors had an alveolar structure and were composed of atypical pale cells with vesicular nuclei and distinct nucleoli, they showed moderate cell polymorphism, and some giant cells with curiously shaped hyperchromic nuclei and many atypical mitotic figures were seen. The picture corresponded to a nonpigmented (epithelial-like) melanoma (Fig. 1a).

Strain Mel-1 originates from cell line MeWo. This strain was transplanted into animals after 15-18 days. It consists of densely intertwined bands of moderately polymorphic, fusiform cells with long, oval nuclei. Massive areas of necrosis of the tumor could be seen in the central parts of the nodule. At the light-microscopic level no signs of the presence of

pigment could be detected. This was the appearance of the tumor at the first passage. At the 83rd passage it still preserved its original structure (Fig. 1f).

Electron-microscopic investigation of the tissue of strain Mel-1, Mel-2, and Mel-3 at different passages showed that the structure of the tumors was identical as regards cell composition in all preparations studied. Poorly differentiated cells predominated. The cells were round or oval in shape and formed a continuous layer. In the cytoplasm there were many mitochondria, together with microfibrils and microtubules. The cell nuclei were oval, sometimes with deep invaginations of the nuclear membrane; the nucleoli were large and clearly formed. Cells with many microprocesses were visible. Sometimes there were junctions between the cells. Quite often cells were in a state of mitosis. Among undifferentiated cells, large electron-dense cells with many pigment granules in their cytoplasm were present. In areas with predominantly "dark cells" there was a fairly large quantity of material in the form of collagen fibers and amorphous material.

All four strains of human melanoma thus obtained, incidentally, are nonpigmented at the light-optical level. Although in these cases the sources of the strains were human tumors containing a large quantity of pigment, cells of the MeWo line also contain melanin [2]. In all four cases a sharp decrease in pigment production was observed in the cells of melanomas transplanted into nude mice obtained on the basis of line BALB/c, and in nude rats, which are albinos. Only on electron-microscopic investigation could a certain number of cells containing pigment granules be found in the transplants. We know from other investigations that in melanomas transplanted into nude albino mice the quantity of melanin is much smaller than in the original melanomas from a patient or from tissue culture [3]. On this basis it can be postulated that there is a deficiency of the enzyme tyrosinase, which is necessary for melanin formation [1], in melanomas transplanted into nude albino animals, or else that a certain factor inhibiting melanin formation is present.

All four strains of melanoma obtained, transplantable into nude mice and rats, consist mainly of human cells, as shown by the five peaks of the lactate dehydrogenase isozyme appearing on electrophoresis. These strains can be used successfully to compare the properties of human melanomas and for objective evaluation of various methods of their treatment, for the use of a set of strains of tumors of the same type enables the investigator to avoid the risk of mistaken conclusions due to the individual properties of single tumors.

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