Rapid melanization of Bomirski amelanotic melanoma cells in cell culture

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Transfer of Bomirski amelanotic melanoma cells from in vivo to in vitro growth conditions results in occurrence of rapid melanization in their cytoplasm. The melanized cells from primary cell culture initiate tumours in hamsters, which do not contain traces of melanin and resemble typical amelanotic melanoma.

Melanoma cells in tissue culture frequently show variations in their melanin content. If the cultured cells are not subjected to any treatment, the changes consist usually in a decrease or loss of melanization (Hu et al., 1967), whilst treatment with different factors may result in an increase in pigmentation (Silagi, 1969; Wong & Pawelek, 1973; Kreider et al., 1975; Giotta et al., 1978). All the tissue cultures examined by the above authors were initiated with the cells of melanotic melanomas, containing variable amounts of melanin. Clear-cut information is not available on how the system of melanogenesis would behave in cells from amelanotic melanomas after their transfer to in vitro growth conditions. One such tumour is the Bomirski hamster amelanotic melanoma (Bomirski A-Mel). It arose in 1963 by a spontaneous alteration of transplants of the Bomirski melanotic melanoma and since then has been maintained by serial passages on hamsters (Bomirski et al., 1966). The Bomirski A-Mel is a malignant tumour, i.e. dedifferentiated, fast-growing, and metastasizing. During 20 years of in vivo maintenance, melanin has never been detected in the Bomirski A-Mel by histological, histochemical, ultrastructural, or electron spin resonance (e.s.r.) spectroscopy methods (Bomirski, 1977; Zbytniewski et al., 1972). To be sure, melanin was found in the Bomirski A-Mel by means of a gravimetric method (Borovansky, 1978), but this result seems to be doubtful as it has not been confirmed by any other method of melanin detection. In as much as tyrosinase activity is present in this tumour, the absence of melanin is presumed to be caused by a lack of premelanosomes (Bomirski, 1977). When Bomirski A-Mel cells were placed in cell culture, a rapid melanization occurred unexpectedly. Description of this process is the subject of the present study.

Materials and Methods

The source of cells for primary cell cultures was the Bomirski A-Mel transplants belonging to the passages 401-408 of the Ab line. The cells were isolated from solid tumours by means of a non-enzymatic method. The viable tumour tissue was dissected free of
necrotic and connective tissue and rinsed several times in Eagle's Minimal Essential Medium (MEM; Biomed, Poland). Then the tissue was cut thoroughly with scissors, suspended in MEM, and filtered under positive pressure, exerted by means of a syringe, via a nylon mesh installed in place of a millipore filter in the Swin-Lok Membrane Holder (Millipore Corp., USA). The filtrate, containing the suspension of single cells, were centrifuged for 5 min at 800 g, and the pellet was resuspended in MEM, after which the cells were counted in a haemacytometer.

The primary cell cultures were started with $10^6$ cells, suspended in 10 ml of culture medium consisting of Eagle's MEM, supplemented with 10% calf serum (Biomed), 100 units/ml penicillin, and 100 µg/ml streptomycin, and placed in Petri dishes of 80 mm diameter. The cultures were kept in an incubator (Assab, Sweden) under the following conditions: 37°C, 5% CO₂ in air, 80% humidity. The culture medium was changed after 1 and 3 days of the culture. Each culture was run simultaneously in 3-10 Petri dishes to enable parallel examinations of cell morphology and measurements of e.s.r. In total 12 cell cultures were performed.

For histological examination the cells freshly isolated from solid tumours, those cultured in vitro, were centrifuged, and the pellet of the cells was fixed in Bouin's fluid and processed further by the paraffin method like a solid tissue. The cells growing as a monolayer were trypsinized prior to processing. Microtome sections of cell pellets were stained with haematoxylin and eosin, Fontana-Masson method for melanin (Bancroft, 1975), or mounted unstained for examination by phase-contrast microscopy.

The e.s.r. examinations were performed at room temperature using a Varian E-4 spectrometer, equipped with 100-kHz magnetic field modulation and working in X band (9.5 GHz). The sample of $4 \times 10^6$ cells/0.3 ml of 0.9% NaCl was placed in a quartz Aqueous Solution Sample Cell, type E-248, and inserted into the type TE-102 cavity. Free radical signals were recorded with a microwave power of 10 mW and a modulation amplitude of 8 G.

The ability to initiate tumour growth in animals by the pigmented cells from day 5 of the cell culture was assayed by subcutaneous reimplantation of $10^6$ cells into hamsters. The presence of melanin in the tumours was examined by histological and histochemical methods as mentioned above.

Results

In solid tumours and during the first 2 days of the culture no pigment was visible in the cells stained with haematoxylin-eosin or ammoniacal silver, or viewed by phase-contrast microscopy (Fig. 1). At day 3 in some cells brown pigment began to be visible in the melanoma cell cytoplasm, and on the following days it almost filled all the cells. The pigment became black after staining with ammoniacal silver according to the Fontana-Masson technique (Fig. 2). The increase in the pigmentation of the cells was accompanied by an intensive, spontaneous detachment of the cells from the glass sub-stratum.
Fig. 1. Bomirski amelanotic melanoma cells isolated from solid tumours and stained by the Fontana-Masson method for melanin. The cytoplasm of the cells is devoid of melanin. 630 x.

Fig. 2. Bomirski amelanotic melanoma cells from day 5 of cell culture, growing upon glass, trypsinized, and stained by the Fontana-Masson method for melanin. The cytoplasm of the cells contains melanin granules. 630 x.
The e.s.r. examination showed lack of free radical signal in the cells isolated from solid tumours and during the first 2 days of the cultures. By day 3, the signal appeared in the cells detached from and attached to the glass substratum (Fig. 3). In the following days the amplitude of the e.s.r. signal rose and reached maximum at day 5-6, being larger in the cells floating in the culture medium than those attached to the glass (Fig. 4).

Reimplantation of melanized cells from day 5 of the cell culture into 12 hamsters resulted in growth of tumours, which did not contain traces of melanin and resembled typical amelanotic melanoma.

Discussion

The above observations demonstrate that in the cytoplasm of Bomirski A-Mel cells grown in conditions of primary cell culture a brown pigment readily appears. The reaction of this pigment with ammoniacal silver and the parallel appearance of a free radical signal in the e.s.r. spectroscopy prove that the pigment is melanin (Bancroft, 1975; Blois, 1977).

At present, it is impossible to determine a direct cause of the melanization of Bomirski A-Mel cells cultured in vitro. So far, a similar process has not been found in any other transplantable amelanotic melanoma in which lack of melanin was proved by electron microscopy.
The Bomirski A-Mel arose from the Bomirski melanotic melanoma as a result of a mutation (Bomirski, 1977). Rapid melanization of Bomirski A-Mel cells in vitro indicates that this mutation involved a regulator gene or genes, but not the structural genes which were responsible for the process of melanin synthesis. Moreover, the melanization of Bomirski A-Mel cells in vitro and the ability of the melanized cells to initiate tumours of an appearance typical for the amelanotic melanoma prove that in vitro conditions derepress the biochemical apparatus responsible for the melanogenesis, which is repressed in vivo.

Melanin synthesis is a process of cell differentiation. The cells of the Bomirski amelanotic melanoma lost their ability to synthesize melanin 20 years ago and, since then, have continuously remained dedifferentiated in serial passages in vivo (Bomirski, 1977). The above-described restoration of melanin synthesis in the cells transferred to in vitro growth conditions indicates that such a fundamental property of the malignant cell as dedifferentiation may be reversible in some cases.
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References


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