Establishment of Pigmented Melanocyte Culture Strain from Harding-Passey Melanoma

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TAKEMATSU, H., MARUYAMA, T., KATAOKA, S. and SEIJI, M. Establishment of Pigmented Melanocyte Culture Strain from Harding-Passey Melanoma. Tohoku J. exp. Med., 134 (3), 339-340 — A cultured cell strain of melanotic melanoma was established from Harding-Passey mouse melanoma. The established cells proliferated as a monolayer culture and actively produced melanin pigment. Under an electron microscope, these cells showed the typical characteristic features of the melanocyte. When a cell suspension was inoculated into Swiss mice, they developed typical melanocytic tumors which closely resembled the original melanomas. —— mouse melanoma; pigmented strain; melanocyte culture strain.

Studies on melanogenesis and pigment degradation are facilitated in a cell culture permitting precise control of environmental conditions (Kato and Seiji 1980). Among the available cell lines established from mammalian melanoma, however, only a few retain the capacity for melanin pigment formation after a long cultivation in vitro. Harding-Passey melanoma has been used for the biochemical studies as melanin-forming cells (Seiji et al. 1966). The cultured cell line of melanotic Harding-Passey melanoma should no doubt be very useful for investigating the biological and immunological mechanisms of depigmentation processes in vitro. The present paper is to describe the procedure for establishing a pigmented cell strain and to report the morphological characteristics of the established strain.

The melanoma was excised from a Swiss mouse 21 days after transplantation. The tumor was cut into small pieces and treated with 0.5% trypsin in Hanks' solution. After rinsing in a growth medium (75% Ham's F12 + 25% fetal calf serum), the cell suspension was passed through a 50 μm pore filter and were dispersed in the growth medium. All cells were grown in the growth medium routinely in culture dishes (Falcon) in a humidified atmosphere of 5% CO₂ and 95% air at 37°C. During the culture, the pigmented cells increased gradually in number and they formed colony-like aggregates in fibroblasts in the bottom of the dishes. Using a micromanipulator attached to a phase contrast microscope, most of the fibroblasts were removed from the dishes. The pigmented cells were transferred to dishes by the micromanipulator and cultured in the growth medium. The same procedure was repeated three times. Thus, pure melanoma cells were obtained and cultured in the present culture system.

The mean population generation time was 4 days in the growth medium and it was maintained at almost constant rate. The melanogenic activity was well maintained in culture for 12 months with stable growth. This strain had an appearance shown in Fig. 1.
Pigmented cells mainly appeared on or near the net structure. Under an electron microscope, cultured cells possessed the characteristic features of melanocytes described by Seiji and Ohtaki (1971). Melanosomes, in various developmental stages, were scattered throughout the cytoplasm of the melanocytes (Fig. 2). The shape of melanosomes was roughly spherical. Melanin was deposited on the internal matrix of the melanosomes. When Swiss mice were given subcutaneous injections of cell suspension all the mice developed fully melanotic tumors. The histological sections of the original tumor and those of the tumors produced by the inoculated cultured cells appeared to be similar (Fig. 3a, b).

These results revealed that the newly established cell strain from Harding-Passey mouse melanoma still retained a pigment-forming ability after a long cultivation in our culture system. Even after cloning melanogenic cell lines, fluctuation and loss of pigmentation have been observed for some unknown reason (Oikawa et al. 1972). In this respect this cell strain should be very useful for studies on melanogenesis and pigment degradation, and the comparison with other melanoma cells in vitro may allow for analysis of melanosome formation.

References


