Short Report

Efficient Culturing of Human Melanocytes from Suction Blisters

YASUSHI TOMITA, HIROAKI YAMAMOTO, CHIKARA SATO, TAKUJI TAKEUCHI* and HACHIRO TAGAMI

Department of Dermatology, Tohoku University School of Medicine and *Biological Institute, Tohoku University, Sendai 980

TOMITA, Y., YAMAMOTO, H., SATO, C., TAKEUCHI, T. and TAGAMI, H. Efficient Culturing of Human Melanocytes from Suction Blisters. Tohoku J. exp. Med., 1985, 147 (2), 219-220 — The culturing of normal human melanocytes from the roof of suction blisters of adult oriental volunteers was carried out. Since the epidermal roofs of blisters did not contain any fibroblasts, and since keratinocytes did not attach to the culture dishes in the presence of PMA (phorbol 12-myristate 13-acetate), many melanocytes were obtained which grew well in the presence of PMA. This method is a very simple and easy way to establish pure melanocyte cultures.

Melanocytes from normal human skin were not successfully cultured until the recent discovery that PMA supported the proliferation of normal melanocytes in vitro (Eisinger and Marko 1982). Contamination with fibroblasts, however, remained a formidable obstacle to the establishment of pure melanocyte cultures from whole skin. Eisinger and Marko tried to separate melanocytes from fibroblasts by preferential detachment of fibroblasts after a short trypsinization (Eisinger and Marko 1982). Halaban and Alfano reported that geneticin could selectively kill contaminating fibroblasts in cultures of human melanocytes (Halaban and Alfano 1984). However, the yield of pure melanocytes was very low by either of the above procedures. We describe here an easy method for establishing pure melanocytes in culture, in high yield, without using cloning methodology or cholera toxin.

Pure epidermis was aseptically obtained from the roof of suction blisters on the forearms of oriental volunteers. Suction blisters were produced using 1.5 cm-diameter syringes connected to a vacuum pump; pressure within the syringe was maintained at 200 mm Hg below atmospheric pressure for about 1.5 hrs. Two or three epidermal roofs from blisters were incubated in 0.5% trypsin at 37°C for 30 min. Fetal calf serum (GIBCO Laboratories, Grand Island, NY, USA) was added to stop the trypsin action, and a single-cell suspension was prepared by pipetting. The epidermal cells were collected and were placed in tissue culture flasks (25 cm², Falcon Oxnard, CA, USA), and grown in Eagle’s minimal essential medium (GIBCO Laboratories, Grand Island, NY, USA) 10% fetal calf serum and 10 ng/ml PMA (Sigma Chemical Company, St. Louis, Mo, USA). The cells were kept in a humidified incubator with 5% CO₂ at 37°C.

Dendritic melanocytes (about 1 × 10⁵ cells from one blister) attached to the dishes within two days after plating, and unattached cells were removed by changing the medium every 3 or 4 days. Usually the epidermal roofs of suction blisters contain melanocytes and keratinocytes but not any fibroblasts, since blister formation occurs at the dermoeipidermal...

Received May, 24 1985; accepted for publication July 8, 1985.
junction (Kistala and Mustakallio 1967). We never used the roofs from bleeding suction blisters, since fibroblast contamination has occurred when we used bleeding ones. Keratinocyte attachment to the culture dishes was completely inhibited by PMA as already reported (Eisinger and Marko 1982), and melanocytes grew well as shown in the figure.

We have repeatedly obtained pure melanocytes from the epidermal roofs of suction blisters, and the yield of pure melanocytes by our method was far higher than the methods already reported. This simple method of culturing melanocytes will greatly contribute to research in dermatology and melanocyte biology.

References

