Culture of Nail Matrix Cells

Jae Hak Yoo, M.D., Hee Chul Eun, M.D.*, Yoon Ho Lee, M.D.**

Department of Dermatology, Kangbuk Samsung Hospital
Department of Dermatology and Plastic Surgery**, College of Medicine, Seoul National University, Seoul, Korea*

Background **: In vitro epidermal keratinocytes(EKs) culture systems are widely used in studying skin biology and differentiation. Recently a monolayer culture method of nail matrix cells(NMCs) has been developed.**

Objective **: This study was designed to establish a method of culture of NMCs and compared its characteristics with those of EKs.**

Methods **: Human NMCs were isolated and cultured in a defined medium. Phase contrast microscopic examination, growth rate and ketatin expression were evaluated.**

Results : In in vitro monolayer culture, NMCs had a similar growth pattern and morphological features as EKs. The synthesis of hard keratins was detected.

Conclusion **: These observations suggest that the NMCs culture model may be useful for the study of nail properties and the effects of drugs. (Ann Dermatol 9:(4):242~245, 1997)**

Key Words : Hard keratins, Nail matrix cells

Nail matrix is a specialized germinative epithelial structure that gives rise to a fully keratinized multilayered sheet of cornified cells, the nail plate. Nail matrix keratinization differs from epidermal keratinization because nail matrix cells(NMCs) undergo complete keratinization without formation of keratohyalin granules; cell differentiation occurs in the keratogenous zone[1].

The growth of isolated NMCs may permit investigation on cell differentiation as well as nail diseases and activity of different drugs. Kitahara et al.[2] cultured NMCs and clarified that NMCs are composed of two types of keratinocytes; one produces both "hard" or hair-type and "soft" or skin-type keratins, and the other, only soft keratin. Since then, the cellular characteristics of NMCs have been studied using cultured cells[3-5], but there has been no report of culture of human NMCs in Korea.

In this study, human NMCs and epidermal keratinocytes(EKs) were cultured and biologic properties of both cells were compared by phase contrast microscopic examination, evaluation of growth characteristics and keratin synthesis pattern.

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Reprint request to : Jae Hak Yoo, M.D., Department of Dermatology, Kangbuk Samsung Hospital, 108, Pyung-Dong, Jongro-Ku, Seoul, Korea 100-634 Tel : 82-2-739-3211 Fax : 82-2-737-1186

MATERIALS AND METHODS

Medium and culture conditions

NMCs and EKs were cultured in keratinocyte growth medium(KGM, Clonetics Corp., San Diego, Calif., USA) composed of MCDB 153 medium supplemented with epidermal growth factor (10 ng/ml), bovine pituitary extract (70 μg/ml), hydrocortisone (0.5 μg/ml), 0.15 mM Ca, insulin (5 μg/ml) and antibiotics. Cells were grown in 35 mm plastic culture dishes at 37°C in a humidified 5% CO_2/95% air atmosphere.

Primary cultures

Nail matrices were obtained from amputated fingers of patients with polydactyly under operative microscopy and human epidermis were obtained from foreskins. Nail matrix specimens were cleaned of excessive nail cornified layers and epidermis. Nail matrix and epidermis were washed twice in phosphate buffered saline(PBS), plus an-

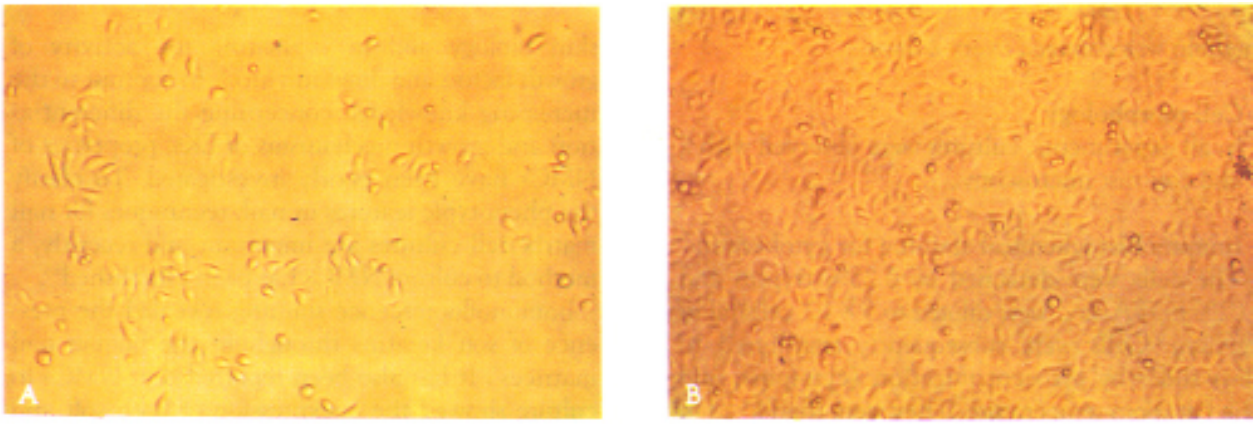

Fig. 1. NMCs in a dispersed cell culture after third(A, ×100) and tenth(B, ×100) day.

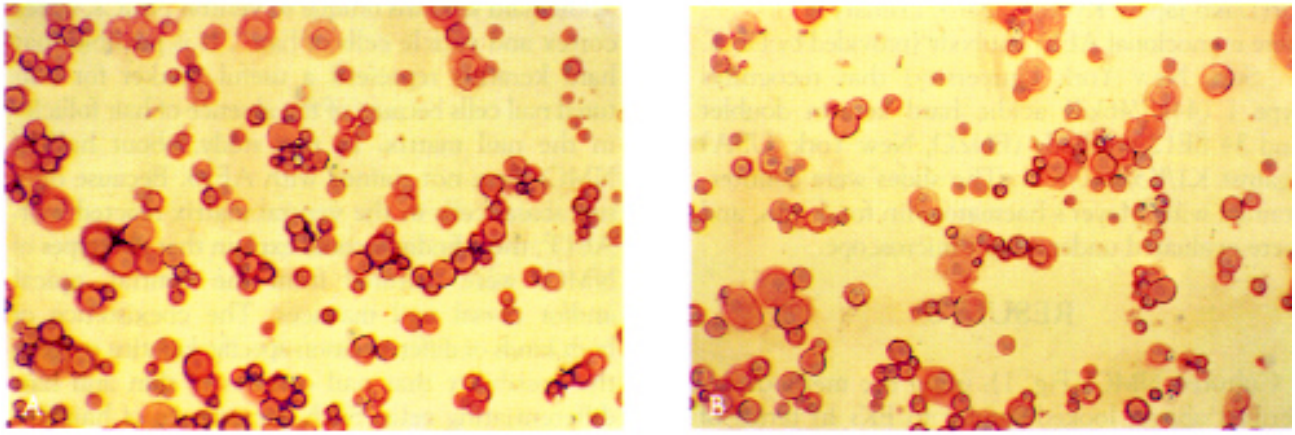

Fig. 2. Cytocentrifuge preparation of cultured NMCs(A, ×100) and EKs(B, ×100) stained with 34 ßE12. Nearly 100% of both cells are stained.

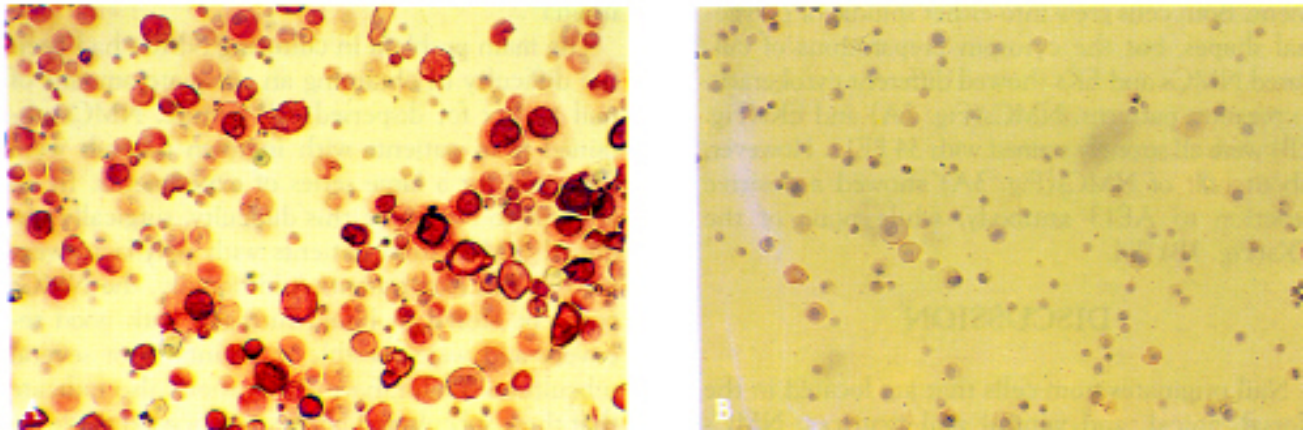

Fig. 3. Cytocentrifuge preparation of cultured NMCs(A, ×100) and EKs(B, ×100) stained with AE13. About half of NMCs were positive reaction with AE13 antibody, while any EKs did not stained.

tibiotics, cut in small pieces and cells isolated by subsequent changes in 0.05% trypsin/0.02% EDTA solution in PBS for 30 minutes at 37°C with shaking. The cells released were plated into culture dishes and cultured in KGM. When the cells formed colonies about 3 mm in diameter, they were passaged. The

medium was changed every 2 days.

Cell morphology

In all experiments, cultures were observed with a phase contrast microscopy.

Immunohistochemical staining for cytokeratins

The cells were detached by 0.25% trypsin and 0.02% EDTA, and were diluted to 5×10^4 cells/ml in medium. Cells were subsequently transferred to glass slides by cytocentrifugation (cytospin), and dried at room temperature. They were then fixed in cold acetone for 10 min, and processed for immnohistochemical staining using the LSAB kit(Dako Japan, Kyoto, Japan). Primary antibodies were monoclonal AE13 antibody (provided by Dr. T. T. Sun, New York University) that recognizes type I (44kd/46kd) acidic hard keratin doublet and 34 ßE12 antibody (ENZO, New York, USA) against K1/K5/K10/K11. The slides were counterstained with Mayer's haematoxylin for 5 min, and were evaluated under a light microscope.

RESULTS

Cultured NMCs(Fig. 1), using the methods described above, looked similar to EKs in terms of morphological features or in growth pattern. Cultured NMCs and EKs both proliferated rapidly and formed colonies with a paving stone like arrangement. Both cells grew into either spindle or polygonal shapes, but the cytospin preparations of cultured NMCs and EKs showed different cytokeratin expression patterns. NMCs(Fig. 2A) and EKs(Fig. 2B) were all strongly stained with 34 ßE12. However, about half of NMCs(Fig. 3A) showed a positive reaction to AE13 antibody, while none of the EKs(Fig. 3B) did.

DISCUSSION

Nail originates from cells that are located in the dorsal, apical, and ventral nail matrices. NMCs differentiate terminally into a hard keratin rich nail plate without formation of keratohyalin granules in the tissue[1]. NMCs, as well as EKs, divided in the basal cell layer and finally developed into cornified cells that are composed mainly of keratin filaments, filament matrix proteins[6].

Cultures of EKs are commonly used in studing skin biology and in evaluating the activity of growth factors and drugs in vitro[7,8]. In contrast to the increasing knowledge concerning the differentiation and growth mechanisms of EKs, properties of NMCs have been poorly investigated. To clarify the phenotypic features in nail, techniques for nail matrix cell cultures are important and recently, a method to culture NMCs has been established[2,5].

Immunofluorescence staining revealed the presence of soft keratins throughout the human nail matrices[9]. It has also been reported that NMCs in culture showed the coexpression of both soft and hard keratins[4]. Monoclonal murine antibody AE13 has been shown to specifically recognize the acidic hard keratins unique to ventral NMCs, upper cortex and cuticle cells of hair[10]. It is possible that hard keratins represent a useful marker for cultured nail cells because of the absence of hair follicles in the nail matrix. In this study, about half of NMCs were not stained with AE13. Because only suprabasal cells in the ventral matrix reacted with AE13[2], these findings could explain that two types of NMCs were cultured from the ventral, apical and/or dorsal nail matrices. The coexistence of both kinds of differentiation-specific keratins suggests the possibility that nail consists of skin and hair differentiating cells. Such coexistence of hair differentiation-specific keratins together with other epithelial keratins has also been described in reference to the hair follicle[11,12] and dorsal tongue epithelia[13].

The main problem in culturing NMCs had been the difficulty in obtaining an adequate amount of nail matrix for dispersed cell culture. NMCs obtained from patients with ingrown toenails were too small for a large series of experiments. In an attempt to overcome this difficulty, surgically obtained NMCs from patients with polydacty were used in this experiment. Additionally, the NMCs could be stored in liquid nitrogen with good recovery and were possible to maintain for several subcultures. Preliminary observations also indicate that these subcultured NMCs were a good model to evaluate the effects of drugs such as systemic antifungal agents(data in press).

Picardo et al.[4] reported that the cultured NMCs obtained from ingrown toenails appeared to be larger and had a higher growth rate than EKs. However, we have observed no significant difference in growth characteristics between NMCs ob-

tained from polydactyly and EKs in this study. We think this discrepancy may be due to the relative low degree of proliferation activity of polydactyly nail matrix.

In this study, it was shown that the culture of NMCs represents a reproducible system that may be useful in the study of the growth and differentiation of NMCs. Therefore this culture system could be a useful model to evaluate the effect of drugs and for studying the differentiation and biology of the nail in vitro.

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