Experimental

Effects of Subepithelial Fibroblasts on Epithelial Differentiation in Human Skin and Oral Mucosa: Heterotypically Recombined Organotypic Culture Model

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The stratified squamous epithelia differ regionally in their patterns of morphogenesis and differentiation. Although some reports suggested that the adult epithelial phenotype is an intrinsic property of the epithelium, there is increasing evidence that subepithelial connective tissue can modify the phenotypic expression of the epithelium. The aim of this study was to elucidate whether the differentiation of cutaneous and oral epithelia is influenced by underlying mesenchymal tissues. Three normal skin samples and three normal buccal mucosa samples were used for the experiments. Skin equivalents were constructed in four ways, depending on the combinations of keratinocytes (cutaneous or mucosal keratinocytes) and fibroblasts (dermal or mucosal fibroblasts), and the effects of subepithelial fibroblasts on the differentiation of oral and cutaneous keratinocytes were studied with histological examinations and immunohistochemical analyses with anti-cytokeratin (keratins 10 and 13) antibodies. For each experiment, three paired skin equivalents were constructed by using single parent keratinocyte and fibroblast sources for each group; consequently, nine (3 × 3) organotypic cultures per group were constructed and studied. The oral and cutaneous epithelial cells maintained their intrinsic keratin expression. The keratin expression patterns in oral and cutaneous epithelia of skin equivalents were generally similar to their original patterns but were partly modified exogenously by the topologically different fibroblasts. The mucosal keratinocytes were more differentiated and expressed keratin 10 when cocultured with dermal fibroblasts, and the expression patterns of keratin 13 in cutaneous keratinocytes cocultured with mucosal fibroblasts were different from those in keratinocytes cocultured with cutaneous fibroblasts. The results suggested that the epithelial phenotype and keratin expression could be extrinsically modified by mesenchymal fibroblasts. In epithelial differentiation, however, the intrinsic control by epithelial cells may still be stronger than extrinsic regulation by mesenchymal fibroblasts. (*Plast. Reconstr. Surg.* 112: 784, 2003.)

The stratified squamous epithelia covering or lining body surfaces differ regionally in their patterns of morphogenesis and differentiation, and regionally specific structural patterns persist throughout life. Some reports suggested that the adult epithelial phenotype was an intrinsic property of the epithelium.[1,2] However, in heterotypic recombination experiments, it was demonstrated that subepithelial connective tissue could modify the phenotypic expression of the transplanted epidermis.[3] Therefore, the intrinsic epithelial phenotype may be modified by subepithelial connective tissue even after birth.

Similarly, site-specific keratin expression is considered to be endogenously rather than exogenously regulated,[4] although there is increasing evidence that keratinocyte differentiation is also regulated by mesenchymal factors.[5–9] It was reported that production of palmoplantar-specific keratin (keratin 9) by nonpalmoplantar keratinocytes was induced by cocultured palmoplantar fibroblasts.[6] Using organotypic cultures, we demonstrated that production of nail matrix-specific hard keratin by non–nail-matrical keratinocytes was induced by cocultured nail-matrical fibroblasts.[9]

The possible use of mucosal epithelial cultures as a source of epithelial sheets for skin repair was reported,[10,11] and more recently, the

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use of prefabricated flaps of oral mucosa or tissue-engineered mucosa for the reconstruction of intraoral defects was reported.[12–16] These methods were generally successful, but the question of the cellular origin of regenerated epithelia and the question of whether the differentiation of regenerated epithelia is influenced by underlying mesenchymal tissues remain. The aim of this study was to elucidate the effects of subepithelial fibroblasts on the differentiation of oral or cutaneous keratinocytes.

The epidermal pattern of orthokeratinization is accompanied by the suprabasal expression of keratins 1 and 10,[17–19] whereas in nonkeratinized sites, such as buccal mucosa, the predominant suprabasal keratins are keratins 4 and 13.[20,21] In this study, we examined whether the expression patterns of site-specific cytokeratins could be modified by cocultured fibroblasts, with immunohistochemical analyses of heterogeneous organotypic cultures with anticytokeratin (keratins 10 and 13) antibodies.

MATERIALS AND METHODS

Cell Isolation and Cell Cultures

Three normal abdominal skin samples and three normal buccal mucosa samples were obtained during plastic surgical procedures (Fig. 1, *above*). Informed consent was obtained from all patients. Human keratinocytes and buccal mucosal epithelial cells were cultured and treated separately, with a modification of the method reported previously.[9,22] Briefly, the specimens were washed three times with phosphate-buffered saline, finely shredded with scissors, and incubated with 0.25% trypsin and 0.02% ethylenediaminetetraacetic acid, in phosphate-buffered saline, for 16 to 24 hours at 4°C. The epithelium was separated from the dermis (or mucosal lamina propria) with forceps and was isolated from the subepithelial side. Keratinocytes and mucosal epithelial cells were grown in a modified, serum-free, keratinocyte growth medium (Kyokuto Seiyaku, Tokyo, Japan), which consisted of MCDB153 with high concentrations of amino acids, transferrin (final concentration, 10 μg/ml), insulin (5 μg/ml), hydrocortisone (0.5 μg/ml), phosphorylethanolamine (14.1 μg/ml), and bovine pituitary extract (40 μg/ml). The final concentration of Ca^{2+} in the medium was 0.03 mM. Human fibroblasts were isolated from separated subepithelial tissue (dermis and mucosal lamina propria) and grown in fibroblast growth medium, which consisted of Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 0.6 mg/ml glutamine.

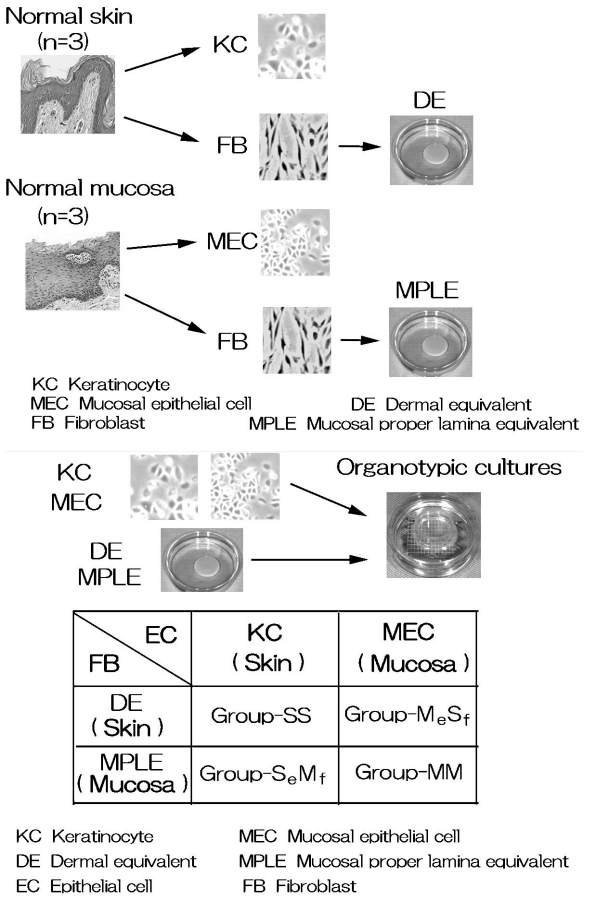

FIG. 1. (*Above*) Design of the study. (*Below*) Design of the organotypic cultures.

Organotypic Cultures

Organotypic cultures were prepared by using the method reported previously.[9] Epithelial cells were cultured in a three-dimensional manner at the air-liquid interface on top of a dermal equivalent consisting of type I collagen and fibroblasts (Fig. 1, *below*). The third cultures of fibroblasts were used for experiments. Dermal equivalents (and mucosal lamina propria equivalents) were constructed by casting fibroblasts into pigskin type I collagen solution and pouring the cells into a 60-mm Petri dish, at 10 ml/dish. The solution was allowed to gel and contract for 7 days. The final concentrations of collagen and fibroblasts were 1 mg/ml and 120,000 cells/ml, respectively.

Preconfluent third cultures of keratinocytes and mucosal epithelial cells were trypsinized

and seeded at 400,000/cm^2, inside a glass ring (16 mm in diameter), on the surface of dermal equivalents (or mucosal lamina propria equivalents). Organotypic cultures were maintained in 60-mm tissue culture dishes supplemented with 10 ml of medium (a 1:1 mixture of keratinocyte growth medium and Dulbecco's modified Eagle's medium plus 10% fetal calf serum, in which the Ca^{2+} concentration was adjusted to 1.8 mM). From the fourth day, the medium was reduced to the level of the epithelial cell sheet, so that the epithelial cells were grown at the air-liquid interface. Every other day, the medium was removed and replaced with fresh medium.

Organotypic cultures were constructed in four ways, with different combinations of two fibroblast lines and two keratinocyte lines. Mucosal epithelial cells were cocultured with fibroblasts from mucosal lamina propria (group MM), mucosal epithelial cells were cocultured with skin fibroblasts (group M_eS_f), keratinocytes from skin were cocultured with fibroblasts from mucosal lamina propria (group S_eM_f), and keratinocytes were cocultured with skin fibroblasts (group SS). The specimens were harvested at 2 weeks, and histological and immunohistochemical examinations were performed. Three normal skin samples and three normal buccal mucosa samples were used for the experiments. For each experiment, three paired skin equivalents were constructed by using single parent keratinocyte and fibroblast sources for each group; consequently, nine (3 × 3) organotypic cultures per group were constructed and studied.

Histological Examinations

Skin and mucosa equivalents (and normal skin and mucosa samples, as control specimens) were fixed in 4% paraformaldehyde and embedded in paraffin according to standard techniques. Tissues were mounted in blocks, cut into 4-μm vertical sections, and stained with hematoxylin and eosin. The sections were mounted and observed with a microscope (Microphot-FXA; Nikon Corporation, Tokyo, Japan). A specimen was defined as orthokeratinized when more than two layers of stratum corneum, with denucleation, were observed in the epithelium.

Immunohistochemical Examinations

For immunohistochemical staining, mouse monoclonal antibodies to human keratin 10 (keratin RKSE60) and keratin 13 were purchased from ICN Pharmaceuticals (Costa Mesa, Calif.) and Sigma-Aldrich (St. Louis, Mo.), respectively. The specimens were frozen in liquid nitrogen and stored at −70°C until used. Frozen sections (5-μm thick) were prepared in a cryostat at −30°C and were examined with an indirect biotin-avidin-horseradish peroxidase method. The sections were lightly counterstained with hematoxylin, mounted, and observed with a microscope (Nikon Microphot-FXA). In negative control samples, phosphate-buffered saline was substituted for the primary antibody.

RESULTS

Histological Examinations with Hematoxylin and Eosin Staining

Normal skin and normal buccal mucosa. In all normal mucosa samples, intact mucosa covered by nonkeratinized, stratified, squamous epithelium was observed. Orthokeratinization was not evident in any of the specimens. In all skin specimens, intact skin covered by orthokeratinized, stratified, squamous epithelium was observed. The epithelium was more stratified and epithelial cells were smaller in buccal mucosa than in skin (Fig. 2, *above*, *right* and *left*).

Organotypic cultures. The numbers of specimens defined as orthokeratinized or nonkeratinized are presented in Table I. The epithelia were composed of smaller epithelial cells (mucosal epithelial cells) and were more stratified in groups MM and M_eS_f, compared with groups S_eM_f and SS (Fig. 2, *center* and *below*). In group MM, nine of nine organotypic cultures demonstrated many layers but lacked terminal differentiation of the stratum corneum. In groups S_eM_f and SS, however, five of nine organotypic cultures supported a well-differentiated epithelium with a stratum corneum. In group M_eS_f, a well-differentiated epithelium with a denucleated stratum corneum was observed in three of nine organotypic cultures, although mucosal epithelial cells were used as the epithelial component (Fig. 2, *center*, *right*).

Immunohistochemical Examinations with Anti-Keratin 10

Normal skin and normal mucosa. In the negative control samples (in which phosphate-buffered saline was substituted for the primary antibody), positive staining was not observed. Antibody to keratin 10 demonstrated a consis-

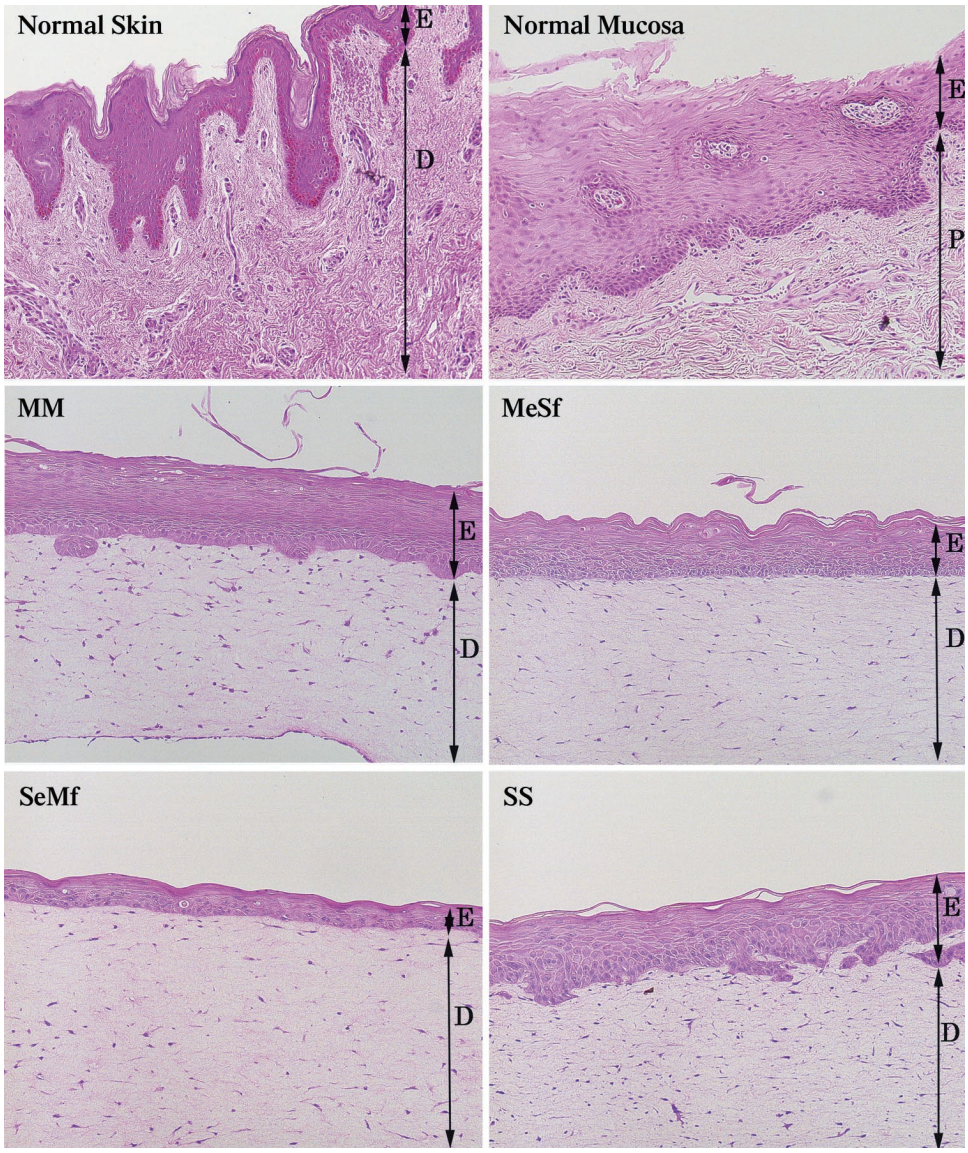

FIG. 2. Histological assessment of normal skin, normal buccal mucosa, and organotypic cultures, with hematoxylin and eosin staining. *E*, epithelium; *D*, dermis (*above, left*) or dermal equivalent (*center, left* and *right*, and *below, left* and *right*); *P*, mucosal lamina propria. (*Above, left*) Normal skin. Intact skin covered by well-keratinized, stratified, squamous epithelium with rete process was observed. (*Above, right*) Normal buccal mucosa. Intact mucosa covered by nonkeratinized, stratified, squamous epithelium was observed, and orthokeratinization was not evident. The epithelium was more stratified and epithelial cells were smaller in buccal mucosa than in skin. The epithelia were composed of smaller epithelial cells (oral keratinocytes) and were more stratified in groups MM and M_eS_f (*center, left* and *right*) than in groups S_eM_f and SS (*below, left* and *right*). (*Center, left*) Group MM. All organotypic cultures had many layers but lacked terminal differentiation of the stratum corneum. (*Center, right*) Group M_eS_f. Well-differentiated epithelium with a denucleated stratum corneum was observed in three of nine organotypic cultures. (*Below, left*) Group S_eM_f. Five of nine organotypic cultures supported a well-differentiated epithelium with a stratum corneum. (*Below, right*) Group SS. Five of nine organotypic cultures supported a well-differentiated epithelium with a stratum corneum. Magnification, ×100.

tent pattern of suprabasal staining in normal skin samples, whereas positive staining was not observed in any normal buccal mucosa samples (Fig. 3, *above, left* and *right*).

Organotypic cultures. In all organotypic cultures in groups SS and S_eM_f, strong staining was observed throughout the suprabasal layer (Fig. 3, *below, left* and *right*). Positive staining was not

TABLE I
Keratinization of Specimens

Group	No. of Specimens	
	Orthokeratinized	Nonkeratinized
Normal skin	3/3	0/3
Normal mucosa	0/3	3/3
Group MM	0/9	9/9
Group M_eS_f	3/9	6/9
Group S_eM_f	5/9	4/9
Group SS	5/9	4/9

observed in any specimen in group MM. In all specimens in group M_eS_f, positive staining was observed consistently in the suprabasal or upper layer, although mucosal epithelial cells were used as the epithelial component (Fig. 3, *center*, *left* and *right*).

Immunohistochemical Examinations with Anti-Keratin 13

Normal skin and normal mucosa. In the negative control samples (in which phosphate-buffered saline was substituted for the primary antibody), positive staining was not observed. Antibody to keratin 13 demonstrated a consistent pattern of basal staining in normal skin samples, whereas positive staining was distributed throughout the suprabasal layer in normal buccal mucosa samples (Fig. 4, *above*, *left* and *right*).

Organotypic cultures. Antibody to keratin 13 demonstrated a consistent pattern of suprabasal staining in all organotypic cultures in group MM, whereas positive staining was observed in the basal and upper layers in all specimens in group M_eS_f (Fig. 4, *second row*, *left* and *right*). In group S_eM_f, positive staining was observed in three patterns, namely, in the upper and basal layers (Fig. 4, *third row, left*), in all epithelial strata (Fig. 4, *third row, right*), and in the suprabasal layer (Fig. 4, *below, left*). In all specimens in group SS, positive staining was observed in all epithelial strata (Fig. 4, *below, right*).

Discussion

This study demonstrated that the differentiation of epithelial cells could be modified by cocultured fibroblasts. The expression patterns of site-specific cytokeratins in oral and cutaneous keratinocytes were exogenously modified by the topologically different fibroblasts.

There are two keratin subfamilies; the type I keratins (keratins 1, 3, 4, 5, 6, and 8) are rather acidic, and the type II keratins (keratins 10, 12, 13, 14, 16, and 18) are neutral to basic.[23] The type I and type II keratins are expressed in pairs, with one particular keratin being coexpressed with one defined keratin of the complementary type.[24] The type I and type II keratins are maintained in equimolar amounts, although the appearance of a type II keratin may precede that of its partner during differentiation.[25] Keratins 1 and 4 are paired with keratins 10 and 13, respectively. Therefore, keratins 10 and 13 were used as differentiation markers in our study.

Normally, the buccal mucosa is nonkeratinized and keratin 13 is expressed suprabasally in the oral epithelium and basally in the epidermis. In the normal skin and buccal mucosa samples used in our study, keratin 13 was expressed in the normal patterns. Keratin 13 is not the ideal marker for our study, because it is observed in both cutaneous keratinocytes and mucosal epithelial cells. We used keratin 13, however, because the normal expression patterns of keratin 13 differ between buccal mucosa and skin and no other keratin was more suitable for our study. Keratin 10 is a specific marker of terminal differentiation in squamous epithelia and is expressed in the suprabasal layers of the epidermis. The expression of keratin 10 is not normally observed in buccal mucosa.[23,26] Several studies demonstrated that small subpopulations of suprabasal cells express keratin 10 in buccal mucosa, although the epithelium is not keratinized,[19,27] but keratin 10 was not expressed in the epithelium in any normal buccal mucosa samples used in our study.

The epidermis of skin equivalent is not identical to natural human epidermis; the distributions of some antigens differ, and differentiation markers appear in an altered form.[28] In group MM, with a combination of mucosal epithelial cells and mucosal fibroblasts, the epithelial phenotype (many layers, without terminal differentiation of the stratum corneum) and the expression patterns of keratins 10 and 13 were quite similar to those in normal buccal mucosa. In group SS, however, with a combination of cutaneous keratinocytes and fibroblasts, the expression pattern of keratin 10 was similar to that in normal skin but the expression pattern of keratin 13 was different; expression of keratin 13 was observed in all epithelial strata, and a denucleated stratum corneum was observed in only five of nine organotypic cul-

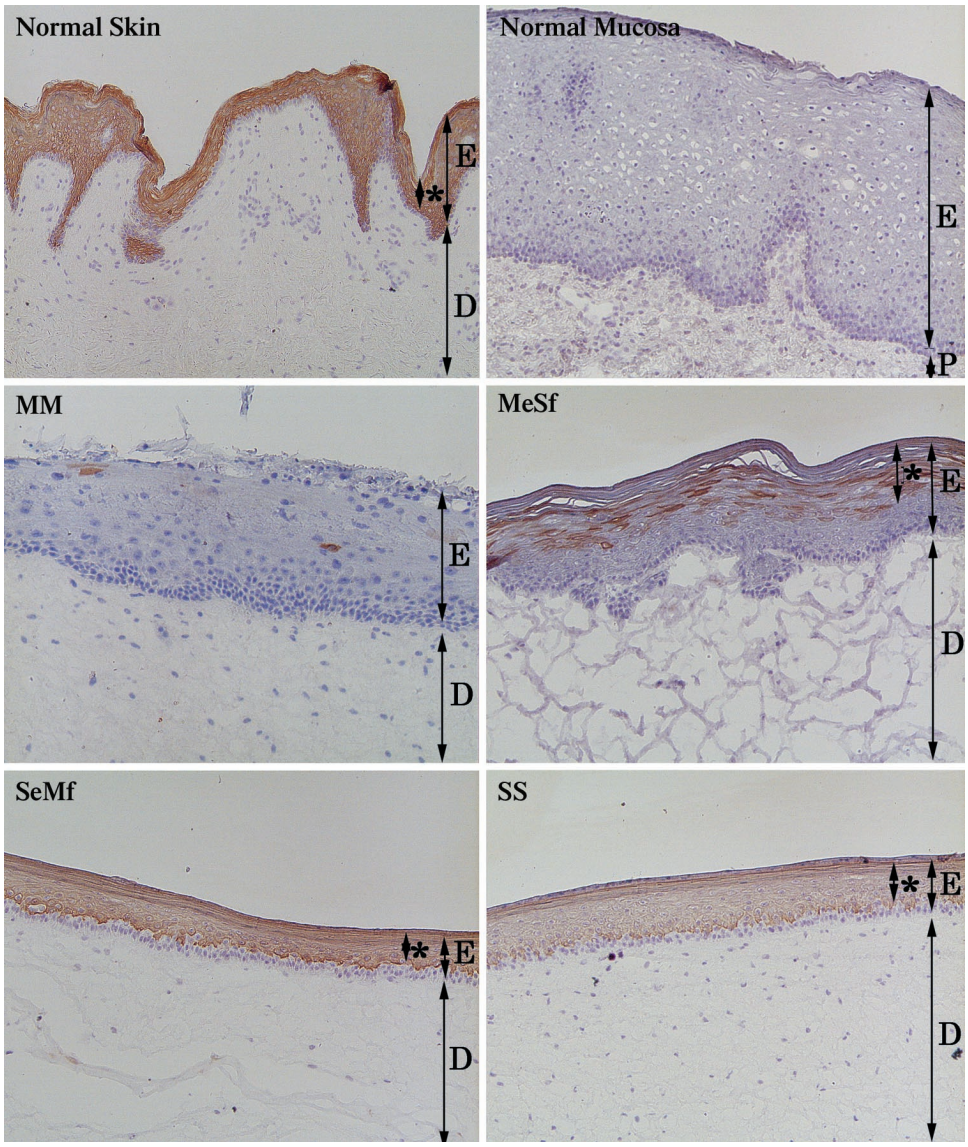

FIG. 3. Immunohistochemical assessments of normal skin and buccal mucosa and organotypic cultures with anti-keratin 10 antibody. *E*, epithelium; *D*, dermis (*above, left*) or dermal equivalent (*center, left* and *right*, and *below, left* and *right*); *P*, mucosal lamina propria (*above, right*). (*Above, left*) Normal skin. In normal skin samples, antibody to keratin 10 demonstrated a consistent pattern of suprabasal immunohistochemical staining (*asterisk*). (*Above, right*) Normal buccal mucosa. Positive staining was not observed in any normal buccal mucosa samples. (*Center, left*) Group MM. Positive staining was not observed. (*Center, right*) Group M_eS_f. Positive staining was consistently observed in the suprabasal or upper layer (*asterisk*). (*Below, left*) Group S_eM_f. Strong staining was observed throughout the suprabasal layer (*asterisk*). (*Below, right*) Group SS. Strong staining was observed throughout the suprabasal layer (*asterisk*). Magnification, ×100.

tures. In our laboratory, skin equivalents composed of cutaneous keratinocytes and dermal fibroblasts have been used for various studies, and the skin equivalents did not always support orthokeratinization under the conditions described above.

Although mucosal epithelial cells were used as the epithelial cells in group M_eS_f, moderately differentiated epithelium was observed. In particular, three of nine organotypic cultures demonstrated denucleated stratum corneum. Immunohistochemical examinations revealed keratin 10 expression in nine of nine specimens, although the expression patterns in group M_eS_f were somewhat different from those in groups SS and S_eM_f; in group M_eS_f, keratin 10 was expressed mostly in the granular layer and upper prickle cell layer, rather than

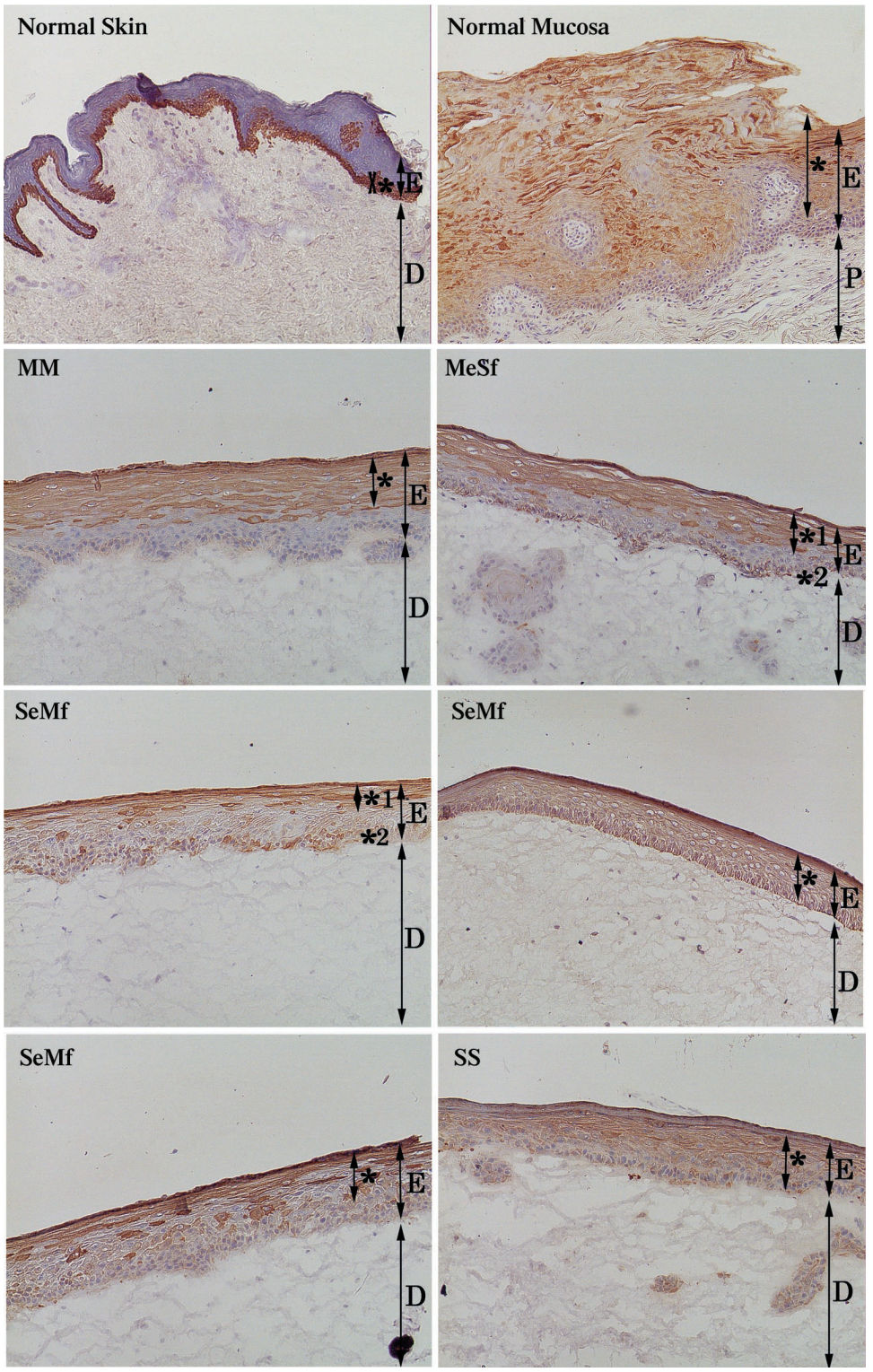

FIG. 4. Immunohistochemical assessments of normal skin, normal buccal mucosa, and organotypic cultures with anti-keratin 13 antibody. *E*, epithelium; *D*, dermis (*above, left*) or dermal equivalent (*second row, left* and *right*, *third row, left* and *right*, and *below, left* and *right*); *P*, mucosal lamina propria (*above, right*). (*Above, left*) Normal skin. Antibody to keratin 13 demonstrated a consistent pattern of basal staining (*asterisk*). (*Above, right*) Normal buccal mucosa. Positive staining was distributed throughout the suprabasal layer (*asterisk*). (*Second row, left*) Group MM. Antibody to keratin 13 demonstrated a consistent pattern of suprabasal staining (*asterisk*). (*Second row, right*) Group M_eS_f. Positive staining was observed in the upper (*asterisk 1*) and basal (*asterisk 2*) layers in all specimens. Group S_eM_f. Positive staining was observed in three patterns, in the upper (*asterisk 1*) and basal (*asterisk 2*) layers (*third row, left*), in all epithelial strata (*asterisk*) (*third row, right*), and in the suprabasal layer (*asterisk*) (*below, left*). (*Below, right*) Group SS. Positive staining was observed in all epithelial strata (*asterisk*). Magnification, ×100.

the suprabasal layer. These findings suggested that orthokeratinization, with keratin 10 expression, of mucosal epithelial cells was induced by dermal fibroblasts, although the extrinsically induced differentiation in group M_eS_f was less than the intrinsically controlled differentiation in group S_eM_f.

Histological examinations with hematoxylin and eosin staining revealed that the epithelial differentiation in group S_eM_f was comparable to that in group SS; the keratin 10 expression patterns were also similar. These findings suggest that dermal keratinocytes have an intrinsic property of orthokeratinization and express keratin 10 as they differentiate and that this intrinsic property has stronger effects on keratinization than does extrinsic control by fibroblasts. However, the expression patterns of keratin 13 were different in the two groups; keratin 13 was expressed in three patterns (in the upper and basal layers, in all epithelial strata, and in the suprabasal layer) in group S_eM_f, whereas positive staining was spread throughout the epithelial strata in all specimens in group SS.

In our study, three paired skin equivalents for each combination of an epithelial line and a fibroblast line were used, because it was not easy to judge digitally whether a skin equivalent was keratinized. Statistical analyses were not performed for the histological examination results, because the use of three paired specimens is not statistically valid and statistical analyses could not reasonably be performed. Therefore, the differences in the numbers of orthokeratinized specimens in the four groups were not statistically significant. The difference in epithelial layers in hematoxylin/eosin assessments between group MM and group M_eS_f was clearly observed, however.

As stated above, keratin 13 was expressed in both normal mucosa and skin, and the expression patterns of keratin 13 differed between normal skin and group SS. Therefore, the effects of fibroblasts on keratin 13 expression were not as clear as the effects on keratin 10 expression. The comparison between group SS and group S_eM_f did suggest that cocultured fibroblasts could extrinsically modify the keratin 13 expression pattern of keratinocytes, although the intrinsic factor was still stronger.

Ueda et al.[10] repaired donor sites for split-thickness skin grafts with cultured mucosal epithelial sheets. They reported that epithelialization was completed 28 days after grafting and that differentiated epithelium with orthokeratinization was observed in the grafted area. In their clinical study, the true source of regenerated epithelial cells could not be determined. It is possible that the grafted mucosal epithelial sheets were well differentiated by the action of dermal fibroblasts; however, it is also possible that well-differentiated epithelium was regenerated from skin appendages. The use of prefabricated flaps with oral mucosa or tissue-engineered mucosa for the reconstruction of intraoral defects was reported.[12–16] However, the question of whether the differentiation of epithelia is influenced by underlying mesenchymal tissues remains. In this study using organotypic cultures, the cells used were free of contamination. Therefore, our study demonstrated that the differentiation of epithelial cells could be influenced by underlying fibroblasts and that mucosal epithelial cells could be cornified when they were cocultured with dermal fibroblasts.

CONCLUSIONS

The epithelial phenotype and keratin expression could be extrinsically modified by mesenchymal fibroblasts. However, in epithelial differentiation, intrinsic control by epithelial cells may still be stronger than extrinsic regulation by mesenchymal fibroblasts.

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REFERENCES

1. Billingham, R. E., and Silver, W. K. Studies on the conservation of epidermal specificities of skin and certain mucosas in adult mammals. *J. Exp. Med.* 125: 429, 1966.
2. Doran, T. I., Vidrich, A. L., and Sun, T. T. Intrinsic and extrinsic regulation of the differentiation of skin, corneal and esophageal epithelial cells. *Cell* 22: 17, 1980.
3. Mackenzie, I. C., and Hill, M. W. Connective tissue influences on patterns of epithelial architecture and keratinization in skin and oral mucosa of the adult mouse. *Cell Tissue Res.* 235: 551, 1984.
4. Bohnert, A., Horming, J., Mackenzie, I. C., and Fusenig, N. E. Epithelial-mesenchymal interactions control basement membrane production and differentiation in cultured and transplanted mouse keratinocytes. *Cell Tissue Res.* 244: 413, 1986.
5. Konstantinova, N. V., Lemak, N. A., Duong, D. M. T., Chuang, A. Z., Urso, R., and Duvic, M. Artificial skin equivalent differentiation depends on fibroblast do-

nor site: Use of eyelid fibroblasts. *Plast. Reconstr. Surg.* 101: 385, 1998.
6. Yanguchi, Y., Itami, S., Tarutani, M., Hosokawa, K., Miura, H., and Yoshikawa, K. Regulation of keratin 9 in nonpalmoplantar keratinocytes by palmoplantar fibroblasts through epithelial-mesenchymal interaction. *J. Invest. Dermatol.* 112: 483, 1999.
7. Boukamp, P., Breitkreutz, D., Stark, H. J., and Fusenig, N. E. Mesenchyme-mediated and endogenous regulation of growth and differentiation of human skin keratinocytes derived from different body sites. *Differentiation* 44: 150, 1990.
8. Smola, H., Thiekotter, G., and Fusenig, N. E. Mutual induction of growth factor gene expression by epidermal-dermal interaction. *J. Cell Biol.* 122: 417, 1993.
9. Okazaki, M., Yoshimura, K., Fujiwara, H., Suzuki, Y., and Harii, K. Induction of hard keratin expression in non–nail-matrical keratinocytes by nail-matrical fibroblasts through epithelial-mesenchymal interactions. *Plast. Reconstr. Surg.* 111: 286, 2003.
10. Ueda, M., Hata, K., Horie, K., and Torii, S. The potential of oral mucosal cells for cultured epithelium: A preliminary report. *Ann. Plast. Surg.* 35: 498, 1995.
11. Hata, K., Kagami, H., Ueda, M., Torii, S., and Matsuyama, M. The characteristics of cultured mucosal cell sheet as a material for grafting: Comparison with cultured epidermal cell sheet. *Ann. Plast. Surg.* 34: 530, 1995.
12. Carls, F. R., Jackson, I. T., Behl, A. K., Lebeda, R., and Webster, H. Prefabrication of mucosa-lined flap: A preliminary study in the pig model. *Plast. Reconstr. Surg.* 101: 1022, 1998.
13. Rath, T., Tairych, G. V., Frey, M., Lang, S., Millesi, W., and Glaser, C. Neuromucosal prelaminated flaps for reconstruction of intraoral lining defects after radical tumor resection. *Plast. Reconstr. Surg.* 103: 821, 1999.
14. Schlenz, I., Korak, K. J., Kunstfeld, R., Vinzenz, K., Plenk, H., Jr., and Holle, J. The dermis-prelaminated scapula flap for reconstructions of the hard palate and the alveolar ridge: A clinical and histologic evaluation. *Plast. Reconstr. Surg.* 108: 1519, 2001.
15. Lauer, G., Schimming, R., Gellrich, N. C., and Schmelzeisen, R. Prelaminating the fascial radial forearm flap by using tissue-engineered mucosa: Improvement of donor and recipient site. *Plast. Reconstr. Surg.* 108: 1564, 2001.
16. Kunstfeld, R., Petzelbauer, P., Wickenhauser, G., et al. The prefabricated scapula flap consists of syngeneic bone, connective tissue, and a self-assembled epithelial coating. *Plast. Reconstr. Surg.* 108: 1908, 2001.
17. Cooper, D., Schermer, A., and Sun, T. T. Classification of human epithelia and their neoplasms using monoclonal antibodies to keratins: Strategies, applications, and limitations. *Lab. Invest.* 52: 243, 1985.
18. Lane, E. B., Bartek, J., Purkis, P. E., and Leigh, I. M. Keratin antigens in differentiating skin. *Ann. N. Y. Acad. Sci.* 455: 241, 1985.
19. Smedts, F., Ramaekers, F., Leube, R., Keijser, K., Link, M., and Vooijis, P. Expression of keratins 1, 6, 15, 16, and 20 in normal cervical epithelium, squamous metaplasia, cervical intraepithelial neoplasia, and cervical carcinoma. *Am. J. Pathol.* 142: 403, 1993.
20. Clausen, H., Moe, D., Buschard, K., and Dabelsteen, E. Keratin proteins in human oral mucosa. *J. Oral Pathol.* 15: 36, 1986.
21. Morgan, P. R., Shirlaw, P. J., Johnson, N. W., Leigh, I. M., and Lane, E. B. Potential applications of anti-keratin antibodies in oral diagnosis. *J. Oral Pathol.* 16: 212, 1987.
22. Tunenaga, M., Kohno, Y., Horii, I., et al. Growth and differentiation properties of normal and transformed human keratinocytes in organotypic culture. *Jpn. J. Cancer Res.* 85: 238, 1994.
23. Moll, R., Franke, W. W., and Schiller, D. L. The catalogue of human cytokeratins: Patterns of expression in normal epithelia, tumors and cultured cells. *Cell* 31: 11, 1982.
24. Sun, T. T., Eichner, R., Schermer, A., Cooper, D., Nelson, W. G., and Weiss, R. A. Classification, expression and possible mechanisms of evolution of mammalian epithelial keratins: Unifying model. In A. J. Levine, G. F. Vande Woude, W. C. Toppe, and J. D. Watson (Eds.), *The Transformed Phenotype*, Vol. 1. New York: Cold Spring Harbor Laboratory, 1984. Pp. 169–176.
25. Roop, D. R., Huitfeldt, H., Kiklenny, A., and Yuspa, S. H. Regulated expression of differentiation-associated keratins in cultured epidermal cells detected by monospecific antibodies to unique peptides of mouse epidermal keratins. *Differentiation* 35: 143, 1987.
26. Sondell, B., Dyberg, P., Anneroth, G. K., Östman, P. O., and Egelrud, T. Association between expression of stratum corneum chymotryptic enzyme and pathological keratinization in human oral mucosa. *Acta Derm. Venereol. (Stockh.)* 76: 177, 1996.
27. Morgan, P. R., Leigh, I. M., Purkis, P. E., Gardner, I. D., van Muijen, G. N. P., and Lane, E. B. Site variation of keratin expression in human oral epithelia: An immunocytochemical study of individual keratins. *Epithelia* 1: 31, 1987.
28. Asselineau, D., Bernard, B. A., Bailly, C., Darmon, M., and Prunieras, M. Human epidermis reconstructed by culture: Is it "normal"? *J. Invest. Dermatol.* 86: 181, 1986.