SY21 Development of Normal Skin-like Substitutes by Controlling Enzymatic Degradation of Extracellular Matrices

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1. Introduction

Skin forms the continuous external surface of the body and is the largest organ of the body. The outermost layer, the epidermis, is supported and nourished by the dermis and hypodermis. Histologically, the epidermis, dermis and hypodermis are quite different. In particular, the epidermis and dermis are linked by anchoring complex composed of anchoring filaments containing laminin 332, basement membrane (BM) containing type IV collagen and anchoring fibrils containing type VII collagen. These anchoring complex structures are difficult to reconstitute under *in vitro* culture conditions, and in skin equivalent (SE) models the epidermis is easily detached from the dermis.

Skin is always exposed to external and internal mechanical stimuli during bodily movement, changes of facial expression, touching and so on. These stimuli cause skin deformation without separation of the layers, and therefore the elasticity and strength of skin are important functional parameters. Collagens and elastic fibers in the dermis are involved in skin strength and elasticity, and the epidermis is strongly bound to the dermis by the BM and the anchoring structures at the dermal-epidermal junction (DEJ).

SEs are used for many purposes, including safety and efficacy testing, and as a skin substitute for grafting. However, they usually contain degraded collagen fibers and lack elastic fibers in the dermis and BM structures at the DEJ, being quite different from normal skin structure. This may result in a reduced take-rate of grafts. Therefore, we aimed to develop normal skin-like models that have a thicker dermis and well-differentiated epidermis, as well as well-organized basement membrane and anchoring structures.

2. Method

To prepare SEs, human keratinocytes were cultured on top of a dermal equivalent consisting of type I collagen and human fibroblasts in a three-dimensional fashion. The culture was lifted to the air-liquid interface so that only the keratinocyte layer was exposed to air, causing it to form a cornified layer. The medium for SE culture was prepared from a 1:1 mixture of KGM and DMEM with 5% FBS and 2-O- α -D-glucopyranosyl-L-ascorbic acid (AA-2G), which is a stable derivative of ascorbic acid. The SEs were subsequently cultured in the presence or absence of a matrix metalloproteinase (MMP) inhibitor, CGS27023A, human Glu-plasminogen, and/or a serine protease inhibitor, aprotinin or tranexamic acid, and/or a heparanase inhibitor, BIPBIPU.

For the observation of BM structure by transmission electron microscopy, SEs were fixed, dehydrated, and embedded in epoxy resin. Ultrathin sections were cut and stained with 5% uranyl acetate and Reynold's lead citrate. The sections were examined with a transmission electron microscope.

3. Results and Discussion

We found that in SEs, mutual communication between keratinocytes and fibroblasts enhanced the production of several cytokines and enzymes degrading extracellular matrices (ECM), such as MMPs, plasminogen activator, plasmin, and heparanase, an enzyme degrading heparan sulfate (HS) chains of proteoglycans, and consequently SEs showed only a faint BM at the DEJ (1-3). The MMP inhibitor, CGS27023A, improved BM structure and allowed the development of linear and continuous lamina densa-like structures (1), but the addition of plasminogen disrupted the BM structure by increasing plasmin activity. The combination of both CGS27023A and aprotinin improved the disorganized BM and restored the linearity and continuity of BM structure (2). HS-proteoglycan at the DEJ also plays important roles by interacting with HS-affinity growth factors, cytokines and ECM. In SE, we found that HS chains at the DEJ were lost, although the core-protein of HS proteoglycan still remained. The degradation of HS chains was due to increased heparanase activity. The combination treatment with inhibitors of heparanase and MMPs strikingly improved the BM structure and resulted in formation of anchoring complexes including the anchoring fibrils (type VII collagen), which were hardly observed in the skin models without inhibitors (3). The BM and anchoring complexes affect the polarity of basal keratinocytes in the epidermis and the dermal stability by controlling MMP production, thereby normalizing the epidermal differentiation of epidermis. The resulting SEs appeared similar to normal human skin.

4. Concluding remarks

The use of degradative enzyme inhibitors in skin-equivalent models resulted in improvement of BM structure, formation of anchoring complexes and recovery of functional HS chains at DEJ. These features are essential for development of improved human skin-like models, serving to normalize the balance between proliferation and differentiation in the epidermis and to enhance the structural formation of ECM components.

References

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