Mini Review

Epidermal regeneration by keratinocyte-alien mesenchymal cell interactions

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Mesenchymal support plays crucial roles in both normal and cancer tissue development. In the skin, dermal mesenchymal fibroblasts provide keratinocytes with many cytokines as critical crosstalk molecules through epithelial-mesenchymal interactions. A number of reports have demonstrated that mesenchymal stem cells and myofibroblasts participate in skin repair. Although the importance of both mesenchymal support and the mesenchymal cell phenotype has been recognized, the relationship between the mesenchymal cell phenotype and the paracrine/autocrine action remains unclear. Moreover, the organ that supplies these supportive mesenchymal cell types within the injured tissue is still unclear. In the skin, it is also unknown whether non-skin organ-derived mesenchymal cells, other than the bone marrow and adipose tissue, affect epidermal regeneration through mesenchymal-epithelial interactions. Here, we highlight recent findings regarding the paracrine/autocrine action and phenotypic characters of non-skin-derived mesenchymal cells. We also briefly present our preliminary data regarding the supporting effect of cancer-associated fibroblasts on normal tissue repair.

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Introduction

Mesenchymal-epithelial interactions play crucial roles in pre-natal and postnatal tissue development and homeostasis¹. In the skin in particular, Rheinwald and Green demonstrated that epidermal keratinocytes depend on the presence of fibroblasts for efficient growth in tissue culture². It is well known that decubitus

ulcer patients suffer from poor wound healing because of their unhealthy granulation tissue. This unhealthy granulation tissue is believed to delay epidermal repair through mesenchymal cell dysfunction, especially inappropriate productions of cytokines. In general, dermal mesenchymal fibroblasts provide keratinocytes with a number of cytokines, such as keratinocyte growth factor,

stromal-derived factor-1 and interleukin-6, as critical crosstalk molecules³⁻⁶).

Bone marrow- and adipose-derived mesenchymal stromal cells contain multipotent stem cells that are capable of differentiating into numerous cell types, including fibroblast, bone, cartilage and muscle (skeletal and smooth) cells^{7,8}. These mesenchymal stem cells are not lineage-restricted and produce functional non-hematopoietic cell types when transplanted into foreign tissues. At the same time, the available reports present a paradox, since the cells originally attracted attention because of their stem cell-like properties, but frequently repair injured tissues without much evidence of either engraftment or differentiation⁹.

Based on wound-healing studies, it has long been known that α -smooth muscle actin (α -SMA)-positive myofibroblasts start to appear in the granulation tissue around the mid phase of wound healing. This timing coincides with the strong induction of contractile properties, so that the cells become aligned in a parallel manner to the mechanical tension that is building up in the granulation tissue. Wound-associated myofibroblasts have been focused upon for their physical properties^{10,11}, while the paracrine/autocrine action of these myofibroblasts remains unclear.

Mesenchymal stem cells and myofibroblasts show strong propensities to ameliorate tissue damage in response to injury and disease. It is well established that bone marrow-derived mesenchymal stem cells produce a variety of cytokines and adhesion molecules that regulate effective tissue repair^{9,12}, but it is still unclear whether non-skin organ-derived mesenchymal cells, other than the bone marrow and adipose tissue, provide these essential cytokines for epidermal repair. In addition, the relationship between the mesenchymal cell phenotype and the paracrine/autocrine action remains unclear.

Here, we highlight the recent findings regarding the supporting effect and phenotypic characters of non-skin-derived mesenchymal cells in epidermal regeneration.

Bone marrow- and adipose tissue-derived mesenchymal cell support skin regeneration

We previously demonstrated that bone marrow- and subcutaneous adipose tissue-derived mesenchymal stromal cells promote active epidermal regeneration in a skin reconstruction model, by affecting the growth and apoptosis of keratinocytes¹³. Bone marrow stromal cells (BMSCs), subcutaneous preadipocytes and dermal fibroblasts clearly promoted the stratification of keratinocytes, resulting in the formation of an epidermal layer consisting of basal, prickle cell, granular and cornified layers

(Fig.1). In contrast, keratinocytes without these mesenchymal supports formed only a thin epidermal layer, underwent apoptosis and disappeared from the culture assembly after 14 days. These results suggest that mesenchymal support is critical for the survival, growth and fundamental differentiation of keratinocytes, and support other studies regarding fibroblast-keratinocyte interactions^{2,3,14}. Interestingly, these mesenchymal cell types showed cell type-specific effects on the reorganization of epidermal structures, including rete ridge-like, epidermal ridge-like and stratified structures. These results suggest that BMSCs and preadipocytes may play more important roles in the integration of native epidermal structures during cutaneous wound healing than dermal fibroblasts and subcutaneous mature adipocytes through keratinocyte-mesenchymal cell interactions.

In general, BMSCs and preadipocytes may not be the major cell type components of normal skin. However, BMSCs and preadipocytes clearly promote epidermal regeneration, as reported in our previous study. Recently, several studies¹⁵⁻¹⁷ have shown: i) that marrow-derived endothelial progenitor cells exist in the adult circulation; and ii) that the progenitor cells differentiate into endothelial cells after homing to neovascularization sites in experimental animal models, including models of hind limb ischemia, myocardial infarction and tumor growth. In addition, preadipocytes actively develop from mature adipocytes under adipocyte-endothelial cell interactions¹⁸, suggesting that preadipocytes may participate in the wound healing of cutaneous deep ulcers. Taken together, these results suggest that homing of BMSCs and preadipocytes may contribute to the epidermal reorganization in cutaneous wound healing through epithelial-mesenchymal communication. It also seems likely that these cells may constantly participate in the homeostasis of the skin structure.

Heart-, spleen-, lung-, liver- and kidney-derived mesenchymal cells support epidermal regeneration

It has been widely recognized that bone marrow- and adipose tissue-derived mesenchymal cells play crucial roles in tissue regeneration. In the skin, our study^{13,19} and others¹⁰ have demonstrated that organ-derived mesenchymal cells support epidermal regeneration. Although every organ has an abundant mesenchymal component, the various organ-derived mesenchymal cell types other than the aforementioned counterparts are not considered to be involved in epidermal regeneration.

To clarify this important issue, we investigated the effects of five mesenchymal cell types other than BMSCs and adipose tis-

Fig.1

Structures of the epidermis regenerated by keratinocytes cultured with or without mesenchymal-epithelial interactions. Bone marrow stromal cells (BMSCs), preadipocytes and dermal fibroblasts promote the stratification of keratinocytes and result in the formation of epidermal layers made up of basal, prickle cell, granular and cornified layers, whereas keratinocytes alone form only a thin epidermal layer. BMSCs cause keratinocytes to reorganize a rete ridge-like structure (arrowheads). Preadipocytes induce an epidermal ridge-like structure in the epidermal layer (arrows). Scale bars: 50 μ m.

Fig.2

Structures of the epidermis regenerated by keratinocytes cultured with heart-, spleen-, lung-, liver- and kidney-derived mesenchymal cells (MCs). Heart-, spleen-, lung-, liver- and kidney-derived MCs promote the stratification of keratinocytes and result in the formation of differentiated epidermal layers. Scale bars: 50 μ m.

sue-derived mesenchymal cells on epidermal regeneration using a skin reconstruction model. We chose to investigate the mesenchymal cell types of the following organs with or without an epithelial cell component: i) heart; ii) spleen; iii) lung; iv) liver; and v) kidney. The heart- and spleen-derived mesenchymal cell types were utilized as representatives of epithelial cell-non-containing organ-derived mesenchymal cell types, while the lung-, liver- and kidney-derived mesenchymal cell types were used as representatives of epithelial cell-containing organ-derived mesenchymal cell types. Our study demonstrated for the first time that these five mesenchymal cell types almost equally promoted epidermal regeneration by keratinocytes through mesenchymal-epithelial interactions using a skin reconstruction model²⁰. All

the mesenchymal cell types derived from the above-mentioned organs promoted the stratification of keratinocytes, resulting in the formation of a regenerated epidermis consisting of basal, prickle cell, granular and cornified layers (Fig.2).

Interestingly, our study showed that the vast majority (97.8-99.7%) of the mesenchymal cells derived from the individual organs were commonly positive for vimentin. The appearance ratios of α -SMA-positive myofibroblasts, which play a role in wound healing, ranged widely from 4.5% (spleen-derived mesenchymal cells) to 63.2% (heart-derived mesenchymal cells). The mesenchymal cells derived from the five organs showed low positivities for the mesenchymal stem cell markers of stage-specific embryonic antigen (SSEA)-4 (0.0-0.8%), CD105 (0.2-

Fig.3

Phenotypic characterization of non-skin organ-derived mesenchymal cells. The vast majority (97.8-99.7%) of the mesenchymal cells derived from the individual organs were commonly positive for vimentin. The appearance ratios of α -SMA-positive myofibroblasts ranged widely from 4.5% to 63.2%. The mesenchymal cells derived from the five organs showed low positivities for the mesenchymal stem cell markers of stage-specific embryonic antigen (SSEA)-4 (0.0-0.8%), CD105 (0.2-1.2%) and CD90 (0.0-1.3%). Positive staining for CD44 was present in varying proportions ranging from 1.5% to 67.7%. Most of the mesenchymal cells derived from the five organs constantly express the common mesenchymal cell marker vimentin. However, they exhibit heterogeneous expressions of mesenchymal stem cell and myofibroblast markers.

Fig.4

Structures of the epidermis regenerated by keratinocytes cultured with cancer-associated fibroblasts (CAFs). (a) CAFs (arrows) promote the stratification of keratinocytes and result in the formation of differentiated epidermal layers. (b) p63-positive nuclei (green) within a basal layer of the regenerated epidermis with CAFs. Scale bar: 50 μ m.

1.2%) and CD90 (0.0-1.3%). Positive staining for CD44, a stromal progenitor marker, was present in varying proportions ranging from 1.5% (heart-derived mesenchymal cells) to 67.7% (liver-derived mesenchymal cells). We did not detect any common expression patterns of the myofibroblast and mesenchymal stem

cell markers among the cell surface markers examined in the heart-, spleen-, lung-, liver- and kidney-derived mesenchymal cell types (Fig.3).

Our current and previous studies suggest that not only skin-localized mesenchymal cells but also non-skin-localized mesen-

chymal cells enable keratinocytes to undergo normal proliferation and differentiation^{13,19,20}. In general, the heart and spleen have no epithelium in their normal states, while the lung, liver and kidney have an epithelium. Consequently, mesenchymal-epithelial interactions do not exist in the heart and spleen, but do exist in the lung, liver and kidney. In addition, the mesenchymal cell-induced epidermal regeneration observed in this study showed no significant differences among the organ types. These findings imply that mesenchymal-epithelial interactions with cross-organ specificity may play critical roles in epidermal regeneration, even at the adult stage.

Taken together, our findings suggest that the vimentin-positive phenotype of mesenchymal cells derived from the heart, spleen, lung, liver and kidney is critical for epidermal regeneration in which the mesenchymal stem cell and myofibroblast phenotypes of the mesenchymal cells may not be involved, at least *in vitro*.

Can cancer-associated fibroblasts support non-cancer epidermal regeneration?

Recent studies have suggested that cancer development is dependent on signals received from closely apposed tumor-associated mesenchyme. Mesenchymal cells provide continuous support for cancer cell survival through cancer-mesenchymal cell interactions²¹⁻²³. In general, mesenchymal cells may act as a barrier to developing tumorigenesis by resentencing cancer cells, while the same mesenchymal cells evolve over time to actively support cancer growth in response to molecular signals derived from the tumor cells. These cancer-affected mesenchymal cells are called cancer-associated fibroblasts (CAFs). It has become clear that there are close interactions between cancer cells and CAFs, but little is known about the epithelial-mesenchymal interactions between CAFs and non-cancer epithelial cells.

To clarify this important issue, research into the paracrine/autocrine action of CAFs is now in progress in our laboratory. Our preliminary data suggested that CAFs isolated from a poorly differentiated adenocarcinoma in a male gastric cancer patient in his seventies supported epidermal regeneration in a skin reconstruction model (Fig.4a). p63-positive epidermal progenitor cells were detected in the basal cells of the regenerated epidermis under the culture conditions used (Fig.4b).

Although further research is needed, these findings suggest that CAFs themselves may be involved in the tissue regeneration of non-cancer cells. Based on this point, it is necessary to study the cross-organ specificity of the mesenchymal cell biology, especially for future regenerative medicine.

Footnote

The study was approved by the Saga University Ethical Committee, and the samples were handled according to medical ethical guidelines.

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