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CROSS-TALK BETWEEN BONE MARROW AND TISSUE INJURY: NOVEL REGENERATIVE THERAPY FOR SEVERELY DAMAGED TISSUES BY MOBILIZING BONE MARROW MESENCHYMAL STEM CELLS *IN VIVO*

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Abstract
In the epidermis, resident stem/progenitor cells are involved in the complete renewal of the stratified squamous epithelium every 2-4 weeks and thus contribute to tissue homeostasis. In patients with the inherited chronic blistering skin disorder, recessive dystrophic epidermolysis bullosa (RDEB), however, the epidermal stem cell pool is continually depleted as a result of detachment of full-thickness epidermis due to lack of the basement membrane protein, type VII collagen (Col 7). Nevertheless, the epidermis in RDEB continues to be renewed, suggesting there may be a mechanism to supplement epithelial stem/progenitor cells from extracutaneous sources. Recently, we reported that the detached epithelium from a Col 7-null mouse model of RDEB will release abundant high mobility group box 1 (HMGB1), which mobilizes a sub-population of non-hematopoietic cells from bone marrow into the circulation to repair skin and restore Col 7 expression. These bone marrow-derived epithelial stem/progenitor cells are derived from a lineage-negative, platelet-derived growth factor alpha-positive mesenchymal stem cell pool in bone marrow, which represents less than 0.3% of the total bone marrow cell population. In addition, systemic administration of HMGB1 to wounded wild-type mice leads to faster wound healing with recruitment of these bone marrow cells to the wounded skin. This study identifies a suitable cell population for cell therapy approaches in individuals with RDEB, but also has broader implications for clinical medicine in identifying a means of mobilizing and recruiting a key population of bone marrow cells germane to tissue repair.

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Key words: mesenchymal stem cell; high mobility group box 1; epidermolysis bullosa

Introduction

Bone marrow (BM) has been shown to contain cells which can generate epithelial cells, i.e. keratinocytes, *in vitro* and *in vivo*[1-13]. Previous studies involving transplantation of sex-mismatched or genetically tagged BM cells have shown that keratin-positive bone marrow-derived cells can be found in skin epidermis[14]. In humans who have undergone BM transplantation (BMT), donor cells that have differentiated into keratinocytes can be detected in the epidermis for at least 3 years[5]. With regard to skin injury, both embryonic and post-natal transplantation of BM cells into mice lacking cutaneous basement membrane components, such as type VII collagen (Col 7) and type XVII collagen, have demonstrated the capacity of BM to promote skin wound healing and to correct the intrinsic basement membrane defect[15-17]. Most recently, a clinical trial of allogeneic whole BMT in humans lacking Col 7, who have the inherited blistering skin disorder, recessive dystrophic epidermolysis bullosa (RDEB), has demonstrated that BM cells can repair fragile skin and restore Col 7 expression in skin basement membrane[18]. These animal and human studies suggest that BM stem cells have significant potential for

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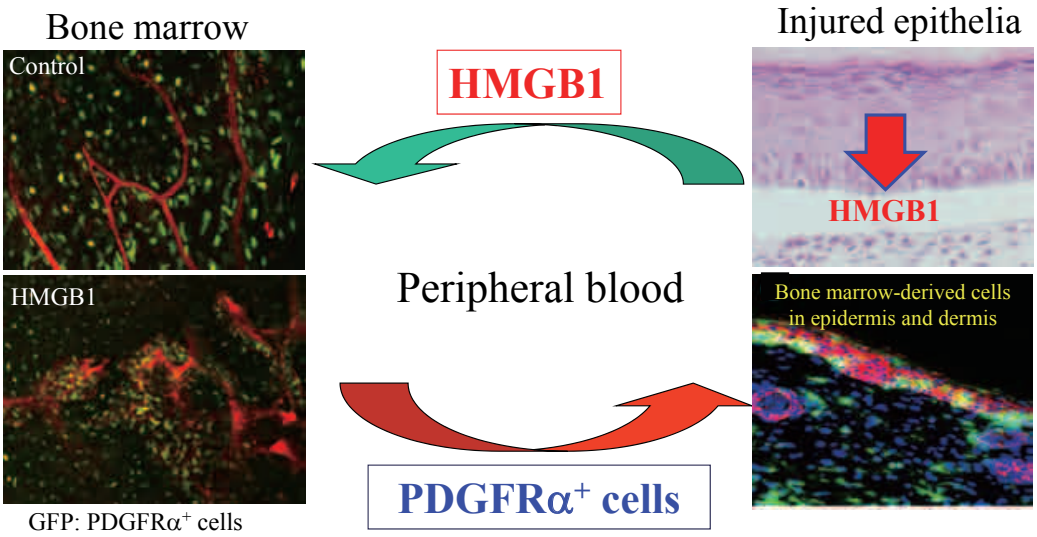

Crosstalk mechanism between bone marrow and injured epithelia via HMGB1.
1. Injured epithelia release HMGB1into peripheral blood. 2. Increased HMGB1 in peripheral blood mobilizes bone marrow PDGFRα+ cells into the circulation. 3. Circulating PDGFRα+ cells migrate into the injured skin possibly by HMGB1 attraction and regenerate injured skin.

therapeutic application on inherited epithelial disorders. In this review, our recent findings on cross-talk between bone marrow and injured skin to promote regeneration of injured epithelia by recruiting bone marrow mesenchymal stem/progenitor cells will be summarized[19], and then future perspective for application of the disclosed mechanism on treatment of tissue injury will be discussed.

Epithelial regeneration by bone marrow cells

Contribution of BM-derived cells to epithelial regeneration was compared in murine skin wounds and skin graft[19]. Precisely, the mice selected for wounding had received lethal dose irradiation followed by GFP-BMT, allowing to evaluate the contribution of GFP-BM cells to skin regeneration following injury. At 4 weeks after the injury, GFP-positive keratinocytes were not obvious in the regenerating epithelia of the wound. The same GFP-BMT mouse model was then used to examine a different form of skin repair, skin grafting, and significant numbers of GFP-positive cells expressing skin-specific keratin 5 were shown to form epidermal proliferative unit (EPU)-like clusters in the epidermis of the skin graft at 4 weeks after the engraftment. These data showed that a sub-population of BM cells contributes to epithelial regeneration and maintenance in skin graft, but not in skin wound[19].

PDGFRα-positive bone marrow cells has the potential for epithelial differentiation

PDGFRα-positive non-hematopoietic BM cell population contains ectoderm-derived mesenchymal stem cells (MSCs) [20, 21]. The PDGFRα+ BM cells were then shown to exclusively generate BM-derived epithelial cells expressing keratin 5 in culture[19]. PDGFRα- cell population also contained adherent and proliferative cells in culture, but none of these cells showed differentiation into keratin 5-positive keratinocytes[19]. These findings suggested that the BM-derived keratinocytes are originated from a specific sub-population of PDGFRα+

BM cells[19]. This hypothesis was confirmed by observation that PDGFRα$^+$ cells in BM contributed regeneration of the injured epithelia of the skin engrafted on the back of GFP$^+$/PDGFRα$^+$ BMT mouse[19]

The PDGFRα$^+$ cells in bone marrow seems to contain different or overlapped mesenchymal subpopulations, including recently identified PDGFRα$^+$ Sca-1$^+$ CD45$^-$ TER119$^-$ (referred to as PαS cells) [21]. Nevertheless, although these PαS cells display multi-potency, they still seem to be a heterogeneous population. The PDGFRα$^+$ cells may also share some characteristics with a less frequent sub-population of cells, "termed muse cells" (multi-lineage differentiating stress-enduring), that can generate cells with characteristics of endoderm, mesoderm and ectoderm[22]. Muse cells have been identified in BM stromal cells, BM aspirates and amongst skin fibroblasts[22], but their relationship with the PDGFRα$^+$ sub-population of cells is not currently known. In addition, although some of the PDGFRα$^+$ population includes cells of neuro-epithelial lineage, it remains to be determined if these or other specific sub-populations of PDGFRα$^+$ cells are directly implicated in epidermal repair and maintenance. Delineation of specific sub-populations of MSCs with the capacity to differentiate into keratinocytes is a key objective.

HMGB1 mobilizes PDGFRα$^+$ cells from bone marrow to regenerate injured epithelia

Subsequent studies clarified the mechanism through which the transplanted skin graft is able to recruit PDGFRα$^+$ cells from the BM[19]. The skin graft was shown to release high mobility group box 1 (HMGB1) into circulation for this mechanism. HMGB1, also known as amphoterin, is a nuclear protein that can regulate chromatin structure and gene expression[23]. It is also released from necrotic cells and some apoptotic cells and acts as an inflammatory regulator. Other studies, however, have indicated that HMGB1 may also act as a local chemo-attractant for various hematopoietic and non-hematopoietic cells that can regulate tissue remodeling[24]. Our study clearly demonstrated that the skin graft-derived HMGB1 in circulation stimulated PDGFRα$^+$ BM cells to mobilize them into the circulating blood, and the circulating PDGFRα$^+$ cells were then recruited by HMGB1 in the skin graft to regenerate the injured epithelia of the graft[19].

In the study, a marked increase in HMGB1 serum levels was observed 3 days after grafting, the timing of generating focal necrosis in the epithelia of the skin graft[19]. Of note, however, no increase in serum HMGB1 was noted in mice with full thickness wounds. One fundamental difference between the wound and the skin graft is that only the latter provides epithelium in the wound site to release HMGB1 into the circulation. Furthermore, epithelial differentiation of PDGFRα$^+$ BM cells was shown to occur only in epithelial tissue in the grafted skin, suggesting a requirement for cell-cell and cell-matrix contact to keratinocytes and basement membrane, respectively. This structural microenvironment is present in the skin grafted tissue but not in the wound. These unique features therefore seem to provide a preferential milieu to raise PDGFRα$^+$ BM cell-derived keratinocytes in the skin graft.

Future perspective of HMGB1-mediated regenerative medicine for intractable tissue injury

As described above, PDGFRα$^+$ cells from BM significantly contribute to the regeneration of the injured epithelia *in vivo*, and one biological repair mechanism involves the key cells being mobilized in response to elevated HMGB1 levels in serum, the source of which is the skin graft. PDGFRα is not expressed by hematopoietic stem cells but by mesenchymal cells including MSCs in bone marrow that can give rise to mesenchymal lineage cells as well as neuro-

epithelial and neural crest lineage cells[20, 21].

In situations in which there is significant necrotic damage to the epidermis, at least some of the PDGFRα$^+$ BM cells have the plasticity to become BM-derived epithelial cells to generate and sustain new keratinocytes in the injury, suggesting that the allogenic PDGFRα$^+$ cells can correct the intrinsic molecular defect in inherited epithelial disorders if transplanted to the skin. Moreover, HMGB1, which is rapidly released from the injured epithelia, mobilizes PDGFRα$^+$ BM cells into the circulation and accelerate regeneration of the injured skin by recruiting these cells to raise BM-derived epithelial cells and BM-derived mesenchymal cells in the epidermis and dermis, respectively. These findings provide future perspectives that systemic administration of HMGB1 may be a possible therapeutic option of treatment of the epithelial diseases including inherited keratinizing disorders as well as inherited blistering diseases, such as epidermolysis bullosa .

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