PRESS RELEASE

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Mouse Experiments Give Promise For Regenerative Dermatologic Therapy

It has been established previously that bone marrow can contribute several cells to the skin and that these may have roles in various aspects of skin biology, including development and wound healing. It has also been shown that bone marrow cells can give rise to cells within the epidermis (keratinocytes) but that this is an extremely rare occurrence.

To examine epithelial repair and the role of bone marrow cells in more detail, we have studied a different type of wound healing, skin grafting. Our research addresses the questions which bone marrow cells contribute to skin repair and how is this population of cells recruited to damaged skin and how the cells are mobilized from the bone marrow?

The key bone marrow cells are non-hematopoietic and make up <0.22% of the total bone marrow population. The population is Lineage-negative, platelet-derived growth factor receptor (PDGFR) alpha-positive.

The advances were made using a mouse model in which full thickness skin from one wild-type mouse was grafted onto another wild-type mouse that had been lethally irradiated to destroy its bone marrow and then recovered using green fluorescent protein (GFP) bone marrow (Fig. 1).

We were able to demonstrate GFP-positive cells in the regenerating epidermis (as well as dermis and follicular epithelium). Moreover, GFP-positive cells were still present in the regenerated epithelium after 5 months, with evidence of GFP-positive epidermal proliferation units – indicating that the bone marrow cells contribute to maintenance of the new epidermis (Fig. 2). In contrast, we did not see such bone marrow cells in non-grafted wound healing.

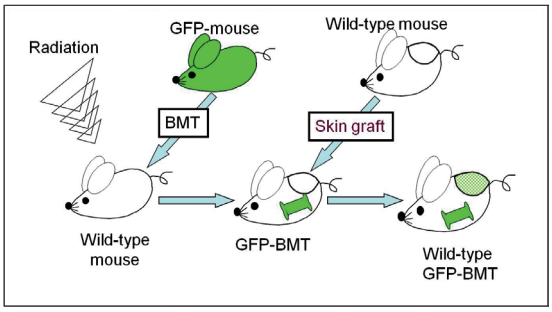

Fig. 1. Bone marrow-derived cells contribute to epidermal and follicular renewal in skin grafts, but not in skin wounds. Schematic outlining details of the skin engraftment of GFP-BMT mice.

To see whether these bone marrow-derived epithelial progenitors might have functional relevance we also grafted skin from mice lacking the basement membrane protein, type VII collagen (a model of the human disease recessive dystrophic epidermolysis bullosa, RDEB). We observed an even greater number of GFP-positive cells within the regenerating epidermis as well as restoration of type VII collagen protein expression in skin basement membrane (Fig. 3).

Both of these observations are interesting – it suggests that skin grafting – a widely used technique in medicine, may not simply have therapeutic benefits through closing a skin defect – it may actually act as a "bioreactor" to recruit a population of bone marrow cells to assist in tissue repair. In situations where there is additional epidermal damage, such as in RDEB, the bone marrow may also supply epithelial progenitors to evoke functional repair. The work showed that such cells were numerous and persisted for several months (i.e. for several renewals of the epidermis).

Further transplantation work in mice then showed that the PDGFRα⁺ cells in bone marrow did contain a population of epithelial progenitor cells.

Cell sorting of whole bone marrow showed that PDGFRα⁺ cells make up about 0.73% of all bone marrow cells. To characterize the key repair cells in more detail, a further mouse model in which GFP fluorescence accumulates within the nuclei of PDGFRα⁺ cells was used. This showed the relevant cells were Lineage-negative and thus, taken together, Lin-/PDGFRa+ cells represent the bone marrow cells that provide the epithelial and mesenchymal progenitors in the RDEB skin; Lin-/PDGFa+ cells comprise about 0.22% of total bone marrow cells.

Although transplantation of whole bone marrow has been shown very recently to benefit patients with RDEB, the new data suggest that the critical cells in the skin repair may be the Lin-/PDGFRα⁺ non-hematopoietic cells that represent

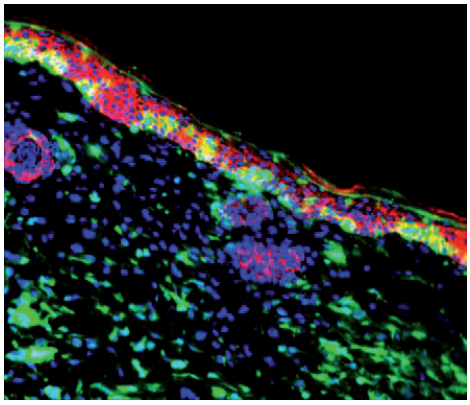

Fig. 2. Several weeks after grafting, GFP-BM cells are present in the epidermis and dermis.

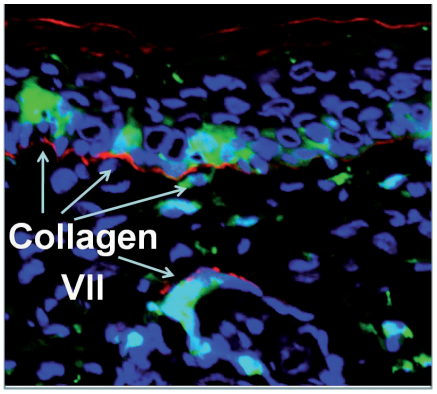

Fig. 3. After grafting of Collagen VII-null skin onto the GFP-BMT mice there are GFP cells within the epidermis and evidence of new collagen VII protein adjacent to these cells at the dermal-epidermal junction and around hair follicles.

about 1 in every 400 bone marrow cells. The Lin-/PDGFRα⁺ cell population is still heterogeneous but the new findings identify a new direction for subsequent clinical trials of bone marrow therapy in RDEB.

Our work subsequently looked at how these Lin-/PDGFRα⁺ cells are mobilized from bone marrow. The key factor in the mobilization of the marrow stromal cells is high mobility group box 1 (HMGB1).

The experimental objective was to identify how a transplanted skin graft can recruit Lin-/PDGFRα⁺ cells from the bone marrow. This was done in Boyden chamber migration assays using the skin conditioned buffer and subsequent characterization of the "recruitment factor" identified HMGB1 as the principal chemoattractant. The rationale was that detached skin should release the key factor(s) that trigger recruitment of this population of bone marrow cells.

HMGB1, previously known as amphoterin, is both a nucleo-cytoplasmic protein involved in chromatin regulation and a secreted protein that is released into the extracellular milieu when cells are stressed. Its cytokine activities are mediated via various receptors including RAGE, TLR2/4, thrombomodulin and syndecan and it can have pro- and anti-inflammatory activities.

The next phase was to see how the systemic HMGB1 functions in normal/RDEB-skin-transplanted mice and in RDEB patients. We were able to demonstrate the following:

1) HMGB1 is rapidly released from a piece of excised skin, especially from RDEB mouse epithelium (blister roof) placed in phosphate buffered saline;
2) Fluid taken from intact blisters in human subjects with RDEB contains high level of HMGB1;
3) HMGB1 levels in the sera of skin-engrafted mice and RDEB individuals are about 20 to 60-fold greater than in control individual sera;
4) Systemic administration of HMGB1 in mice, at levels comparable to those in the sera of humans with RDEB, can mobilize Lin-/PDGFR alpha+ bone marrow cells into the blood circulation;
5) Intra-vital two-photon imaging demonstrates that HMGB1 can mobilize PDGFRα⁺ cells, allowing them to congregate around blood vessels in marrow sinuses for entry into the circulation;
6) Systemic administration of recombinant HMGB1 in mice does not cause any local or generalized adverse effects.

This research study has several implications for translational medicine.

- For patients with RDEB, Lin-/PDGFRα⁺ bone marrow cells have potential clinical utility as local or systemic cell therapy. The extensive damaged tissue in RDEB may be sufficient to recruit the cells because of the high levels of HMGB1 associated with extensive epithelial damage.
- For patients with other forms of more localized injuries (including non-RDEB disorders) it is plausible that local release of HMGB1 from damaged tissue is insufficient to reach a threshold to recruit Lin-/PDGFRα⁺ cells from bone marrow. However, systemic administration of recombinant HMGB1 may offer a new therapeutic means of mobilizing these tissue repair cells into the blood circulation. HMGB1 released from the damaged site (with or without additional recombinant HMGB1 administered to that site) could then recruit the cells to minimize further damage and to invoke local cellular and non-cellular repair processes. We believe our findings have very significant implications for wound healing and tissue regeneration strategies that will have clinical impact in the not-too-distant future.

Read the article:

Tamai K, Yamazaki T, Chino T, Ishii M, Otsuru S, Kikuchi Y, et al. PDGFRα-positive cells in bone marrow are mobilized by high mobility group box 1 (HMGB1) to regenerate injured epithelia. PNAS 2011: 108: 6609–6614.