Published in final edited form as:

Nat Med. 2009 April ; 15(4): 367–369. doi:10.1038/nm0409-367.

Neuropeptide beckons cells that heal

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Abstract

Experiments in rodents identify a factor that causes the release of multipotent cells into the circulation after injury. These cells contribute to tissue repair.

In the early 1900s, the renowned hematologist Alexander Maximow observed a curious population of cells in the circulation. Unlike most types of blood cells, these cells could adhere to plastic culture dishes and had many of the cellular habits of fibroblasts¹. Other researchers subsequently found that these blood-borne fibroblasts are similar, but not identical to, bone marrow stromal cells (BMSCs, also known as ‘mesenchymal stem cells’)^{2,3}. Some of these circulating cells seem to be multipotent, but their mere existence remains controversial, especially in humans. Even murkier is information on where these cells come from, what they do and what the signals are that cause them to appear in the circulation.

In this issue of *Nature Medicine*, Hong *et al.*⁴ provide more evidence that *bona fide* circulating stromal-like stem cells do exist and report that substance P, a neuropeptide, elicits the appearance of these enigmatic cells in peripheral blood. The authors also show that these cells participate in wound repair⁴.

From the clinical perspective, determining the signals that mobilize cells with potential reparative properties into the circulation and fully characterizing their biological properties and tissue origins is highly relevant. Human trials around the world are testing for the clinical utility of systemic infusion of different cell populations after *ex vivo* isolation and, often, expansion⁵. But it would be highly preferable to stimulate a naturally occurring process to liberate reparative cells through administration of appropriate factors, thereby abrogating the need for harvest of cells and their subsequent *ex vivo* manipulation.

Using a corneal injury model, Hong *et al.*⁴ identified substance P as such a factor. The authors homed in on the peptide after finding that its concentration in the circulation increased after injury and was dependent on the size of injury⁴. The authors then queried whether injection of substance P alone, or corneal injury alone, could elicit the appearance of stromal-like cells into the circulation, as assessed by the number of plastic-adherent cells that also expressed a marker characteristic of connective tissue and stromal cells, CD29⁺ ($\alpha_1\beta_1$ integrin subunit)⁴. Both scenarios substantially increased the number of these cells, providing support to the concept that the injured cornea emits at least one signal, substance P, that increases the number of circulating CD29⁺ cells.

These cells did, in fact, resemble BMSCs, on the basis of their expression of a series of markers, their ability to form cartilage *in vitro* and their ability to form bone and support blood formation

upon *in vivo* transplantation by established procedures. Unexpectedly, both the CD29⁺ cells and BMSCs expressed keratin-3, previously thought to be a corneal marker.

Corneal injuries treated either with substance P alone or with autologous CD29⁺ cells healed at a faster rate than untreated controls, indicating that there is a physiologically relevant effect of elevated substance P concentration and CD29⁺ cell number in the circulation. Furthermore, prelabeled CD29⁺ cells were incorporated into the regenerating corneal epithelium (expressing keratin-3) and stroma (expressing α -smooth muscle actin), up to three weeks after injury.

Owing to the similarity of substance P-mobilized CD29⁺ cells to BMSCs, and to the finding that labeled substance P injected into mice binds adherent CD29⁺ cells from flushed bone marrow, the authors speculated that these cells originate from bone marrow stroma⁴. On the basis of this assumption, they sought to address not only how substance P might mediate BMSC mobilization into the circulation but also whether it has a role in maintenance of BMSCs within the bone marrow, in the face depletion owing to their mobilization after injury⁴.

In *in vitro* experiments, they found that substance P induced migration of human BMSCs, increased the expression of several key matrix metalloproteinases (thought to play a part in cell mobilization of hematopoietic cells⁶) and stimulated proliferation of BMSCs mediated by the extracellular signal-related kinase-1 and extracellular signal-related kinase-2 (ERK-1/2) signaling pathway⁴. Lastly, substance P mediated translocation of β -catenin from the cytoplasm to the nucleus, suggesting that the TCF/LEF- β -catenin pathway is active in repopulating the bone marrow stroma. This pathway has been implicated in stem cell self-renewal⁷, but its role has not been rigorously demonstrated for the subset of stem cells present in the BMSC population.

Taking all of the data together, the authors conclude that substance P can induce mobilization of a stromal-like cell that homes to a site of injury and promotes healing⁴. They further suggest that these cells originate from the bone marrow stroma and are able to transdifferentiate into epithelial cells, given the persistence of infused cells in the corneal epithelium and their continued expression of a corneal marker, keratin-3 (ref. 4 and Fig. 1).

Before this study, the presence of circulating cells with characteristics similar to BMSCs was definitively shown in a number of animal species, although they are exceedingly rare in humans for reasons that are as yet unclear³. The relationship of these distinctly adherent, stromal-like cells to another population that has been isolated from human blood by FACS with an antibody specific for osteocalcin (a bone matrix protein) is not yet clear, as these osteocalcin-positive cells have a different cell surface character compared to BMSCs and have been reported to increase in number during pubertal growth and after fracture⁸. But other studies have failed to show the existence of these stromal-like cells, most likely owing to differences in methods of isolation and culture (reviewed in ref. 9).

The data presented by Hong *et al.*⁴ clearly support the notion that circulating stromal-like cells do exist. There are many reports of mobilization of mesenchymal stem cells into the circulation by a variety of factors (reviewed in ref. 10). However, in most cases, the determination of these cells as mesenchymal stem cells has relied on *in vitro* assays that can be extremely artifactual¹¹, whereas Hong *et al.*⁴ have shown the true biological character of the substance P-induced CD29⁺ cells by *in vivo* transplantation, the gold standard by which to judge differentiation capacity.

However, it is unlikely that substance P is the sole factor involved in the appearance of CD29⁺ cells in the circulation after corneal injury, and more complete studies are necessary to determine the full repertoire of factors that participate in this phenomenon. Furthermore, it is yet to be determined whether this reaction to injury is specific to the cornea or whether injury

of other tissues requires substance P to liberate stromal-like cells with the same character as those identified by Hong *et al.*⁴

The major unanswered question relates to the origin of the CD29⁺ cells. The authors provide circumstantial evidence that they arise from bone marrow stroma⁴, and other studies indicate that mesenchymal stem cells can be mobilized from bone marrow by a number of factors and conditions. However, definitive proof is lacking in this study and in others. Part of this irresolution relates to the lack of rigorous criteria applied to the evaluation of the origins of circulating stromal-like cells. To date, it has not been possible to generate animal models in which BMSCs are endogenously and specifically labeled to follow their fate. Currently, there is no BMSC-specific promoter that can be used in lineage-tracking studies to definitively answer the question.

There is a great deal of variability between animal species in the numbers of circulating stromal-like cells, even in the case of injury. How the results of animal studies presented by Hong *et al.*⁴ relate to humans will ultimately determine the potential clinical utility of substance P.

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Figure 1.

Substance P helps in healing. In experiments performed by Hong *et al.*⁴, corneal injury releases the neuropeptide substance P (SP) into the circulation. This release, or direct injection of substance P, elicits the appearance of CD29⁺ (β_1 integrin subunit) cells into the circulation. These blood-borne cells become adherent in plastic culture dishes and are able to form cartilage *in vitro* and bone *in vivo*. These cells also participate in healing of the cornea.