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The Stem Cell Niche Should be a Key Issue for Cell Therapy in Regenerative Medicine

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Abstract Recent advances in stem cell research have highlighted the role played by such cells and their environment (the stem cell niche) in tissue renewal and homeostasis. The control and regulation of stem cells and their niche are remaining challenges for cell therapy and regenerative medicine on several tissues and organs. These advances are important for both, the basic knowledge of stem cell regulation, and their practical translational applications into clinical medicine. This article is primarily concerned with the mesenchymal stem cells (MSCs) and it reviews the current aspects of their own niche. We discuss on the need for a deeper understanding of the identity of this cell type and its microenvironment in order to improve the effectiveness of any cell therapy for regenerative medicine. Ex vivo reproduction of the conditions of the natural stem cell niche, when necessary, would provide success to tissue engineering. The first challenge of regenerative medicine is to find cells able to replace and/or repair the lost function of tissues and organs by disease or aging and the trophic and immunomodulatory effects recently found for MSCs open up for new opportunities. If MSCs are pericytes, as it has been proposed, perhaps it may explain the ubiquity of these cells and their possible role in miscellaneous repairs throughout the body opening for new chances for extensive tissue repair.

Keywords Stem cell niche · MSC · Pericyte · Cell therapy · Regenerative medicine · Tissue engineering

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Stem Cells and Stem Cell Niche

Adult stem cells (SCs) have been shown to be involved in natural tissue renewal. These cells maintain both the capacity of self-renewing and certain degrees of multipotency in the adult organism [1, 2]. From the early 70´s, it has been known that SCs obtained from an embryo (embryonic SC) form chimeras when injected into a host embryo [3]. Moreover, embryonic SC grafting in adult tissues leads to teratoma formation [4]. These data suggest that SCs require a specific tissue environment to develop their intrinsic potency [3].

A Stem cell niche is defined as a complex, multifactorial local microenvironment required for the maintenance of the SC biology. The SC niche consists of SCs, non-SCs, an extracellular matrix and molecular signals. Inside the niche, the SC can divide asymmetrically giving rise to both new SCs and proliferating progenitor cells. These proliferating cells give rise to a cell population that undergoes differentiation. Recent scientific advances have lead to a substantial increase in the amount of information regarding SC niche data. Some of the best-characterized SC niche models are *Drosophila* germarium or testis, vertebrate

hair follicle, intestinal crypts, bone marrow, and brain subventricular/subgranular zones [5, 6]. In vitro culture conditions of single stem cells from intestinal crypts can give rise to organoids which may behave as self-organizing structures in the absence of other non-epithelial cellular niche components [7]. All these studies are providing information on SC niche biology and SC dependence on this tissue microenvironment in several organisms along the Animal Kingdom [see 8].

Defining Equivalence and Non-Equivalence

Studies in animal models have suggested the interesting concept of non-equivalence. Two different cells are considered equivalent when they can substitute each other without altering development [see 9]. Baguñá et al., (1989) showed that neoblast-enriched cell extract from 10 different specimens of planaria were able to restore the renewing and regenerative capacity of a single planaria after X-ray irradiation [10]. This indicates that neoblasts (planarian SC) may interact with differentiated cells and extracellular matrix from the irradiated animal to re-establish its tissue renewal potency. These authors suggested that cell migration and position-specific cell recognition would mediate this cell behavior [10]. In order to reconstruct a mosaic-like organism, neoblasts would migrate to positions in the host, which are identical to the original positions in the donors. During this hypothetical event, neoblasts would differentiate in their original positions. Irrespective of their origin, an alternative regeneration-like hypothesis suggests that totipotent neoblasts could specify each new position as previous to planaria reconstruction. Under this second hypothesis, totipotent neoblasts substitute the original neoblasts at different positions and should be considered equivalent to them. For a correct definition of equivalence, the molecules involved in planaria reconstruction should be thoroughly examined. The same could be applied to SCs.

All the reviewed references on stem cells support molecular non-equivalence for both stem cells and stem cell niches. This concept could also be applied to autologous human mesenchymal stem cell (MSC) therapy. MSCs from bone marrow grafted into a different bone (irradiated or severely fractured) can reconstruct the tissue. However, grafted MSCs are molecularly dissimilar to resident bone MSCs. Thus, donor's and host's bone marrow cells might be functionally equivalent but molecularly non-equivalent. Future studies might clarify functional equivalence and transcriptional non-equivalence, given that both processes are useful for a complete definition of cell therapies. For instance, MSCs from a donor's iliac ridge could be used to heal a fracture non-union in a humerus, autologously or in an immunocompatible recipient. This would lead to humerus reconstruction with iliac ridge tissue. When existing, bone type markers should help verifying this concept of molecular non-equivalence. Once this technique is available, regenerative medicine should use it to restitute molecular equivalence to the patients during cell therapies in order to "regenerate" their original organs. Available techniques are only partially reparative, and no regenerative treatments can be applied nowadays.

Cell Therapy for Healing

The goal of SC-based therapies is the in vitro expansion of tissue-specific cells to heal damaged tissues. The finding that the MSCs administration to patients produced recovery through trophic and immunomodulatory effects has prompted the development of numerous clinical trials that may hopefully lead to new healing therapies [11–14]. Such effects are owed to the action on the resident cells rather than to the action on the implanted cells themselves. Nevertheless, the recovery mediated by these trophic and immunomodulation functions of SCs is still far from the real goal of regenerative medicine, which is the replacement of the lost cells in the damaged tissue with new healthy ones, which are also specific of that particular tissue. So far, few studies have demonstrated the exact fate of the implanted MSCs in a specific injured tissue. The participation of the MSCs in the formation of the callus in fracture healing is a fact, however, the number of differentiated cells in the repair tissue does not justify the quality of the response. Technical difficulties or lack of a comprehensive space-time tracking of implanted cells may be the cause of this discrepancy. Very recently, it has been shown that MSCs systemically injected in a model of fracture healing in mice are directly responsible for the formation of callus by the in situ expression of BMP2 [15]. They also show that MSCs modulate the injury-related inflamatory response, seen through the release of several cytokines between days 1 to 7 after bone fracture. Thus, in recent years the number of results showing that MSCs have the capacity to influence the immune response mediated by T cells, interact with dendritic cells of the host, as well as being able to migrate to areas of ischemia or inflammation in different locations is increasing significantly [12, 13]. All these findings make these cells a clear promise of clinical application in diseases that go beyond the field of single regenerative medicine. The use of MSCs to treat neurodegenerative pathologies which, although diverse, have a common pathological feature is no longer a utopian proposal, but a reality at hand (14).

The combination of the functions of MSCs such as paracrine secretion, immunomodulatory capacity, low immunogenicity and lost cells replacement, should be the objective of efficient regenerative medicine. If we add the

real possibility of ex vivo genetic manipulation, MSCs may be powerful therapeutic tools for a variety of pathologies.

Hematopoietic stem cells (HSCs) have been the subject of intense research for almost 50 years, from the pioneering work of Till and McCulloch [16]. Most modalities of cell therapy should recapitulate previous knowledge in HSC research. Current bone marrow transplant, a therapy for blood cell diseases, represents an optimal strategy involving both SC and the SC niche. In this therapeutic procedure every immature cell of the patient is inactivated or destroyed through chemotherapy and then replenished with transplanted HSCs and progenitor cells from an immunocompatible donor, which repopulate the preserved host SC niche. This cell therapy together with the trophic and immunomodulatory effects of MSCs, open an encouraging door for an allogeneic MSC therapy, even for genetic deficiencies, autoimmune diseases, etc. [17]. MSCs as "universal donors" for the treatment of autoimmune diseases may be a substantial clinical advantage. Although discrepancies in the effects of MSCs on T lymphocyte proliferation have been recently shown, in a murine model [18], the use of these cells in treating autoimmune diseases is a reality not free from problems [19–22]. Type 1 diabetes [23], autoimmune encephalomyelitis [24], systemic lupus erythematosus [25], autoimmune glomerulonephritis [26] and acute graft-versus-host disease [27], are only a few recent examples of MSC therapies.

Genetic deficiency are another group of diseases that can benefit from MSC transplantation. Allogeneic or autologous MSCs, in this case after genetic correction, when injected systemically are also developing as a therapeutic possibility. Osteogenesis imperfecta is one of the main focuses of activity [28–32].

The success of HSC therapy has given rise to extensive research on hematopoietic SC niche that has led to a detailed knowledge of its cellular and molecular components [6, 33–35]. An endostheal and a vascular component have been described in this niche, and differentiated cells such as osteoblasts, stromal cells, and even fibroblasts are proposed to constitute the non-equivalent hematopoietic SC niche. Osteoblast lineage of the HSC niche in vitro rapidly expands after treatment with cyclophosphamide/granulocyte colony-stimulating factor [36]. The resulting osteoblasts do not lose their functional qualities as far as the regulation of HSC is concerned [36]. We must assume that these differentiated elements of the HSC niche are not affected by chemotherapy treatment. Similar to hematopoietic progenitors transplant, bone autografts have long been used in orthopaedic surgery**.** Once again, the great success of such type of operations comes from the use of non-equivalent SCs and SC niches in the area that is to be repaired.

All the above empirical findings support the notion that cell therapy should use undifferentiated cells better than differentiated ones. One can predict that using terminally differentiated cells, therapy would expire quickly; it would restore defects fleetingly, without restoring tissue renewal ability in the long term. A cell therapy that is based on stem and progenitor cells would be perhaps a much more lasting, permanent solution. This argument is contradicted by the fact that undifferentiated cells could suffer uncontrolled proliferation into the host that could go towards cell transformation. But this will not happen when using cells coming from adults, even after in vitro culture, providing that the number of passages is not high. Although the mechanisms controlling MSC proliferation, senescence or transformation remain unclear, some significant developments have recently been made [37–41]. Several reports have shown certain human MSC transformations when they are cultured in vitro, even proposing certain implicated molecular mechanisms. All these works seem to rule out the tendency of MSCs to transform spontaneously, because they seem to mediate the presence of certain "contaminating" tumor cells that would trigger the proliferation in culture, after MSC senescence. Moreover, the presence of active MSCs seems to prevent the proliferation of tumor cells in such cultures [39], as indeed has been demonstrated in other situations [42–44]. What remains true in any case is that human MSCs cultures have a high rate of aneuploid cells, although these crops do not seem to generate tumors when inoculated into immunocompromised animals (A. Bernad, pers. comm.).

So, ideally, any new cell therapy trial should follow the mentioned successful strategy, in which both SCs and SC niches are taken into account. With some exceptions (tendon reconstruction [45], skin tissue engineering [46] and bone marrow transplant), there is not enough knowledge yet about other therapies where SCs and the SC niche are considered. Understanding SC niche basic biology seems to be an important scientific goal in order to develop efficient cell therapies.

On the Origin of SC for Cell Therapy

Protocols for adult SC in vitro amplification have been developed in the scientific community seeking for a source of SC for therapies. Indeed, there are concrete sources of stem cells ready to be used in clinical trials. Epidermis and certain dermis elements can be grown in vitro and then grafted back into the patient [47], but MSCs are one of the most used in several cell therapies.

Mesenchymal stem cells can be isolated, grown as adherent monolayers or as 3D cultures, and induced to form bone, cartilage and other tissues [48–50]. However,

their location in adult tissues is still elusive. Bone marrow MSCs have been considered to be stromal cells, and called 'multipotent stromal mesenchymal stem cells', 'mesenchymal stromal cells' or just 'stromal stem cells' [51–57]. Stromal cells, as differentiated cells of the bone marrow, are part of the HSC niche. Other differentiated mesenchymal cell types have also been considered MSCs, such as in vitro cultured dedifferentiated chondroblasts [58] and fibroblasts, although, as regarding to fibroblasts, this hypothesis was dismissed [59].

Recently, pericyte, cells of the perivascular space, have been identified as MSCs [51, 59, 60]. MSCs/pericytes stabilize blood vessel walls and support tissue and immune system homeostasis. Pericytes also proliferate and differentiate to reconstruct injured blood vessel walls and injured tissue, and they induce resident SCs to differentiate in the surrounding tissues. Indeed, evidence of pericyte to chondrocyte transition during fracture healing was described long ago [61]. Similarly, blood vessel microenvironment has been postulated to be a key component of the adult stem cell niche at the subventricular zone (SVZ) in the brain [62].

The picture of the MSC niche is just emerging. Caplan (1994) proposed that mesenchymal formation occurs at the bone marrow during the process he named "mesengenesis" [63]. Bone marrow "mesengenesis" was proposed to be the source of all progenitor cells needed for mesenchyme-derived tissue homeostasis in the adult organism. These bone marrow derived progenitors would reach their target tissues through the blood stream. Authors supporting this idea suppose that active molecules coming from the injured places and released into the blood stream would reach the MSCs in the bone marrow. These active molecules would change adhesion molecules expression allowing MSCs to escape from their niche [64]. Although some authors have reported evidence of MSCs in systemic circulation [65], there is no evidence for real MSC homing in humans, except for cases of local homing, i.e. homing from a short distance to the injured site (local homing) [52]. The recent demonstration of pericyte as a MSC supports the ubiquitous existence of MSCs at ubiquitous vascular SC niche [51, 60, 66, 67] ruling out the necessity of a circulating stem cell. There is a higher chance for MSCs to migrate to local or distant tissues in response to systemic influences if MSCs are resident in a perivascular niche throughout the body. Following these results, Caplan (2009) argues in favor of a perivascular location of the MSC niche, "sit as a functioning pericyte on vasculature in every tissue of the body (except avascular cartilage)" [11]. All those arguments may probably explain natural tissue repair and homeostasis long life span. They may also explain the use of cultured stromal vascular fraction of adipose tissue as a source of MSCs amplified for cell therapy. Resident cells within the walls of fat tissue blood vessels may originate MSCs [68]. However, this hypothesis does not properly explain the nature of MSCs coming from cultures of bone marrow stromal fraction. Although stromal cells in the bone marrow do not form the vasculature of this organ, molecular identification should provide tools for comparison between MSCs and bone marrow stromal cells. A close relationship, and even identification, between multipotent bone marrow stromal cells and perycites has been demonstrated in a mouse model [69]. They showed how stromal cells selected from bone marrow through a maker of pericytes, the smooth muscle actin-α, stimulated endothelial cells to form tube-like structures and subsequently robust vascular networks in a 3D culture system.

Except in avascular tissues, pericytes would form a network of mesenchymal stem cells and MSC-niches through the body. Pericytes at the injured tissue would both proliferate and release molecular signals to activate resident stem cells in repairing processes. Those functionally equivalent MSCs secrete a wide array and a great amount of growth factors to induce tissue-specific stem cells proliferation and differentiation into specific cells to repair damaged tissue [70]. As stated above, equivalent cells may not be genetically but functionally identical. MSCs/pericytes are site-regulated, depending on tissue damage (bone fracture, tendon lesions, miocardic infarct, brain vascular accident, etc.) and they secrete several factors to facilitate protection and regeneration [11].

All the above considerations would also lead to reconsider the mechanism of self-renewal in avascular tissues, because vascular availability and a certain density in blood vessels are necessary for a good skeletal repair or regeneration. Among avascular skeletal tissues, the articular cartilage is the more reluctant to be repaired, when natural tissue renewal fails. This could easily be understood by the absence of MSC niches at the vasculature, but, however, stratified epithelium stem cells are induced to proliferate and differentiate under the influence of MSC/pericytes which are mobilised in the neighboring vascular dermis [51].

The regeneration of tissues such as articular cartilages would depend on a neighboring synovial tissue, as it has been proposed by several authors [50, 71–73]. This interaction could depend on either neighbor synovial MSCs or resident stem cells, as it occurs in the epidermis. Perhaps, these cartilage resident stem cells might be activated by factors coming from MSCs associated to the vascular niche in the neighboring synovial tissues. The special cellular, extracellular matrix and even the architectural features of the articular cartilage have prevented further tissue engineering progress [74]. Until now, the different ways to repair the articular cartilage (mosaicplasty, cell therapy and several tissue engineering procedures) have produced new tissue that is far from the hyaline cartilage and is not

well integrated into the host tissue [75]. This establishes a functional and structural discontinuity between the complex 3D architecture of the cartilage matrix and the new tissue matrix. This leads often to the release of "scar tissue". Therefore, we must keep working on finding the right cells and how to bring them to defect. Undifferentiated cells versus differentiated or predifferentiated chondrocytes will be the choice. A permanent solution will come when the new tissue that is built in the defect is of the same nature and is perfectly integrated in the whole structure regardless of the type of pathology or age. Only then, structure and function will be fully recovered. Maybe we should deepen the knowledge of "articular cartilage niche", where the physical-chemical environment of the cells is very important for their behavior [76, 77]. Since it is easy to understand that the articular cartilage remains unchanged throughout life, a certain degree of tissue renewal should be taken into consideration. Future studies should establish if this renewal is intrinsic and/or from surrounding tissues.

Concluding Remarks and Future Perspectives

In conclusion, if the SC niche is the necessary microenvironment controlling SC fate, a cell therapy trial that does not take into account such component would not be an optimal therapy. The stem cell niche should be active in the restoring area. In such conditions, SC activity would better mimic self-repair occurring in healthy tissues. Tissue engineering should provide both cells and adequately functionalized biomaterials in order to restore the non-equivalent elements of the SC niche. Incorporating signals into the biocompatible materials that can promote desired cell functions in a spatially and temporally controlled manner; redesigning an artificial SC niche, seems to be the most suitable option [78–80]. Ex vivo reproduction of the conditions of the natural SC niche, when necessary, would make tissue engineering successful. Very recently, autologous respiratory epithelial cells and bone marrow MSCs were seeded and cultured onto a de-cellularized human donor tracheal matrix and then, the new trachea was implanted in the patient. One year later, both graft and patient are healthy, and biopsies confirm angiogenesis, viable epithelial cells and chondrocytes [81, 82]. Such experiments, although they are very complex and difficult to be interpreted because they cannot study the "internals" of experience, are very useful in order to advance in this field. Controlled experiments, in vitro and in vivo, should be made to determine the exact nature of the niche of stem cells. The ability of fragments of bone to rebuild, not only bone marrow with hematopoietic progenitors, but also the ability of such fragments to attract MSC capable to form new bone [83] has been discovered very recently. This allows us to infer that if stem cells find the appropriate niche they can differentiate properly and rebuild even organs with a certain complexity.

When regenerative medicine started several years ago, the main goal was for the implanted cells to directly participate in the reconstruction of the damaged tissue. But now, after the reported paracrine effects in several MSCs therapies we know that MSCs, far from building those tissues, they exert immunomodulatory functions, secreting, in addition, several bioactive molecules that inhibit apoptosis and scarring at sites of injury, and stimulate angiogenesis and mitosis of tissue-specific progenitors [84–86]. These actions have been found either when the implanted MSCs coming from bone marrow or adipose derived in adherent cell cultures [87] or when the mononuclear fraction of bone marrow was infused. We cannot predict the extent of the paracrine effect, immunomodulatory, or if the effective replenishment of differentiated cells can be assigned, in each case, to the MSCs, or whether these effects have some degree of integration between them. We cannot even know if all those effects can influence the surrounding tissue positively during regeneration, but perhaps negatively towards the pathogenesis of cancer and metastasis [40]. For now, we can only say that in many cases, these actions have a certain synergy to the purpose they claim. In the future, it might be useful to know the responsibility of each action in the regenerative process in order to control it appropriately. All this indicates the necessity to highlight again the importance of a tight control over the stem cell culture method in order to define the cell products for transplantation properly, according to the specific functional outcome sought.

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