CELL THERAPY OF PRIMARY MYOPATHIES

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INTRODUCTION

Through the years, the work of several laboratories brought to the identification of a myogenic potential in a large number of cells. Spontaneous activation of a myogenic differentiation program in cells of mesodermal origin cocultured with muscle cells or injected into regenerating muscle in vivo has been known for many years (9). Myogenic conversion, as this phenomenon is called, is rare and inefficient under normal conditions, although it has been observed in cells derived from non-muscular tissues as diverse as the central nervous system (17), adipose tissue (38), synovia (12) and bone marrow (16, 19).

Searching for the origin of the bone marrow cells that contribute to muscle regeneration, it has been identified, by clonal analysis, a progenitor cell derived from the embryonic aorta that shows similar morphology to satellite cells and express several myogenic and endothelial markers expressed by satellite cells. In vivo aorta-derived myogenic progenitors participate in muscle regeneration and fuse with resident satellite cells (13). These newly identified, vessel associated stem cells, the mesoangioblasts, participate in postembryonic development of the mesodermal tissues (24). Mesoangioblasts can be isolated not only from embryonic dorsal aorta, but also from other embryonic or postnatal vessels of the mouse and attempts to isolate the same cells from postnatal human tissues are in progress. As mesoangioblasts can be expanded indefinitely, are able to circulate, can efficiently differentiate in skeletal muscle and are easily transduced with viral vectors, they appeared as a potential novel strategy for the cell therapy of primary myopathies (31).

PHENOTYPE AND PROPERTIES OF MESANGIOBLASTS

The name "mesoangioblasts" was chosen to denote a common progenitor for extra-vascular and vascular mesodermal derivatives (9). Freshly established mesoangioblast clones in culture consistently express several early endothelial markers, such as Flk-1, SCA1, CD34, and VE-cadherin, but do not express late ones, such as Von Willebrand factor. In each clonal strain, a variable proportion of cells consistently

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express α smooth muscle actin (α-SMA) (24), which is known to be absent in endothelial cells, but is present in skeletal myoblasts, smooth muscle precursors and pericytes (34, 33, 20). Exposure to BMP-2 induces expression of alkaline phosphatase and other osteoblastic traits in mesoangioblasts, and adipogenesis is induced by exposure to adipogenic cocktails. Mesoangioblast cell lines maintained in long-term culture down-regulate expression of certain endothelial markers (such as VE-cadherin and c-kit) do not express either Myf5 or MyoD, are not spontaneously myogenic, but remain inducible to myogenesis upon co-culture with myoblasts. Likewise, they are inducible to cardiomyogenesis when co-cultured with cardiomyocytes (6) and turn on osteoblast-like traits upon stimulation with osteo-inductive factors. Expression of α-SMA is maintained in clonal mesoangioblast lines, which are able to differentiate into mature smooth muscle upon treatment with TGF-β (2).

Clonal cell lines of mesoangioblasts were recently compared among themselves and against a line of embryonic fibroblasts by microarray analysis (35). Interestingly, mesoangioblasts express receptors of the TGF-β/BMP family and several SMADs. This observation can explain their ability to differentiate into smooth muscle cells and into osteoblasts in response respectively to TGF-β and BMP-2. Mesoangioblasts express several pro-inflammatory genes, cytokines and cytokine receptors. This is consistent with a role of these cells in tissue regeneration and first inflammatory response to damage. Furthermore, we have recently observed that mesoangioblasts respond to HMGB1, a nuclear protein released by necrotic and by inflammatory cells (32), which is present at high levels in regenerating and dystrophic muscle. They proliferate, migrate through the endothelial layer and accumulate in vivo around beads soaked with HMGB1 and implanted into skeletal muscle after cell delivery trough the femoral artery (26).

CELL THERAPY APPROACH FOR THE TREATMENT OF MUSCULAR DYSTROPHY

Muscular dystrophies are caused by progressive degeneration of skeletal muscle fibres. The lack of proteins that form a link between the cytoskeleton and the basal lamina increases the probability of damage during contraction and leads to fiber degeneration. In Duchenne muscular dystrophy, the most severe case of primary myophaty, respiratory and cardiac functions are affected, leading to respiratory failure and premature death (15). Fiber degeneration is counterbalanced by regeneration of new fibers at the expense of satellite cells (23). However in the most severe forms, the pool of satellite cells is exhausted. Moreover, the satellite cells of the patients with muscular dystrophy carry the same mutation responsible for the fiber degeneration observed in these patients. At the moment the only effective treatment available consists in the administration of antiinflammatory drugs, like corticosteroids (4) with relatively modest beneficial consequences and several side effects. For this reason novel experimental approaches are currently under study. They can be schematically grouped in three major areas (i) new pharmacological strategies, (ii)

in vivo gene therapy and (iii) cell therapy. Outstanding advances in each of these therapeutical approaches have been recently reviewed (11).

The first cell therapy approaches for the treatment of muscular dystropy reported in literature started with satellite cells and cell lines derived from them. (27, 28). Myogenic cell transplantation was continued and many efforts were made by different laboratories for optimizing the protocols (21). Nevertheless the major problem still faced by this approach is the very limited migratory capacity of injected cells, which renders difficult to reach an even distribution within the whole muscle. This might be overcome by using circulating stem or progenitor cells wich are able to cross the endothelial layer and distribute widely within the muscle. This perspective became possible, at least from a theoretical point of view, when it was demonstrated that murine bone marrow contains transplantable progenitors that can be recruited to an injured muscle through the peripheral circulation and can participate in muscle repair by undergoing differentiation into muscle fibers (16). It was susequently reported that a fraction of bone marrow cells, the SP (side population) cells would give rise to dystrophin-positive fibers in the mdx mouse following bone marrow transplantation (18). Recently, retrospective analysis in a Duchenne patient that had undergone bone marrow transplantation confirmed the persistence of donor-derived skeletal muscle cells over many years (19). In all these studies, the extent of colonization by donor cells was very small. During the last two years several reports have convincingly confirmed that bone marrow SPcells can be recruited to dystrophic or regenerating muscle and can differentiate into skeletal muscle; moreover, a fraction of SP cells localizes to a position (between the basal lamina and the sarcolemma) that is typical of satellite cells and expresses markers of satellite cells (1, 22, 29). Two independent groups showed that the progeny of a single SP cell can reconstitute the hematopoietic system of a mouse following bone marrow transplant and can also contribute cells to regenerating muscle (3, 7). Interestingly cells expressing the hematopoietic marker AC133 that can differentiate into dystrophin-positive fibers in vivo are present in the human circulation, suggesting that strategies developed in murine models might later be transferred to patients (37).

Evidence that SP cells are not the only mesoderm progenitor that can differentiate into skeletal muscle comes from several studies showing that different CD45 negative cells, such as multipotent adult progenitors (MAPs) (29), mesoangioblasts (24) or muscle-derived stem cells (MDSCs) (5), can differentiate into skeletal myotubes in vitro or in vivo when delivered to regenerating or dystrophic muscle. The expression of CD45 in SP cells clearly defines the hematopoietic nature of these cells; the lack of CD45 in these other types of stem cells, which are generally associated with the vascular niche in bone marrow, skeletal muscle (5, 36) or other tissues (12), identifies them as non-hematopoietic and probably belonging to the endothelial or pericyte lineages (9).

Two alternatives cell therapy approaches can be followed: (i) using cells obtained from a healthy donor, which express the normal copy of the mutated gene but induce an immune rejection unless the patient is permanently immune suppressed; or (ii) using cells obtained from the patient, which do not require immune suppression but

must be 'genetically corrected' in vitro to restore the expression of the mutated protein. In order to avoid the side effects derived from a permanent immuno suppression the second approach seems to be preferable. In principle, the most attractive scenario for clinical application requires the possibility to isolate cells from an easily accessible anatomical site, the possibility to expand them in vitro without the loss of both self-renewing capacity and myogenic differentiation ability; the possibility to correct them genetically by inserting a functional, appropriately regulated copy of a missing gene (i.e. dystrophin) and then the possibility to transplant them in sufficient numbers by an appropriate delivery route without inducing a immunological response despite the presence of a new protein (coded by the therapeutic gene) (10).

SP cells are still difficult to expand in vitro and the frequency of myogenic differentiation appear to be low, far from any hope of clinical benefit (29). The potential to contribute to muscle regeneration in dystrophic muscle by intra-vascular delivery of most CD45 negative stem cells has not yet been precisely evaluated with the exception of mesoangioblasts. Mesoangioblasts can be isolated from postnatal tissues and maintained in culture for > 60 passages without entering senescence. Moreover they are easy to transduce ex vivo with lentiviral vectors, which can encode therapeutic genes under the control of an appropriate promoter, which should guarantee a long-term expression. Finally, when injected into the blood circulation, mesangioblasts are able to circulate, accumulate in the first capillary filter they encounter and, in presence of inflammation; they can migrate outside of the vessels and significantly contribute to the regeneration of dystrophic muscle (Fig. 1). Indeed, intra-arterial delivery of wild type mesangioblasts in the α-sarcoglycan knockout mouse (14), a model for limb-girdle muscular dystrophy, corrects morphologically and functionally the dystrophic phenotype of all the muscles downstream of the injected vessels. Furthermore, mesoangioblasts isolated from a α-sarcoglycan knockout mouse and transduced with a α-sarcoglycan expressing lentiviral vector, injected into the femoral artery of KO mice seem to be efficacious in the amelioration of the dystrophic phenotype similarly to wt cells (31). These data represent the first successful attempt to treat a murine model of limb-girdle myopathy with autologous stem cells.

CONCLUSION AND FUTURE DIRECTIONS

In conclusion, of the many types of stem cells with a myogenic potential only the vessel-associated mesoangioblasts have been shown so far to be effective in restoring to a significant extent the phenotype in a murine models of muscular dystrophy. However, functional correction of a large muscle may be a different problem, probably calling for experimentation in a large animal model like the dystrophic dog. It is likely that cell therapy in this animal will bring further information about the feasibility of similar protocols in dystrophic patients. Mesoangioblasts express many but not all of the proteins that leukocytes use to adhere to the endothelium and extravasate (35). This may explain why only 30% of injected mesoangioblasts end up in
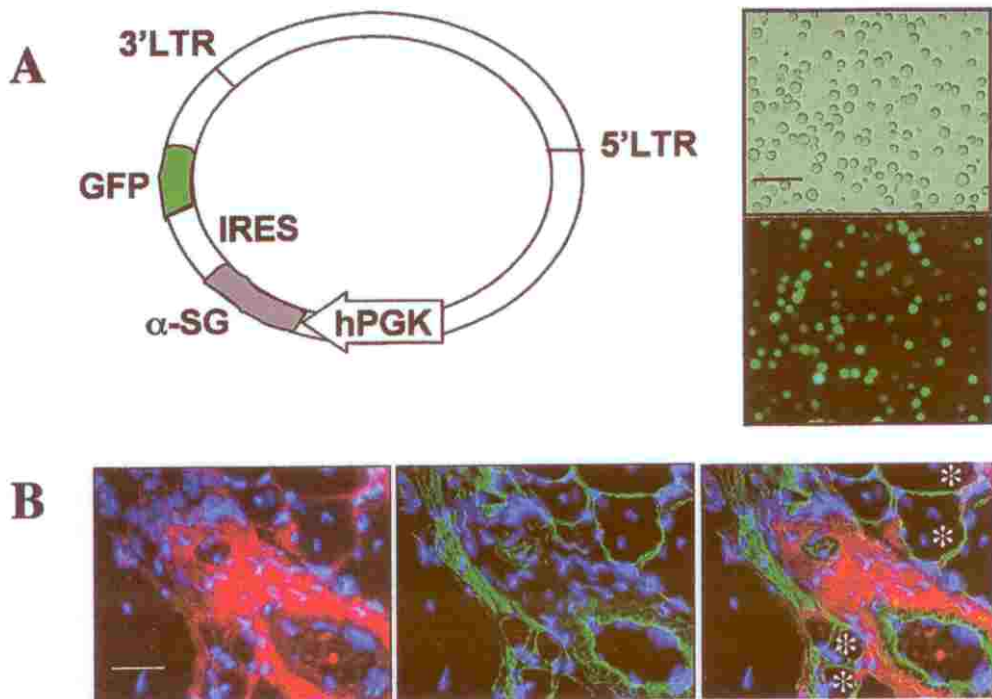
Fig. 1. - *Immunofluorescence analysis of tibialis anterior muscle after autologous cell therapy.*
A, lentiviral vector construct, carrying the GFP reporter and α-SG genes under the control of human PGK promoter (hPGK); in the right upper panel phase contrast of dystrophic mesoangioblasts infected with the lentiviruses and expressing GFP reporter gene (lower panel). B, double staining with anti-laminin (green) or anti α-SG (red); nuclei in blue (hoechst); several cells expressing α-SG are showed after 2 weeks from transplantation through the femoral artery; * = regenerating fibers expressing α-SG, in the merged panel; bar = 50 μm. Methods and analysis see reference 31.

downstream skeletal muscle (31), opening perspectives to optimize this process for future cell therapy protocols in larger animals or in patients, by using molecules that can recruit mesoangioblasts and facilitate their homing to diseased muscle. Finally, preliminary work suggests that mesoangioblasts can be isolated from human patients. If this expectation will be confirmed and the work on large animal models will give encouraging results, the beginning, in relatively few years, of clinical trials with these stem cells could be hypotized.

SUMMARY

Mesoangioblasts are multipotent progenitors of mesodermal tissues. In vitro mesoangioblasts differentiate into many mesoderm cell types, such as smooth, cardiac and striated muscle, bone and endothelium. After transplantation mesoangioblasts colonize mostly mesoderm tissues and differentiate into many cell types of the

mesoderm. When delivered through the arterial circulation, mesoangioblasts significantly restore skeletal muscle structure and function in a mouse model of muscular dystrophy. Their ability to extensively self-renew in vitro, while retaining multipotency, qualifies mesoangioblasts as a novel class of stem cells. Phenotype, properties and possible origin of mesoangioblasts are addressed in the first part of this paper. In the second part we will focus on the cell therapy approach for the treatment of Muscular Dystrophy and we will describe why mesangioblasts appear to be promising candidates for this strategy.

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