

Giulio Cossu, Stem Cell Research Institute, Dibit, HSR. Milan, Italy.  
Mesoangioblast transplantation as a cell therapy for muscular dystrophy

In the last years several types of mesoderm stem cells have been identified and tentatively characterized in the bone marrow and other tissues of the adult, as well as in the developing vessels of the fetus. Most if not all these progenitors appear to be associated with the micro-vascular niche. It is currently unknown how many different mesoderm progenitor/stem cells (mesenchymal stem cells/multipotent adult progenitors/ mesoangioblasts) exist in mammals. Similarly unknown are their reciprocal lineage relationships. In 1998 we reported that the bone marrow contains progenitors able to differentiate into skeletal muscle following bone marrow transplantation (BMT) into lethally-irradiated recipient mice. Searching for the origin of these progenitors, we identified cells that are physically associated with the embryonic dorsal aorta in avian and mammalian embryo and can grow extensively in vitro. We termed these cells “mesoangioblasts”. When transplanted in vivo, mesoangioblasts give rise to multiple differentiated mesoderm phenotypes such as smooth and skeletal muscle, cartilage and bone. Their ability to extensively self-renew in vitro, while retaining multipotency, qualifies mesoangioblasts as a novel class of stem cells. Mesoangioblasts express initially a number of early endothelial markers (Flk-1, Tie-2, CD34 and Kit) but with time in culture they loose expression of many endothelial markers while acquiring markers of pericytes such as Sca-1 and Smooth alpha actin. This suggests that culture conditions select the growth of a cell type probably representing an angioblast in the process of producing a perithelial cell. Thus mesoangioblasts not only emerge as an unexpected source of progenitors for skeletal muscle and a variety of other mesoderm-derived tissues, but also reveal a lineage relationship between progenitors of vascular and extra-vascular mesodermal tissues, with important basic and applied implications. When both wild type or dystrophic, genetically corrected, mesoangioblasts were delivered intra-arterially to dystrophic muscle of - sarcoglycan KO mice (a model for limb girdle muscular dystrophy), they resulted in a dramatic functional amelioration of the dystrophic phenotype. This was due to the widespread distribution of donor cells through the capillary network and to an intrinsic defect of proliferation in the resident satellite cells, a situation that created a selective advantage for donor cells.

In order to proceed towards clinical trials with significant hopes of success it is necessary to address and solve the following issues: 1. to improve the efficiency of homing to skeletal muscle; 2. to isolate and characterize human mesoangioblasts from an accessible source; 3. to test the model in a large, non syngenic animal model.

Homing: Mesoangioblasts, vessel-associated stem cells, can be induced to transmigrate through endothelium in presence of myotubes or cytokines, among which SDF-1 or TNF- $\alpha$  appeared as the most potent. GFP labeled mesoangioblasts were delivered through the femoral artery to regenerating

muscles of normal as well as dystrophic (mdx or  $\alpha$ -SG null) mice. By a quantitative PCR analysis their homing to dystrophic muscles in vivo, was measured and found to be quite modest (about 10% of injected cells) although higher than other types of stem cells. Pretreatment of these mesoangioblasts with TNF- $\alpha$  or SDF-1 (and to a lesser extent with other cytokines) induced a two to fourfold increase in homing to muscles. Transient expression of  $\alpha$ 4 integrins or L-selectin, also increased mesoangioblast transmigration and homing to skeletal muscles without affecting their differentiation potency. Therefore, combined pretreatment with SDF-1 or TNF- $\alpha$  and expression of  $\alpha$ 4 integrin generates a potent kind of modified mesoangioblast, 50% of which, after intra-arterial injection in  $\alpha$ -sg null dystrophic mice, home to damaged muscles and differentiate into skeletal muscles expressing  $\alpha$ -sarcoglycan.

We recently isolated human mesoangioblast-like cells from small fragments of interstitial tissues from biopsies of human skeletal muscle or other tissues. Under appropriate culture conditions, human mesoangioblasts can be expanded in culture up to at least 40 divisions while maintaining the same morphology, a diploid karyotype and the inability to form tumors in nude mice. Both at early at late passages these cells express a number of surface antigens that distinguish them from both mesenchymal stem cells or multipotent adult progenitors. A genome wide expression analysis confirmed that human mesoangioblasts represent a unique type of mesoderm stem cell. Moreover these cells are able to differentiate into skeletal muscle when co-cultured with mouse myoblasts or when injected into SCID-mdx mice, where they restore a large percentage of fibers expressing human dystrophin. Mesoangioblasts can also be isolated from biopsies of Duchenne patients and show in vitro an identical behaviour to their normal counterparts. When transduced with a lentiviral vector encoding human mini-dystrophin, dystrophic mesoangioblasts also give rise to dystrophin positive fibers in mdx-SCID mice. Because of these features, human mesoangioblasts represent an excellent example of a human cell population with demonstrated potency to restore successfully dystrophin and an obvious first choice for future cell therapy protocols in patients.

Finally, that intra-arterial delivery of wild type, non DLA matched mesoangioblasts resulted in a partial recovery of muscle morphology and function, dystrophin expression and clinical amelioration, which persisted for at least five months after removal of immune suppression. Treatment at early age was essential to achieve significant results. Delivery of autologous mesoangioblasts expressing human micro-dystrophin did not cause a comparable amelioration, despite widespread micro-dystrophin expression. These results show efficacy of cell therapy in a large, immuno-competent animal and set the rationale for a future clinical trial.