DIFFERENTIATION OF MUSCLE DERIVED STEM CELLS INTO MYOFIBROBLASTS IN INJURED SKELETAL MUSCLE: IMPLICATIONS FOR MUSCLE FIBROSIS

Introduction: Injured muscle can promptly initiate regeneration by activating myogenic cells to proliferate and differentiate into myotubes and myofibers. However, it seems that this prompt regeneration does not significantly help the functional recovery of the injured muscle because its full recovery is often hindered by the development of scar tissue. The question that then arises is what happens to the numerous activated regenerative cells that are present in the injured skeletal muscle. In response to this, we hypothesized that the early-appearing myogenic cells in the injured area differentiate into myofibroblasts and eventually contribute to the development of fibrosis. To investigate this, we transplanted a genetically engineered clonal population of muscle-derived stem cells (MC13 cells) into the skeletal muscle of SCID mice, which were injured 4 weeks post-transplantation. The MC13 cells regenerated numerous myofibers in the non-injured muscle; however, these myogenic cells gradually differentiated into fibroblastic cells upon muscle injury. Our results suggest that the release of local stimuli after muscle injury trigger the differentiation of myogenic cells including muscle stem cells into fibroblastic cells.

Methods: Cell transplantation and development of muscle laceration: Thirty SCID mice were separated into 5 groups. After receiving anesthesia, 1×10^6 MC13 cells were injected into the gastrocnemius muscles (GMs) on both sides of the SCID mice. Four weeks later, the injected muscle from the left legs was injured by laceration. The injected muscles of the right legs were non-injured and used as control. At different time points after transplantation and laceration injury, the GMs from each leg were taken for either preparation for primary cell culture via the preplate technique and/or for assessment by histology and immunohistochemistry. The isolated donor derived muscle cells from the injured and non-injured skeletal muscle: We isolated the injected GMs at different time points post-injury from both the control and injured muscles using a technique previously described. The isolated cells were suspended in medium (DMEM+ 20%FBS) and were seeded onto collagen-coated flasks. Different generations of preplate cells were separated from pp1 to pp6. Normally, the pp1 and pp2 populations of cells are mostly fibroblasts since they are 5-15% desmin positive and some of them express α-SMA. In contrast, the pp4 and pp5 fraction of cells were highly enriched for desmin positive cells (>80%). The isolated cells (pp1/pp2 and pp4/pp5) from both injured and non-injured skeletal muscles were stained for β-galactosidase. For each population of cells (pp1/pp2 and pp4/pp5) isolated from injured and control skeletal muscles, a minimum of 10 cells was analyzed for LacZ expression. Statistical significance was carried out by a Student’s t-test.

Results: 1) Muscle derived stem cells differentiate into myofibers after transplantation in normal non-injured skeletal muscle (control): The injection of MC13 cells within skeletal muscle resulted in the formation of many regenerating myofibers from 6 weeks post-transplantation. At 9 weeks post-implantation, the injected site contained numerous large myofibers expressing the β-galactosidase reporter gene (Fig. 1). 2) Muscle derived stem cells differentiate into myofibroblasts in lacerated skeletal muscle by laceration: The fate of the injected cells was analyzed as described above at 2 and 5 weeks post-laceration, which also represents 6 and 9 weeks post-transplantation, respectively. As in the non-injured skeletal muscle at 6 weeks post-implantation, many LacZ-expressing myofibers were found in the injured sites at 2 weeks post-laceration. However, at 5 weeks post-laceration, the development of a large scar tissue was found in the injured skeletal muscle. Immunohistochemical staining revealed that the β-galactosidase positive scar tissue area colocalized with high levels of α-SMA and vimentin expression (Fig. 1). 3) Gradual differentiation of muscle derived stem cells toward myofibroblast at different time points post-injury: The LacZ positive cells derived from the injured skeletal muscles at 1 week post-injury (5 weeks post-transplantation) were mostly isolated from the pp4/pp5 fraction (Fig. 2). Interestingly, at 3 weeks post-injury (7 weeks post-transplantation) the number of LacZ positive cells in the pp4/pp5 fraction was similar to that of the pp1/pp2 fraction. Finally, at 5 weeks post-injury (9 weeks post-transplantation) most of the LacZ positive cells were found in the pp1/pp2 fraction (Fig. 2). In contrast, the injected cells from the non-injured muscle at 5, 7, 9 weeks post-transplantation were always isolated in the pp4/pp5 fraction (Fig. 2). These results further confirm that upon muscle injury, the MC13 cells gradually differentiate from a myogenic state toward a myofibroblastic lineage.

Discussion: In muscle injuries, the release of growth factors at the injured site is an important step in the healing process. These growth factors can stimulate the growth and differentiation of various muscle-derived cells. In fact, various growth factors have been identified to promote myoblast proliferation and differentiation, which can eventually promote muscle regeneration and healing post-injury. However, some growth factors such as TGF-β1 and PDGF are also highly expressed and are likely to be involved in the development of muscle fibrosis. These growth factors may trigger the differentiation of the MC13 cells into a myofibroblastic lineage after injury. We report here that myogenic cells present in the injured site, including muscle-derived stem cells, can differentiate into fibroblastic cells upon stimulation from muscle injury. However, the loss of numerous regenerating myofibroblasts after muscle injury implies that not only early myogenic precursors, but also myofibroblasts, myotubes and myofibers are triggered to differentiate into fibroblastic tissue. These results may also explain the process of scar tissue formation, which often occurs in disease states such as Duchenne’s muscular dystrophy. These observations may help to shed light on the process by which fibrosis develops after muscle injury and eventually help in the design of biological approaches that can block muscle fibrosis.

Acknowledgements: The authors wish to thank Marcelle Pellerin, Ryan Pruchnic, and Arvydas Usas for technical assistance.

References: