Regeneration and trans-differentiation potential of muscle derived stem cells

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Introduction and objectives

Recent studies have indicated the prevalence and importance of adult stem cells in development, maintenance and regeneration of various tissues. These studies resulted in new insights into major aspects of developmental biology and aging and raised hopes for new approaches for the therapy of degenerative diseases. Muscle stem cells are of special interest as an excellent, easy accessible cell type, with well-characterized markers and transcription factors associated with its various differentiation stages. It is relatively easy to clone and manipulate in culture; thus offering a convenient model system. Muscle progenitor cells are also promising candidates for treating muscle degenerative diseases and perhaps as a source for replacement of other cell types. However, therapeutic approaches for skeletal muscle degeneration, by cell transplantation have been hindered by poor cellular survival rates and restricted cell sources. Major efforts were made to identify the most suitable cells for transplantation. As described below, we have isolated muscle precursor cells from adult skeletal muscle. Our main goal is to further characterize the biological nature of these cells and their capacity to participate in the regeneration of degenerated or injured muscles and other damaged tissues.

Recent findings:

Isolation and characterization of muscle precursor cells that propagate in culture as floating cells (myospheres):

Using the differential plating technique and cell cloning, we have established in the past several myogenic cell lines. Following recent reports on the possible existence of slower adherent stem cells in skeletal muscle cell cultures, we have used a modified differential plating technique to identify and isolate, from mouse skeletal muscles, a very slow adherent cell population, which can be propagated in suspension as unattached clusters of pure populations of muscle precursor cells (called myospheres) (Fig. 1). These cells could be propagated, by serial passages, as suspended myospheres, for at least several months without losing their proliferation capacity. When myospheres grown in gelatin coated plates are left for several days in the same plate, many of them adhere to the plate, start to spread out and form a monolayer of MyoD positive cells. Unlike previously described myogenic cell lines, most of the myosphere cells differentiate, without cell fusion, into thin mononucleated contractile fibers, which express myogenin and skeletal muscle myosin heavy chain (Fig. 1). The presence of Pax-7 in a significant

Fig. 1 Cultures of myospheres (A) Myospheres grown in suspension (B) Outgrowth of myogenic cells from adherent myospheres (C) Myospheres cells grown as an adherent monolayer. Myosphere cells adhere to the plate as rounded or spindle shaped cells. (D) A monolayer of cloned myospheres cells. (E) Magnification of D. (F) After several passages of the adherent cells, foci of cells fusing into thick multinucleated fibers start to appear.
proportion of these cells suggests that they originate from satellite cells. Addition of leukemia inhibitory factor (LIF) to the growth medium of the myospheres enhances proliferation and dramatically increases the proportion of cells expressing Sca-1, a specific marker of several types of stem cells. When inoculated into injured muscle, myosphere derived cells participated in regeneration, forming multinucleated cross-striated mature fibers (Fig. 2).

The trans-differentiation capacity of myosphere cells

Our study also focuses on the open basic question, which is still a matter of controversy, of whether intact cells that are already programmed to a specific differentiation pathway can be reprogrammed into another differentiation fate. In addition to the basic biological interest, this question has also potential important clinical implications.

Exposure of myosphere cells to BMP-4 resulted in suppression of myogenic differentiation and induction of osteogenic markers such as alkaline-phosphatase and osteocalcin. Removal of the BMP-4 resulted in regaining of the myogenic phenotype, and disappearance of the osteogenic markers. Myosphere derived cells also sporadically differentiate to adipocytes. Myosphere cells could not, so far, be induced to trans-differentiate to hematopoietic cells.

For our work on the Duchenne Muscular Dystrophy gene please see the abstract by Nudel et al.

Selected publications:
Sarig, R., Baruchi, Z., Fuchs, O., Nudel, U., and Yaffe, D. Regeneration and trans-differentiation potential of muscle derived stem cells propagated as myospheres. Stem Cells, in press.

Acknowledgements
This research was supported by the Association Francaise contre les Myopathies (AFM)