Abstract: Skeletal muscle of the vertebrate embryo originates from paraxial mesoderm (somites, somitomers and prechordal cephalic mesoderm) (Christ and Ordahl, 1995) and is formed in discrete steps by different classes of myogenic progenitor cells (Cossu and Biressi, 2005). After myotome formation, embryonic myoblasts give rise to primary fibers in the embryo, while fetal myoblasts give rise to secondary fibers, initially smaller and surrounding primary fibers. Satellite cells appear underneath the newly formed basal lamina that develops around each muscle fiber, and contribute to their post-natal growth and regeneration (Bischoff, 1994). In addition to canonical progenitors, evidence accumulated through the years that cells cultured from tissues that do not derive from paraxial mesoderm and do not contain skeletal muscle such as thymus, brain or kidney may differentiate at low frequency into skeletal muscle. Initially dismissed as a tissue culture artifact, the phenomenon came under closer scrutiny when it was unequivocally demonstrated that the bone marrow of adult normal mice contain cells capable of contributing to skeletal muscle regeneration in vivo (Ferrari et al., 1998). In the following years, different types of non-somitic stem-progenitor cells have been shown to contribute to muscle regeneration. The origin of these different cell types and their possible lineage relationships with other myogenic cells as well as their possible role in muscle regeneration is actively studied in these years and will be the subject of this chapter. Finally, the possible use of different non-canonic myogenic cells in experimental protocols of cell therapy will be briefly outlined.

Keywords: Skeletal myogenesis; muscle satellite cells; skeletal myoblasts; mesoangioblasts; muscle regeneration.
Abbreviations: BMP2: Bone morphogenetic protein 2; GFP: green fluorescent protein; HSC: hematopoietic stem cell; MSC: mesoderm stem cell (referred to as non hematopoietic); PKC: protein kinase C; Shh: Sonic hedgehog; SP: side population; TGF β: transforming growth factor β.

1. A BRIEF HISTORY OF UNORTHODOX MYOGENESIS AND OF ITS POSSIBLE SIGNIFICANCE IN REGENERATION

Myogenic progenitor cells, termed myoblasts, have been isolated and cultured since the early 60’ of the last century. Originally isolated from the muscle anlagen of avian embryos, myoblasts were later cultured from muscles of virtually all vertebrates, both embryonic and adult. Removal or consumption of growth factors (often provided as serum or embryo extracts) induces irreversible withdrawal from the cell cycle and terminal differentiation of myoblasts that fuse into multinucleated myotubes. During further maturation, which occurs only partially in vitro, myotubes complete sarcomerogenesis, assemble a functional excitation-contraction coupling system and contract in response to appropriate stimuli (Okazaki and Holtzer, 1966).

Because they are easily recognized morphologically in living cultures, myotubes were occasionally observed in cultures of cells that were not myogenic nor derived from tissues that in vivo contain skeletal muscle. These observations remained anecdotic and largely unpublished, also because they lacked a rational explanation. “Contamination with myogenic cells during isolation” or “tissue culture artifact” represented the easiest interpretations of these data (Cossu, 1997).

Nevertheless papers accumulated through the years, some of which reporting solid and unquestionable data. Perhaps the most striking example is represented by the thymus that is derived from pharyngeal pouches and does not contain any skeletal muscle fiber. In 1975, it was reported the occurrence of striated muscle fiber differentiation in monolayer cultures of adult thymus reticulum (Wekerle et al., 1975). Later it was reported that in the thymus from adult but not neonatal mice, MyoD or myogenin-positive cells are concentrated in the medullary region but do not differentiate within the normal murine thymic environment. However, myogenesis takes place both in vitro, as demonstrated in the original paper, and in vivo, upon transplantation into regenerating muscle (Grounds et al., 1992).

Another example is represented by the so called “myogenic conversion of fibroblasts” originating from dermis and, to different extent, other mesoderm tissues. The first example of this phenomenon was the correction by fibroblast-myoblast fusion of the genetic defect of the mdg mouse mutant muscle fibers (Chaudhary et al., 1989; Courbin et al., 1989). Subsequently, several groups reported that genetically labeled dermal fibroblasts could be incorporated into differentiated myotubes both in vitro and in vivo (Gibson et al., 1995; Breton et al., 1995; Salvatori