



## Myogenic Satellite Cells and Its Application in Animals - A Review

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**ABSTRACT :** Myogenic satellite cells have been isolated and identified by several recently elucidated molecular markers. Furthermore, knowledge about the precise function of these markers has provided insight into the early and terminal events of satellite cells during proliferation, differentiation, transdifferentiation, specification and activation. Recently, quiescent myogenic satellite cells have been associated with possession of Pax 3 and 7 that represent pluripotent stem cells capable of differentiating into other lineages. However, the mechanism by which myogenic satellite cells attain pluripotent potential remain elusive. Later, transdifferentiating ability of these cells to another lineage in the absence or presence of certain growth factor/ or agents has revolutionized the scope of these pluripotent myogenic satellite cells for manipulation of animal production (in terms of quality and quantity of muscle protein) and health (in terms of repair of skeletal muscle, cartilage or bone). (**Key Words :** Satellite Cells, Myogenesis, Plasticity, Transdifferentiation, Adipogenesis)

### INTRODUCTION

Skeletal muscle satellite cells are mononucleated cells that are embedded in the basal lamina of the myofibre. Muscle satellite cells represent a distinct lineage of myogenic progenitors responsible for the postnatal growth, repair and maintenance of skeletal muscle. They are quiescent during most of the adult life of animals, but these cells can be activated in response to diverse stimuli like injury, exercise, stretching, electrical stimulation (Schultz et al., 1985; Appell et al., 1988; Rosenblatt et al., 1994), and oxygen stress (Csete et al., 2001). The total number of quiescent satellite cells in adult muscle remains constant over repeated cycles of degeneration and regeneration which suggests that satellite cell population is maintained by self-renewal (Schultz et al., 1985, Morlet et al., 1989). Satellite cells are the precursors of the myogenic cells that give rise to majority of the nuclei within adult skeletal

muscle cell. After stimulation, satellite cells become activated, proliferate and express myogenic markers and ultimately fuse with existing muscle fibres or fuse together to form myofibres during regeneration of damaged skeletal muscle (Schultz and McCormick, 1994). Satellite cells form a population of mono-potential stem cells distinct from their daughter myogenic precursor cells as defined by biological and biochemical criteria (Bischoff, 1994). Satellite cells have capacity not only for muscle regeneration but they may also contribute to alternative muscle and non-muscle lineages. As a result, satellite cell population has a number of applications in the field of animal production and health. The identification of satellite cells is done on basis of their location by electron microscopy but this method is cumbersome. Other alternative methods used involve the identification of cell surface markers on quiescent and proliferating (activated) satellite cells.

### DISTRIBUTION AND MARKERS OF MYOGENIC SATELLITE CELLS

Satellite cell number is dependent on the species, age, and type of muscle fiber (Bischoff, 1994) Satellite cells constitute 30% of the muscle nuclei in the neonate and decrease with age to 4% in the adult and 2% in the senile mouse (Snow, 1977). The decrease in the percentage of satellite cells with ageing is the result of an increase in

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myonuclei (oxidative and glycolytic myofibers) and a decrease in total number of satellite cells (glycolytic myofibers) (Gibson and Schultz, 1983; Bischoff, 1994; Schultz and McCormick, 1994).

Various transcription factors expressed by satellite cell population are potentially used as markers for their identification and characterization. Myocyte nuclear factor (MNF), a member of helix transcription factor is localized to the quiescent satellite cells in adult skeletal muscle. MNF is present on quiescent and proliferating satellite cells and required for functioning of satellite cells (Garry et al., 1997). The spliced isoforms of MNF termed as MNF- $\alpha$  and MNF- $\beta$  (Garry et al., 1997; Yang et al., 1997; 2000) which are expressed during myogenesis and during muscle regeneration. MNF- $\beta$  is principally expressed in quiescent satellite cells while MNF- $\alpha$  predominates in proliferating satellite cells after muscle injury. Proliferating myogenic precursor cells, the daughter cells of satellite cells express desmin, Myf5, MyoD and other myoblast specific markers (Cornelison and Wold, 1997). MyoD and Myf5 which are muscle specific transcription factors are not expressed by quiescent satellite cells but they are rapidly upregulated in activated and proliferating satellite cells (Musaro, 1999).

M-cadherin (M-cad) is one of the most important and unique cell surface protein located at interface of satellite cells and underlying myofibre. M-cad is a calcium dependant cell adhesion molecule which is expressed not only in quiescent satellite cells, but however, its expression is increased when satellite cells become activated in response to a number of stimuli (Cornelison and Wold, 1997; Beauchamp et al., 2000). Satellite cells are also characterized by c-Met, receptor for hepatocyte growth factor (HGF) which is expressed mainly on quiescent cells. In addition to above, neural cell adhesion molecule (NCAM) and vascular adhesion molecule-1 (VCAM-1) are also potential markers of quiescent cells (Covault and Sanes, 1986; Jesse et al., 1998). These adhesion molecules may function in the adhesion of the satellite cells to the basal lamina of the myofibre and participate in the migration of satellite cells in response to different stimuli. NCAM is expressed in both myofibres and satellite cells whereas VCAM-1 is limited to satellite cells in adult muscle (Covault and Sanes, 1986) and it mediates interaction of satellite cells to leucocytes following injury (Jesse et al., 1998).

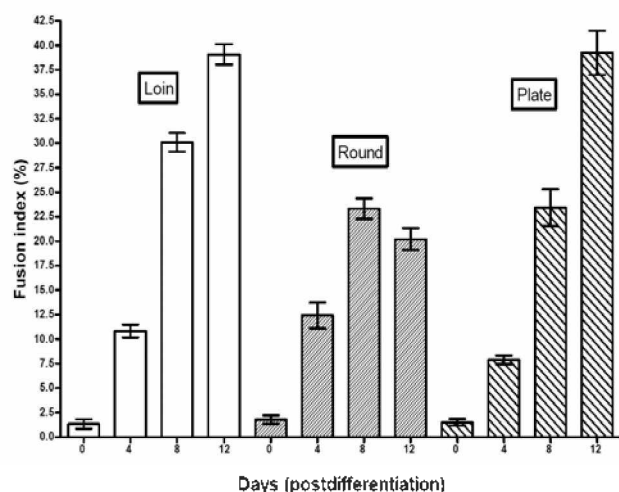
Pax7 is essentially a useful and reliable marker for satellite cells. Pax7 expression is required for formation of satellite cells (Seale et al., 2000). Pax7 belongs to a family of genes that encode paired-box-containing transcription factors involved in the control of developmental processes (Jostes et al., 1990; Schafer et al., 1994). Different members of the Pax-family of transcription factors appear to regulate the development and differentiation of diverse cell lineages

during embryogenesis (Noll, 1993; Strachen and Read, 1994; Mansouri et al., 1996). Pax7 and the closely related Pax3 gene belong to a paralogous subgroup of Pax genes based on similar protein structures and partially overlapping expression patterns during mouse embryogenesis (Jostes et al., 1990; Goulding et al., 1991). Pax3 plays an important role in upregulating the developmental programme of embryonic myoblasts (Maroto et al., 1997; Tajbakhsh et al., 1997). Pax7 and Pax3 proteins bind with identical sequence-specific DNA elements thereby suggesting that they regulate similar sets of target genes (Schafer et al., 1994). Pax7 is expressed in adult human primary myoblasts but not Pax3 (Schafer et al., 1994). Though the Pax7 and Pax3 are structurally similar, their different patterns of expression suggest that they regulate myogenesis in distinct cell types during development. The most recently satellite cells markers identified include Sca-1 (Jackson et al., 2002), CD34 (Beauchamp et al., 2000), glycoprotein Leu-19 (Schubert et al., 1989; Kadi and Thornell, 2000) and anti-apoptotic factor Bcl-2 (Miller et al., 1999; Lee et al., 2000). Sca-1 is present on multipotential stem cells and vascular cells but apparently not on satellite cells.

#### Plasticity of myogenic satellite cells

Plasticity of skeletal muscle is defined as the adaptations to change function, expression of genes and structural phenotype in relation to demand or environmental pressure. These changes are mainly mediated by satellite cells which are considered as the agent of rapid, prolonged and persistent change during muscle development, growth, responses to disease or injury and regeneration. Skeletal muscle plasticity has been described in relation to stem cells, trans-differentiation across the cell lineages and germ layer origins (Vescovi et al., 2002; Bottai et al., 2003; Cossu and Bianco, 2003), cell fusion (Goodell et al., 2001; Jackson et al., 2002; McKinney-Freeman et al., 2002), adaptation in response to exercise, disuse or aging (Caccia et al., 1979; Allen et al., 2001; Harrison et al., 2002; Renault et al., 2002; Thornell et al., 2003), regeneration following injury or degenerative neuromuscular and neurological diseases (Harris, 2003; Martino, 2004; Sohn and Gussoni, 2004), development (Duxson et al., 1986; Brand-Saberi and Christ, 1999; Williams and Ordahl, 2000; Denetclaw Jr et al., 2001; Brand-Saberi, 2005).

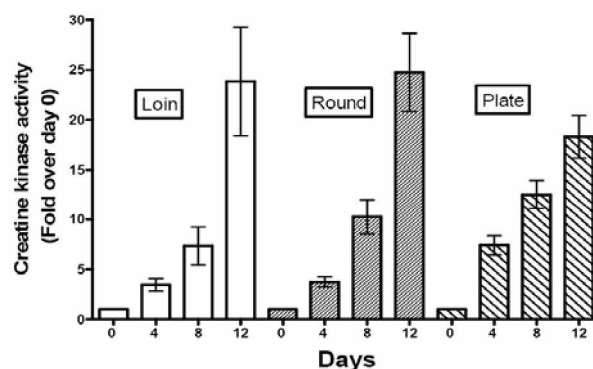
Satellite cells are the operational cash units that play an important role in muscle formation which supports adaptation for functions including contractility and thermogenesis. Satellite cells act as building blocks of muscle in development and regeneration through proliferation. Expression of muscle-specific regulatory genes directs differentiation because satellite cell progeny contribute incremental increase of the transcriptional capacity of a myofibre by fusing to the fibre sarcolemma



**Figure 1.** Fusion index was calculated as the percentage of nuclei incorporated in the myotubes relative to the total number of nuclei. Fusion index indicated myogenic differentiation of satellite cells isolated and cultured from different portion of muscles from bovine. Bars are mean $\pm$ SE.

(Figure 1). Satellite cell progeny bring the inherent developmental program that guides synthesis of proteins, from embryonic to neonatal to adult, mostly recognized in segmental expression of developmental myosin (Pernitsky et al., 1996). Muscle cell regeneration also requires the expenditure of the currency comprising satellite cell progeny. Damage of the fibre either local in trauma (Schultz et al., 1985) or induced by pathology that is intrinsic to fibres (Hoffman et al., 1987; Cooper, 1989; Kunkel and Hoffman, 1989; Lefaucheur et al., 1995) or extrinsic such as ischaemia (Makitie and Teravainen, 1977; Hansen-Smith and Carlson, 1979; Authier et al., 1997) induces myogenic cells to become activated, proliferate and fuse to form new fibres that elongate between mature fibres. New fibres attach to tendons ultimately to perform a function. In cultures of muscle cells (derived from satellite cells) and single muscle fibres, a gradual loss of proliferative capacity occurs with age and muscular dystrophy (Bockhold et al., 1998; Lagord et al., 1998; Renault et al., 2002; Cooper et al., 2003; Jejurikar and Kuzon, Jr, 2003). Satellite cells do not only reproduce a muscle but they represent a heterogeneous population that will express a range of phenotypes. Myogenic differentiation of satellite cells are also characterized with the increase in creatine phosphokinase (Figure 2).

Satellite cells give rise to new generation of progeny with a particular phenotype which may be required for in response to changes in the severity of a disease, environmental toxicity, starvation, temperature change or weightlessness (Karpati et al., 1990; Blaivas and Carlson, 1991; Brooks and Faulkner, 1994; Carlson and Always, 1996; McArdle et al., 2002; Welle, 2002; Conboy et al.,



**Figure 2.** Creatine phosphokinase (CK) activity was determined at different days post-differentiation for satellite cells isolated and cultured from different portion of muscles from bovine. CK activity indicated myogenic differentiation. Bars are mean $\pm$ SE.

2003; Conboy et al., 2005). Satellite cells can serve to manufacture muscle as a patch or alternate contractile or non-contractile tissue (Muller-Ehmsen et al., 2002) or genetically modified to make high levels of a hormone, non-muscle protein or a novel therapeutic protein (such as IGF-1).

Satellite cells serve a plastic role for research investigators as markers of events in skeletal muscle that follow activation from G<sub>0</sub> or quiescence, into cell cycle. The selection of particular genes to use as expression markers has been shown to affect the observed nature of currency and conveyance roles in satellite cell behaviour (Tamaki et al., 2002a; Tamaki et al., 2002b; Tamaki et al., 2003). Particular set of genes denoting stem-like phenotype have used sorting and cell purification based on surface marker expression. The viability of stem cell in their stem-like capacity is observed to decline after tissue dissociation and flow cytometry. The powerful *in vivo* approach to study cell lineages in development has the potential to be achieved for single muscle fibres cultures observed over time, where the satellite cell-fibre complex within the basement membrane is retained similar to conditions *in vivo* (Shefer and Yablonko-Reuveni, 2005).

Single fibre studies have revealed clues about myogenesis and differentiation through the cascade of regulatory gene expression (Bischoff, 1986a; Bischoff, 1986b; Yablonko-Reuveni and Rivera, 1994; Yablonko-Reuveni, et al., 1999b; Shefer et al., 2004; Shefer and Yablonko-Reuveni, 2005; Yablonko-Reuveni and Anderson, 2006) and demonstrated the kinetics, symmetry or complexity of precursor cell division (Beauchamp et al., 2000; Asakura et al., 2001; Tamaki et al., 2003), the effects of deficient expression of particular proteins including syndecans, dystrophin and MyoD (Yablonko-Reuveni et al., 1999a; Cornelison et al., 2000; Cornelison et al., 2004) or changes with age or denervation (Bockhold et al., 1998;

Kuschel et al., 1999; Yablonko-Reuveni et al., 1999b).

Satellite cells are positioned at the external aspect of muscle fibres and therefore, form a part of the direct environment of the fibre. They receive signals from the fibre or the further interstitial or circulating environment and also have potential to receive signals from mononuclear cells (possibly by attracting them to the site of injury), other fibres, the blood stream or the fibre itself. Once activated, satellite cells and myogenic progeny release growth factors (FGF-2, VEGF AND IGF-1) and muscle fibres express the relevant receptors. This means that fibre activity may be mediated in part by the products and signaling of muscle precursors, both activated (cycling) and those in an apparent quiescent state in the direct environment of fibres (intact or damaged). Satellite cells and myogenic progeny have the capability to communicate with one another as well as with the nearby fibre and the complexity of such a myogenic network may have an impact on the eventual capacity for myofibre growth and formation and the satellite cell renewal. The signal trafficking functions that pass in both directions between the fibre and surrounding extracellular matrix environment are a field of research that has evolved since the first discovery of dystrophin (Hoffman et al., 1987; Beggs et al., 1990; Darras et al., 1988; Koenig et al., 1988; Koenig and Kunkel, 1990; Kunkel and Hoffman, 1989). Laminin, integrins and fibronectin at the fibre surface affect fibre processes including calcium signaling and membrane recycling (Rando, 2001; Bansal et al., 2003; Bansal and Campbell, 2004; Kikkawa et al., 2004). Satellite cells may participate in this two way connection between fibres and the environment.

Adhesion of satellite cells is very important in connecting the fibre to its environment. M-cadherin is produced by myogenic cells (Kauffman et al., 1999) and deposited around the whole satellite cell. M-cadherin contribute in defining the satellite cell population in development, regeneration and disease (Irintchev et al., 1994; Cornelison and Wold, 1997; Cooper et al., 1999; Tamaki et al., 2002a).

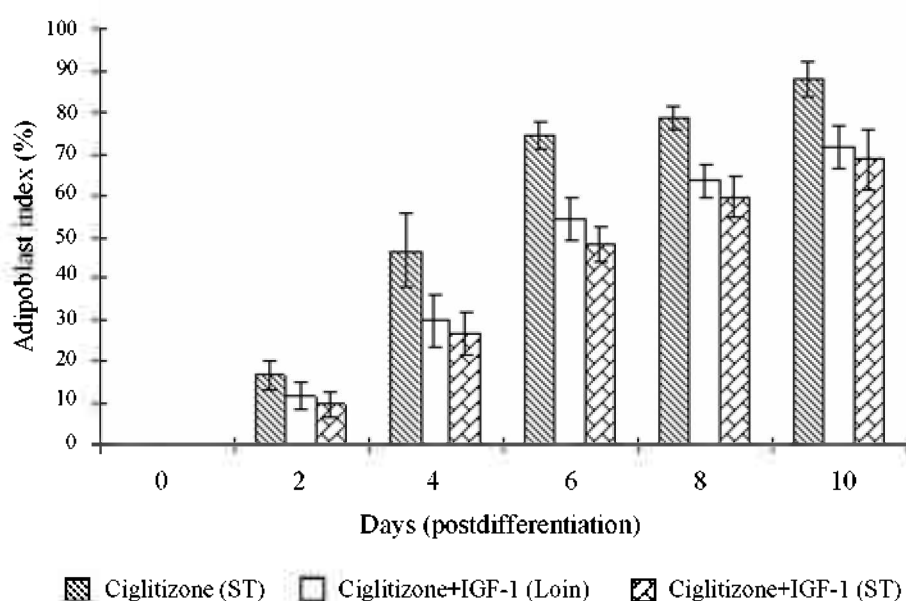
Satellite cells act as filters that can correct or modulate particular signals. Signals that are particularly important for long term fibre adaptations may be read differently from signals essential for an effective regeneration. Satellite cells may take part in directing the metabolic activity of fibre or nearby tissues. Signals critical to muscle may shift satellite cell functions toward quiescence and modulate the muscle response to alter metabolism rather than growth. Vascular, endothelial and interstitial signals may also be received differently by satellite cells than the juxtaposed myofibres and satellite cells may return distinct signals in response. Satellite cell migration along the length and around the surface of fibres is further proof that they are active participants in filtering the stimuli related to muscle

plasticity. Satellite cells will actively travel between adjacent fibres, with an intervening cell cycle that deposits a muscle precursor.

### GROWTH FACTORS AND TRANSDIFFERENTIATION OF MYOGENIC SATELLITE CELLS

Transdifferentiation is defined as an irreversible switch in postnatal life of one type of already differentiated cell to another type of normal differentiated cell (Tosh and Slack, 2002). Transdifferentiation is associated with a discrete change in cellular morphology associated with a change in the programme of gene expression. The cause of molecular transdifferentiation is presumably a change in the expression of a master regulatory gene whose normal function is to distinguish the two tissues in normal development (Slack and Tosh, 2001; Tosh and Slack, 2002). Satellite cells contain a subpopulation of cells with stem-like properties that serve to replenish the satellite cell compartment. Myogenic stem cells arise developmentally as a subpopulation of satellite cells. They originate from stem cells lying outside the muscle fibre and eventually migrate into the satellite cell position beneath the basement membrane of myofibres. Two distinct lines of stem cells extracted from skeletal muscle tissue (McKinney-Freeman et al., 2002). Plasticity of satellite cells can be determined by growing them on isolated myofibres in culture (Beauchamp et al., 2000; Heslop et al., 2001), harvesting the satellite cells and then assessing the capacity of clones to give rise to cells of different lineages. Satellite cells on isolated myofibres in culture can give rise to adipocytes and osteogenic cells (Asakura et al., 2001). Intrinsic lineage plasticity using isolated myofibres occurred in the absence of exogenous inducers that are required to demonstrate plasticity with cultured myoblasts (Zammit and Beauchamp, 2001). Isolated myofibres grown at different oxygen concentrations using co-localization of markers for myoblasts and adipocytes within individual satellite cells also supports that satellite cells can give rise to cells of the adipogenic lineage (Csete et al., 2001).

Myoblasts and adipocytes arise from the same germ layer of the embryo, the mesoderm and it is possible to directly induce the conversion of myoblasts to adipocytes. G8 myoblasts are a tissue-culture model for myogenesis and can differentiate into myotubes when cultured in medium containing fetal calf serum (Singh et al., 2007c). Transcription factors C/EBP $\alpha$  and PPAR $\gamma$  (Figure 5) identified quantitatively by real time PCR when porcine satellite cells were transdifferentiated in the presence of ciglitzone and when expressed in G8 myoblasts, can suppress the muscle-specific transcription factors (Myf5, MyoD, myogenin and MRF4) (Hu et al., 1995).



**Figure 3.** Adipoblast index of porcine satellite cells during differentiation. Postconfluent cells cultured with adipogenic mixture plus ciglitizone showed adipoblasts (adipoblast index). Bars are mean±SE and total of six piglets went into means.

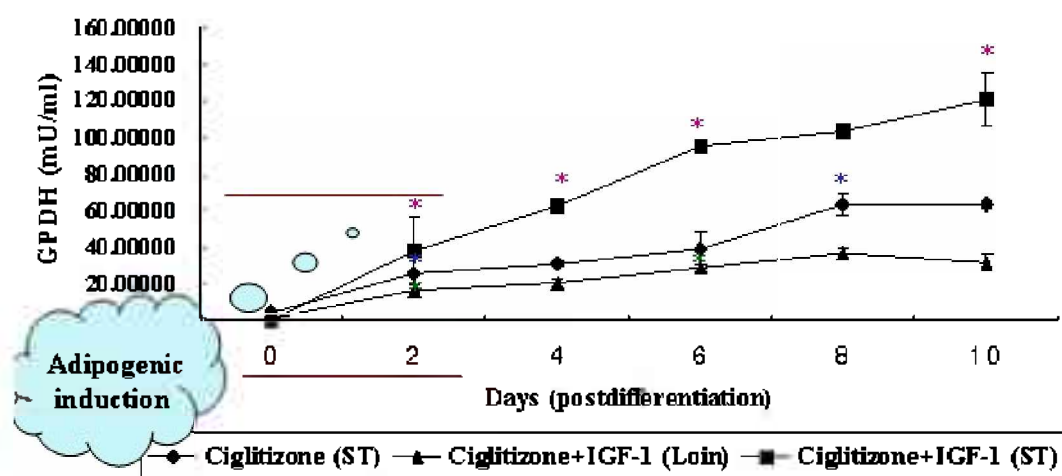
Adipogenesis was also characterized in our study with adipogenic index (Figure 3) and with the increase in the measurement of glycerol-phosphate-dehydrogenase (GPDH) (Figure 4). Markers specific for adipocytes such as aP-2, adiponin, lipoprotein lipase and phosphoenolpyruvate carboxykinase appeared in G8 myoblasts co-expressing both C/EBP $\alpha$  and PPAR $\gamma$ .

The population and age of the satellite cell is an important consideration in cell culture preparations as the response of aged, quiescent satellite cells to growth factor stimulation differs when compared with young, proliferating satellite cells (Tatsumi et al., 1998). The

growth factors are important in the regulation of satellite cell proliferation, differentiation, and motility.

#### Insulin-like growth factors

Skeletal muscle secretes insulin-like growth factors I and II (IGF-I and IGF-II), which are known to be important in the regulation of insulin metabolism (Allen and Boxhorn, 1993; Vierck et al., 2000). In addition, these growth factors are important in the regulation of skeletal muscle regeneration. IGF-I and IGF-II increases proliferation and differentiation of bone marrow progenitors to osteoblast and chondroblast (Singh et al., 2007d) and myogenic satellite



**Figure 4.** Glycerol-phosphate dehydrogenase (GPDH) activity was determined at different days post-differentiation for satellite cells isolated and cultured from different portion of muscles from bovine. GPDH activity indicated adipogenic differentiation. Bars are mean±SE. \* indicate significant level ( $p < 0.05$ ).

cells to adipoblast (Singh et al., 2007c) *in vitro*. IGF-I causes enhanced satellite cell proliferation and increased muscle mass (Chakravarthy et al., 2000a). Exercise results in elevated IGF-I levels, increased DNA content and a compensatory hypertrophy of skeletal muscle (Yan et al., 1993; Adams et al., 1999). IGF-I utilizes multiple signaling pathways for regulation of the satellite cell pool. The calcineurin/NFAT, mitogen-activated protein (MAP) kinase, and phosphatidylinositol-3-OH kinase (PI-3K) pathways are involved in proliferation of satellite cell (Coolican et al., 1997; Chakravarthy et al., 2000b).

#### Fibroblast growth factors

There are nine different isoforms of fibroblast growth factor (FGF) (FGF-1 to FGF-9). FGF-6 is restricted to skeletal muscle (Floss et al., 1997). FGF leads to satellite cell proliferation in culture (Sheehan and Allen, 1999). FGF-1, 2, 4, 6, and 9 stimulated cellular proliferation, whereas FGF-5, 7 and 8 had no mitogenic activity. In addition to an increase in satellite cell proliferation, the FGF family also causes differentiation of satellite cells to myofibers (Sheehan and Allen, 1999; Spizz et al., 1986; Johnson and Allen, 1995; Clegg et al., 1987). Absence of FGF-6 (FGF-6 locus) results in impaired satellite cell proliferation and a subsequent defect in muscle regeneration in response to injury (Floss et al., 1997). The release of FGF-2 from the damaged myofibers, like HGF, is proportional to the degree of injury (Clarke et al., 1993). FGF levels are coordinated with FGF receptor expression. When receptor expression is increased, satellite cells propagated in culture demonstrate an increased proliferation and decreased differentiation (Scata et al., 1999). When receptor expression is diminished, proliferation is decreased and there is a concomitant increase in satellite cell differentiation. During the period of satellite cell activation and proliferation (0-48 h after injury), FGF receptor (FGF-R1) mRNA is increased fivefold, and this increase is further enhanced in the presence of HGF (Sheehan and Allen, 1999). MAP kinase pathway is important in transducing the FGF-induced increase in satellite cell proliferation.

#### Transforming growth factors

Transforming growth factor- $\beta$  (TGF- $\beta$ ) is member of cytokines that includes bone morphogenic protein and growth-differentiation factors. The TGF- $\beta$  family of cytokines transduces their signal through the SMAD family of proteins (Whitman, 1998). Generally, the TGF- $\beta$  family members function to inhibit muscle proliferation and differentiation (Clegg et al., 1987; Zentella and Massague, 1992; Katagiri et al., 1997; Kosamis, 2001) by silencing the transcriptional activation of the MyoD family members (Martin et al., 1992). During muscle regeneration, TGF- $\beta$

receptor levels (TGF- $\beta$  RII) and TGF ligand are reciprocally expressed, resulting in the initial promotion of cellular proliferation followed by enhanced muscle differentiation (Sakuma et al., 2000).

#### Interleukin-6 cytokines

Leukemia inhibitory factor (LIF) and interleukin-6 (IL-6) are members of the IL-6 family of cytokines produced by many different cells, including myoblasts and macrophages. These cytokines share a common receptor component, and their actions are mediated through the same signaling pathways (Pennica et al., 1995; Hibi et al., 1996). IL-6 promotes the degradation of necrotic tissue, synchronizes the cell cycle of satellite cells, and induces apoptosis of macrophages following muscle injury (Cantini and Carraro, 1996). IL-6 expression in injured muscle does not increase satellite cell proliferation (Kami and Senba, 1998). This growth factor appears to play an integral role in skeletal muscle regeneration.

### CHARACTERIZATION OF MYOGENIC SATELLITE CELLS

Western blot immunodetection and immunohistochemistry have been used for identifying the expression of muscle-specific actin, myosin heavy chain, and desmin by proliferating satellite cells. Desmin is a muscle specific intermediate filament protein that was originally believed to be expressed only in terminally differentiated myoblasts in other species (Allen et al., 1991; Lawson-Smith and McGeachie, 1998). However, species differences in the expression of desmin by proliferating satellite cells have been reported. Desmin was expressed *in vitro* by undifferentiated myoblasts from embryonic and adult rats, young chick embryos, and adult human muscle (reviewed in Lawson-Smith and McGeachie, 1998). Allen et al. (1991) detected the expression of desmin in proliferating rat satellite cells but failed to detect similar staining in cultured bovine satellite cells. Skeletal muscle actin and myosin heavy chain are markers of differentiation (Lawson-Smith and McGeachie, 1998). Immunocytochemical staining detects the expression of MyoD1 and myogenin by satellite cells. The expression of myogenin can be confirmed by Western blot, but this was not the case with MyoD1 and could be due to the inability of 5.8A to bind under Western blot conditions. The positive staining seen with 5.8A after reaction with avidin DH-biotinylated horseradish peroxidase complex may simply have been a result of nonspecific binding. However, it is possible that satellite cells that are between 75 and 80% confluent are entering the terminal differentiation phase and express MyoD1 and myogenin. The expression of the cellular adhesion molecule

NCAM is detected by both immuno-cytochemical staining and Western blot in satellite cells and by Western blot in muscle lysate. NCAM is expressed by developing muscle, and many studies indicate that NCAM is essential for signaling myoblast adhesion and fusion (McDonald et al., 1995). It is interesting to note that both satellite cells and muscle from adult dogs expressed NCAM. These cells were between 75 and 80% confluent when stained or harvested and were thought to be actively proliferating. No fusion of nuclei was observed. However, the expression of NCAM by muscle and satellite cells may be important in regulating the speed at which muscle regeneration occurs after injury by enhancing the rate of satellite cell fusion.

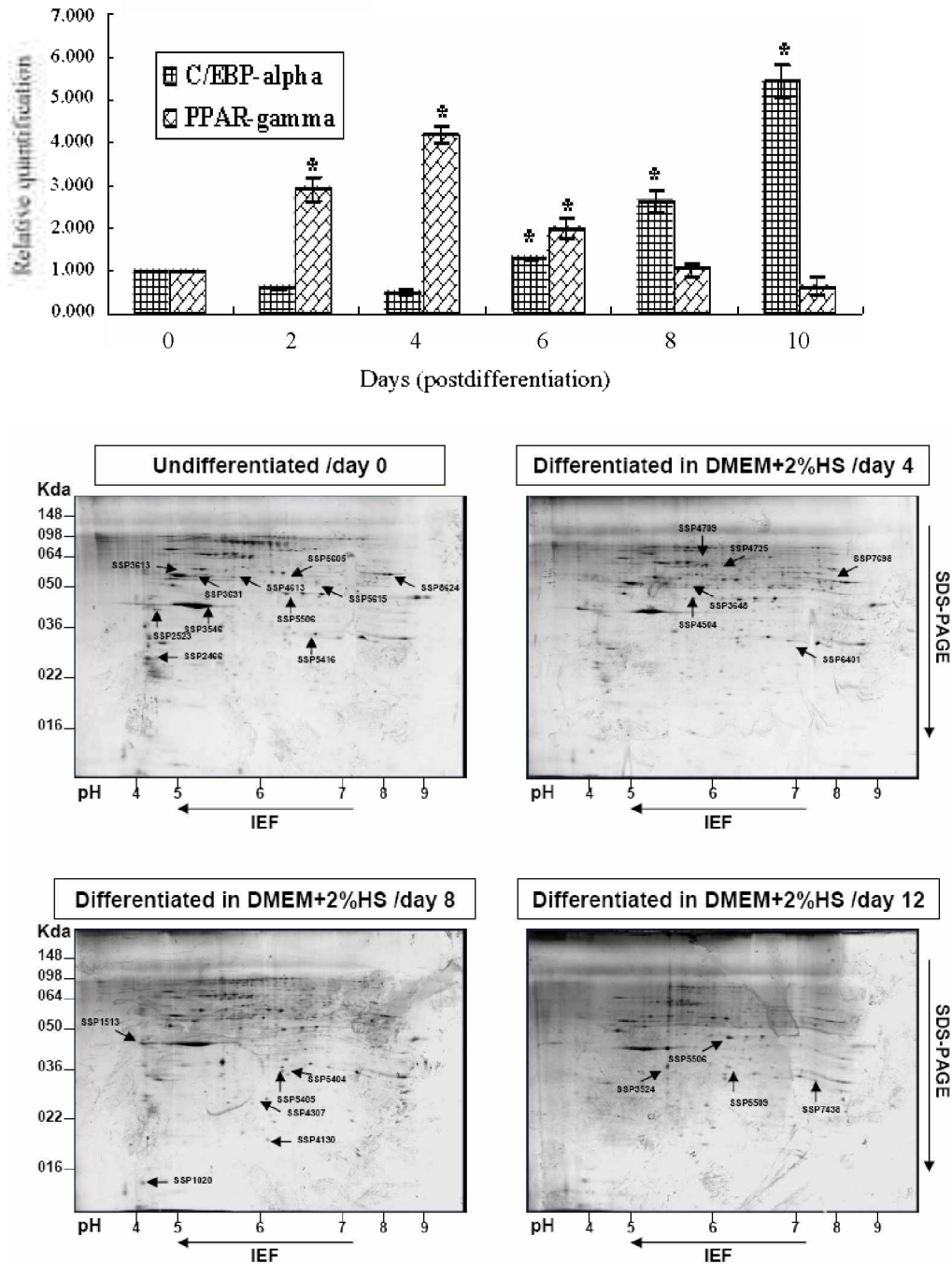
Constitutive expression of MHC class II by satellite cells is not detected by indirect immunohistochemistry or Western blot. Normal muscle cells do not express the MHC class II antigen. Others have reported that there was no constitutive expression of MHC class II molecules by normal human myoblasts (Mantegazza et al., 1996) or muscle fibers (Karpati et al., 1988). However, muscle fibers from patients with inflammatory myopathies expressed MHC class I as well as MHC class II antigens in the absence of inflammatory cell infiltration (Englund et al., 2001). Treatment of human myoblasts with interferon- $\gamma$  induced expression of MHC class II molecules (Mantegazza et al., 1991; Bao et al., 1999). These results suggest that muscle cells may act as antigen presentation cells and play a role in initiating and maintaining muscle autoimmune diseases (Englund et al., 2001). Therefore, it is important to obtain a more complete understanding of the immunopathogenesis of various myopathies in order to accomplish successful myoblast transplantation in diseased muscle.

Recently the phenotypic characterization of myogenic satellite cells has also been performed by few laboratories by flow cytometry. The muscle-derived cell populations could be obtained by the preplate method for quickly identification and isolation. The preplate technique purifies distinct myogenic cell subpopulations expressing CD34 alone (Sca-1 negative) and Sca-1 alone (CD34 negative) and other transcriptional factors as well (not yet reported), but it has been observed that the expression of these transcriptional factors are subject to change with time during cell culture (Jankowski et al., 2001). Flow cytometry will aid in the rapid isolation of specific cell populations for muscle cell which could be of importance in health and production. Skeletal muscle differentiation follows a highly regulated program of gene expression. Several proteins such as myogenic regulatory factors (MRFs), a group of skeletal-muscle specific basic helix-loop-helix (bHLH) transcription factors including of MyoD, Myf5, myogenin, and MRF4 have been documented and linked to myogenic differentiation. These factors are reported to play important

roles in satellite cell activation, proliferation, and differentiation (Rudnicki et al., 1992; Smith et al., 1994; Cornelison and Wold, 1997; Seale and Rudnicki, 2000). Furthermore, the expression of paired-box transcription factor *Pax7* have been shown associated to both quiescent and activated satellite cells. *Pax7* is related to *Pax3*, based on highly similar protein structures and partially overlapping expression patterns during embryonic development (Jostes et al., 1990; Goulding et al., 1991). *Pax3* is key regulator of somatic myogenesis (Maroto et al., 1997; Tajbakhsh et al., 1997). Detail analysis of the distribution of *Pax7* m-RNA using northern blot analysis of by Seale et al. (2000) revealed the essential role of *Pax7* in satellite cell development (Seale et al., 2000). During muscle regeneration, MPC are required to fuse to each other to form syncytial muscle fiber. Classical cadherins, transmembrane proteins mediating cell-cell interactions in a calcium-dependant manner, are thought to play important roles in these processes (Geiger and Ayalon, 1992; Kaufmann et al., 1999).

Therefore, global analysis of protein expression profiles might yield lot of complete awareness of biological outcome such as differentiation, proliferation and evolution of organisms of our interest. Proteomics is systematic study of proteins of gene expression. The entire process involves large-scale protein separation and identification of sensitive proteins (Sperling, 2001). The two important technologies are two-dimensional electrophoresis (2-DE) along with the powerful image analysis software and mass spectrometry associated with searching database (Pennington et al., 1997). 2-DE is the most powerful and widely used technique for the analysis of complex protein mixtures extracts from cells, tissues, organelles or other biological samples (Figure 5). This method which is originally described by O'Farrell (1975) and Klose (1975) allows separation of cellular proteins according to their isoelectric point (pI) in the first-dimension step (IEF) and relative molecular mass (Mr) in the second-dimensional step (SDS-PAGE). Protein identification route start through isolation of the target protein spots on 2-DE gel and achieving peptide mass fingerprint (PMF) using matrix associated laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) or matrix associated laser desorption/ionization time-of-flight/ionization time-of-flight (MALDI-TOF-TOF) tandem mass spectrometry. Identification of protein spots is performed through database searching by matching these PMF with protein sequence database such as SWISS-2D Page (<http://www.expasy.ch>), NCI 2DWG Image Meta-Database (<http://www.lecb.ncifcrf.gov/2dwg>), Human and mouse 2-D database (<http://biobase.dk/cgi-bin/celis/>), Partial list of databases on WWW (<http://www.lecb.ncifcrf.gov/EPTable> 2Ddatabases.html), and Mascot (probability-based MOWSE) database





**Figure 5.** Myogenic satellite cell differentiation and transdifferentiation 2DE map (Mr = 15-150 kDa) from bovines and porcine. About 400 µg of satellite cell proteins were applied onto pH 3-10 nonlinear IPG/12% SDS-PAGE (24-cm) 2-DE gels and then visualized by silver staining. Several protein spots of different expressional levels during myogenesis and adipogenesis has been determined and only few up-up-up regulated proteins have been listed here.

(<http://www.matrixscience.com>). Systematic comparison of changes in the proteome composition of differentiating myoblasts and adipogenesis (transdifferentiation) should also provide insight into mechanisms and pathways of various proteome identified in our lab (few of them are listed in Table 1) that underlie the formation of skeletal muscle.

**APPLICATION OF MYOGENIC SATELLITE CELLS FOR ANIMAL PRODUCTION AND HEALTH**

Myogenic satellite cells provide the opportunity to study the growth and differentiation of individual cells into tissues. Understanding these processes could provide insights into



**Table 1.** List of proteins indicative of expressional level changes in proteome associated with myogenic differentiation and adipogenic differentiation from bovines and porcines

Spot No.	Tissue	Accession No.	Identified protein	Peptide sequence	Theor. Mr.	Theor. pl.	Peptide match	Protein score	Type of analysis
SSP3544	Loin	018840	Actin, cytoplasmic 1 (Beta-actin)	AVFPSIVGRPR	41637.96875	6.03	5	53.7	MS/MS
SSP5509	Loin	Q8RFX6	Probable D-serine dehydratase		49921.85938	6.55	3	41.4	MS
SSP5605	Loin	Q4WPF7	Diphthamide biosynthesis protein 4		22137.93945	5.71	4	33.09	MS
SSP3121	Plate	Q29095	Prostaglandin-H2 D-isomerase precursor transmembrane emp24		21215.59961	6.43	2	31.7	MS
SSP2006	Round	P49020	Domain containing protein 2 precursor	RFFEVR	22175.03906	5.07	4	34.29	MS/MS
SSP2323	Round	P35285	Ras-related protein Rab-22A	RQPSEPK	21788.13086	8.33	4	38.09	MS/MS
SSP5213	Round	Q9PDLO	Chorismate synthase		39852.32031	6.33	4	51.7	MS
SSP5214	Round	Q5HT90	Imidazole glycerol phosphate synthase subunit hisH 1		22726.0293	7.68	5	46.09	MS
SSP9307	Round	PO2587	Troponin C, skeletal muscle		18013.30078	4.06	4	36.4	MS

the cause of birth defects, genetic abnormalities, and other disease states. Stem cells could be used to produce large amounts of one cell type to test new drugs for effectiveness and chemicals for toxicity. Stem cells might be transplanted into the body to treat disease such as spinal injuries in animals. The damaging side effects of cancer treatments (destruction of immune response) might be repaired with stem cell treatment.

Marbling, or intramuscular adipose tissue, enhances juiciness, flavor, and overall desirability of meat and has been the focus of many studies attempting to improve meat quality (Singh et al., 2007c). The muscle growth rate in cattle is an important performance determinant among various beneficial growth factors for evaluating profits, such as daily gain and feed efficiencies among others. Since muscles are mainly composed of myofibers differentiated from the myogenic satellite cells, muscle growth is thought to be associated with increased numbers of myofibers and myogenic satellite cells (Allen et al., 1979). The transcription factors (C/EBP $\alpha$  and PPAR $\gamma$ ) which are responsible for intramuscular adipogenesis have been identified during transdifferentiation of mesodermal stem cells and myogenic satellite cells; however, the mechanism for adipocytic commitment of these cells still remains elusive.

Before myogenic stem cells can be applied to animals, substantial advances in basic cell biology and clinical technique are required. In addition, very challenging regulatory decisions will be required on the individually created tissue-based therapies resulting from stem cell research. Such decisions would likely be made by the center

for biologicals evaluation and research of the food and drug administration (FDA). The potential benefits mentioned above would be likely only after many more years of research. Technical hurdles include developing the ability to control the differentiation of myogenic stem cells into a desired cell type (bone and cartilage) and to ensure that uncontrolled development, such as a cancerous tumor, does not occur. If myogenic stem cells are to be used for transplantation, the problem of immune rejection must also be overcome. Some scientists think that the creation of many more embryonic stem cell lines will eventually account for all the various immunological types needed for use in tissue transplantation therapy. Others envision the eventual development of a universal donor type of stem cell tissue analogous to a universal blood donor.

However, if the SCNT technique was employed using a cell nucleus from the patient, stem cells created via this method would be genetically identical to the patient, would presumably be recognized by the patient's immune system, and this would avoid any tissue rejection problems that could occur in other stem cell therapeutic approaches because of this, many scientists believe that the SCNT technique may provide the best hope of eventually for animal production (in terms of muscle protein improvisation) and for treating patients using stem cells for tissue transplantation.

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