

Our results demonstrate that the decrease of sarcolemmal nNOS represents a molecular marker of muscle atrophy and disuse in a variety of inherited and acquired myopathies. These findings are of substantial clinical significance as the loss of sarcolemmal nNOS and its physiological repercussions may be a contributing factor to myopathic and atrophic features in patients with a variety of inherited and acquired forms of myopathy.

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CELL THERAPY; POSTER PRESENTATIONS

P3.20

Mouse and human skeletal muscle cells differentiate in a temperature-dependent manner

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We have been studying the effects of low temperature on differentiation of mammalian skeletal muscle cells in culture. C2C12 cells and human skeletal muscle cells neither expressed myogenin, one of the myogenic regulatory factors (MRFs), nor fused into multinucleated myotubes at 30 °C. However, the cells formed myotubes at the same level as a culture at 38 °C, the normal temperature condition, when they were cultured in medium containing both insulin-like growth factor-I (IGF-I) and L-ascorbic acid phosphate, stabilized form of vitamin C (VC). This result suggests that myoblasts do not completely lose the capacity to differentiate at 30 °C, but are just inhibited to undergo terminal differentiation in a temperature-dependent specific manner which can be overcome by exogenous IGF-I and VC. At 30 °C, C2C12 cells continuously expressed Id3, which acts as a negative regulator of MRFs and decreases upon the initiation of differentiation at 38 °C, and did not upregulate expressions of muscle-specific microRNAs. These low temperature-induced inhibitions of myogenic differentiation were cancelled by addition of IGF-I and VC into the culture medium. The combination of IGF-I and VC could promote myogenic differentiation, expressions of myogenin and sarcomeric myosin heavy chain, even at 25 °C. Conditioned medium from C2C12 cells differentiated at 38 °C also had an effect on promoting myogenin expression at 30 °C. In addition, we demonstrated that satellite cells of IGF-I overexpression transgenic mice in single-fiber culture expressed myogenin at a higher level than those of wild type mice at 30 °C. Our study suggests that body temperature plays an important role in the regulation of myogenic differentiation in mammals, but the sensitivity to low temperature can be buffered by some physiological factors such as IGF-I and VC in vivo.

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P3.21

Expression of microRNA in muscle resident stem cells

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Skeletal muscle regeneration is an organized process involving activation, proliferation, and differentiation of myogenic precursor cells. Muscle satellite cells are myogenic precursor cells that are largely responsible for muscle regeneration in adult muscle. In addition to satellite cells, side population (SP) cells, muscle resident stem cells, also contribute to muscle regeneration. SP cells exhibit high mesenchymal potential and are a potential cell source for the therapy of muscular dystrophy. The differentiation mechanism of their SP cell into myogenic cells, and the regulation of SP cells is still poorly understood. miRNAs play key roles in modulating a variety of cellular processes through repression of mRNA targets. In skeletal muscle, it is well known that miRNAs are involved in myoblast proliferation and differentiation. Here, to investigate the mechanism of SP cell regulation, we profiled the miRNA expression of SP cells and MP (main population) cells in muscle by using real-time PCR-based expression assays. We identified some miRNAs that were highly expressed in SP cells. One of them had increased expression not only in SP cells, but also in Sca-1(+) CD31(+) cells, Sca-1(-) CD31(-) cells and satellite cells. This miRNA expression was decreased in Sca-1(+) CD31(+) and Sca-1(-) CD31(-) cells after continued culture *in vitro*. However, in satellite cells, the miRNA expression was dramatically increased throughout myogenic differentiation. These results suggest that miRNAs may contribute to the maintenance of quiescent state in SP cells, and regulate muscle differentiation in myogenic cells.

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P3.22

Reprogrammed fibroblasts as a feasible source of cell-based therapy for muscular dystrophy

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The generation of induced pluripotent stem (iPS) cells by introducing four embryonic transcription factors into fibroblasts has made a breakthrough to produce specific lineage cells to replace diseased tissues in regenerative medicine. However, the risk of forming teratomas or cancers originated from pluripotent status remains to be overcome in applying iPS cells for cell-based therapies. MyoD is a myogenic master gene to transform several cultured cells into myogenic cells. Here we show that fibroblasts reprogrammed by MyoD differentiate into slow and fast myofibers *in vitro* without pluripotent status and incorporate into the skeletal muscle of NOD/SCID/IL2Rf β Null (NOG) immunodeficient mice *in vivo*. The transplantation of these transformed fibroblasts derived from green fluorescent protein (GFP) mice restored expression of laminin *f*₂, the protein deficient in merosin-deficient congenital muscular dystrophy (MDC) 1A, or caveolin-3, the protein deficient in limb-girdle muscular dystrophy (LGMD) 1C, in model mice. The restoration of deficient proteins was observed in not only GFP-positive, but also GFP-negative myofibers. Our findings indicate that direct reprogramming of fibroblasts into myogenic lineage cells become a feasible strategy of cell therapy for muscular dystrophy.

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