SAES-422 Multistate Research Activity Accomplishments Report

Project No. and Title: NC1131 Molecular Mechanisms Regulating Skeletal Muscle Growth and Differentiation (NC131)
Period Covered: 10-2006 to 09-2007
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Accomplishments:

It is now well-established, to a considerable extent because of previous work by members of this committee, that satellite cells are crucial to postnatal growth of skeletal muscle in addition to being important contributors to skeletal muscle repair following injury. Without proper vascularization, myogenic cells do not survive. A co-culture of satellite and microvascular fragments has been developed and has been used to show that satellite cells can secrete angiogenic factors such as VEGF and that the satellite-derived VEGF will induce angiogenesis in the co-cultures. The studies indicate that a heretofore unknown aspect of satellite cells is their ability to initiate pro-angiogenic signals. Studies by other stations have shown that HGF (hepatocyte growth factor) induces a cell cycle-arrest of satellite cells, and that this cell cycle arrest requires MEK (MAP kinase kinase) activity. The level of nutrition and muscle type also affects satellite cells. Higher levels of nutrition increase the rate of proliferation of satellite cells obtained from turkey pectoralis thoracicus muscle. Satellite cells obtained from rat soleus muscle had higher numbers of Pax7, myoD, and myogenin-positive nuclei than satellite cells from rat extensor longus digitorum muscle, suggesting that the satellite cells from rat soleus muscle were posed for differentiation. Other studies have measured level of phosphorylation of MAP kinase (ERK 1/2 kinase) to learn whether phosphorylation of this kinase could be used as a marker to objectively measure the response of satellite cells to growth factor stimuli. The results indicated that the degree of phosphorylation of MAP kinase varied in response to different growth factors, although the responses did not correlate with proliferation rates of the individual clones. It is clear from the research done by committee members during the past year that skeletal muscle satellite cells are responsive to a number of external stimuli, but how these stimuli relate to differentiation and proliferation are still unclear. Some preliminary studies suggest that the phospholipid content of satellite cell plasma membranes may be involved in this responsiveness. Future studies by committee members will focus on the signaling pathways involved in satellite cell response to growth factor stimulants. The Arizona, Florida, North Carolina, and South Dakota stations are collaborating on this aspect of the regional project. Postnatal skeletal muscle growth can be altered by administration of either anabolic steroids such as estradiol or β-adrenergic agonists such as clenbuterol or trenbolone acetate (TBA). Clenbuterol administration to mice during lactation resulted in an increased rate of muscle growth of the offspring, but such administration during gestation had no effect on rate of muscle accumulation of the offspring. Studies on bovine animals indicated that estradiol implants increased the rate of muscle growth by increasing satellite cell proliferation and subsequent fusion with the adjacent muscle fiber, whereas TBA implants increased rate of skeletal muscle growth by altering the rates of muscle protein synthesis and degradation. Other studies
showed that estradiol and TBA implants increase the IGF-1 mRNA levels in longissimus muscle of steers but did not affect myostatin, IGFBP-3 (IGF binding protein 3), or hepatocyte mRNA levels in the same muscle. If IGFBP-3 is removed from serum by immunoaffinity chromatography, estradiol and TBA both increased 3H-thymidine incorporation into cultures of bovine satellite cells by ~50% but did not affect IGF-1 mRNA levels. The use of agents that block binding to the estradiol receptor and the androgen receptor suggested that these two steroids are exerting their effects on proliferation by binding to their respective receptors. Zilpaterol, another ²-adrenergic agonist, also did not affect the rate of proliferation in cultures of satellite cells, but altered rates of protein synthesis and degradation. Hence the two agents seem to work by different mechanisms and may be expected to have synergistic effects of rates of postnatal muscle growth. The Illinois, Kansas, and Minnesota stations are collaborating on this part of the regional project.

The NC-1131 committee also is working the role of the extracellular matrix in skeletal muscle growth. This work has focused on the role of the heparin sulfate proteoglycans, syndecan and glypican, in skeletal muscle development. Because so little is known about the role of the extracellular matrix in skeletal muscle growth and development, the experiments have been largely at the observation stage thus far. The cDNAs for glypican-1 and syndecan-4 have been cloned and some of the glycosylation sites on these clones have been mutated to determine the effects of glycosylation on muscle differentiation. Overexpression of glypican-1 in turkey satellite cells increased the FGF2 responsiveness of these cells during proliferation, whereas inhibition of glypican-1 expression with RNAi techniques decreased satellite cell proliferation, differentiation, and FGF2 responsiveness. Mutating three Ser residues that are glycosaminoglycan (GAG) sites reduced the effects of glypican on the proliferation and differentiation of the satellite cells, suggesting that glycosylation has an important role in the effects of glypican-1 on satellite cell differentiation. Overexpression of syndecan-4 decreased satellite cell proliferation and differentiation. Experiments using syndecan mutants have not been completed yet, but the results thus far suggest that glycosylation has little effect on the role of syndecan on satellite cell differentiation. The results indicate that the extracellular matrix has important effects on muscle cell growth and differentiation, although the area remains understudied. The Ohio station is taking the lead on this part of the regional project with assistance from the South Dakota station.

The IGF-binding proteins (IGFBP) have received increasing attention in muscle growth and differentiation as recent results have shown that they have varied, important, and complex roles in skeletal muscle. Of the family of IGF-binding proteins that have been identified, IGFBP-3 is the principal IGFBP in skeletal muscle. IGFBP-3 is involved in facilitating the proliferation-suppressing effects of myostatin and TGF-² on cultured myoblasts, but studies using an IGFBP-3 antibody indicated that IGFBP-3 does not exert its effects through PSmad2, PSmad3, p38, MAP kinase, SnoN, or cyclin because expression of these signaling molecules did not change in the presence of anti-IGFBP-3 even though the ability of myostatin and TGF-² to inhibit proliferation of porcine embryonic myogenic cells (PEMC) was suppressed. Treatment of PEMC with TGF-² results in IGFBP-3 being translocated to the nucleus. Recent studies indicate that the low-density lipoprotein receptor-related protein 1 is involved in the mechanism by which IGFBP-3 facilitates the proliferation-suppressing effects of myostatin and TGF-² on cultured myoblasts. The Minnesota station is leading the efforts in learning how the IGFBPs function in skeletal muscle growth. The Ohio station is also investigating the effects of TGF-² on Samd2/3 phosphorylation and MD and myogenin.
expression in a different cell system, satellite cells.

Although is has been well-documented that turnover of skeletal muscle protein has important effects on both rate and efficiency of skeletal muscle growth in domestic animals, the mechanism by which muscle proteins are turned over metabolically has not been studied. It is difficult to understand how myofibrillar proteins turnover because the myofibrillar structure must remain intact if the muscle is to preserve its contractile function. Yet, the myofibrillar proteins constitute 55-60% of all muscle protein, and it is clear that they turnover metabolically at different rates depending on the physiological state of the animal. At present, there seem to be two possible mechanisms for turnover of myofibrillar proteins: removal of the outer layer of filaments leaving the remainder of the myofibril intact and functional or exchange of individual proteins in the myofibril with newly synthesized or newly released individual myofibrillar proteins in the muscle cell cytoplasm. The NC-1131 committee is working on both possibilities. It is possible to remove ~0.1-0.5% of the myofibrillar protein fraction by gently shearing myofibrils in the presence of ATP. These easily releasable myofilaments (ERMs) are identifiable entities in muscle because once they have been removed, additional ERMs cannot be obtained by shearing the residual myofibrils in the presence of ATP. Gentle treatment with purified calpain increases the amount of ERMs by 5-10-fold, suggesting that the calpains may initiate myofibrillar protein turnover as was first proposed over 30 years ago. Turning over myofibrillar proteins via ERMs, however, cannot explain how different isoforms of the myofibrillar proteins can be exchanged during muscle development. Other studies by NC-1131 members have shown that the subunits of the troponin complex in solution can exchange with troponin subunits in intact myofibrils and that this exchange can be quantitated by measuring rates of binding and dissociation. The extent to which these two mechanisms are used to turnover myofibrillar proteins in living muscle is still unclear, but it seems likely that both are involved. These studies involved collaboration between the Indiana and Arizona stations.

Studies on the role of signaling via the AMP-activated protein kinase (AMPK) in skeletal muscle growth has shown that AMPK signaling is linked to intracellular Ca2+ concentration by a mechanism involving Ca2+/calmodulin-dependent protein kinase kinase; activity of the latter enzyme is dependent on Ca2+ as its name implies, and this enzyme, once activated by Ca2+, then phosphorylates AMPK to activate it. AMPK activity is decreased during hind limb unloading, a procedure that results in pronounced muscle wasting. AMPK activity is also necessary for expression of GLUT4, the principal glucose transporter in skeletal muscle. Other preliminary studies on AMPK indicate that it has a crucial role in adipogenesis in developing muscle. These studies involve the Indiana and Wyoming stations.

Studies on titin and on the response of muscle to eccentric contractions (ECs) have discovered that a rat strain that has an autosomal mutation that alters titin isoform expression expresses a very large polypeptide of ~3,900-kDa; this is significantly larger than the native titin polypeptide of 3,000-kDa. The mutation is in chromosome 1 of the rat and not on chromosome 3 where the titin gene is located. A knockout mouse that lacks all three of the muscle ankyrin repeat proteins has a greater degree of torque loss after a bout of ECs than muscle from control mice but recovered at the same or even slightly faster rate than muscle from control mice (Wisconsin station).

Modern biology is heavily dependent on new technologies, and several stations are working on developing technologies/procedures that can be used by the other stations in the studies described in
the preceding paragraphs. Work has been done to develop a proteomics approach that can be used to characterize expression of different proteins (as opposed to mRNAs for these proteins) in response to different treatments or during different stages of muscle development/growth. This proteomics approach has thus far used protein microarrays and two-dimensional electrophoresis (Nebraska station). Microarrays have also been used to compare mRNA levels in normal and a transgenic mouse that expresses an inhibitor of myostatin activity. Fifty-one different genes are overexpressed in the transgenic mouse compared with the normal mouse. Verification of these genes and their identification are currently underway (Hawaii station). Other microarray studies are comparing expression of genes at different stages of development (57, 70, 90, 105 days of gestation and 1-,3-, 5- and 7-days postnatally) in the pig (Michigan station). A real-time PCR procedure for rapid quantification of the message levels of different myosin heavy chains has been developed and a procedure for accurate quantification of the different myosin heavy chains at the protein level is being developed, so mRNA expression and protein expression can be compared on the same sample (Illinois station). This is important because quantification of mRNAs is becoming increasingly widespread, and some results have suggested that levels of mRNA and levels of actual protein are poorly related in many instances. Other research has focused on developing a technique to enrich cell suspensions for use in implantation into developing embryos. These studies have used chicken cells and fluorescence-activated cell sorting (FACS). Chimeric chickens have been obtained (North Carolina station). Studies on adipocytes have found that subcutaneous-derived mature adipocytes will dedifferentiate to form proliferative-competent adipofibroblasts, but that subsequent redifferentiation is incomplete and that perimuscular-derived adipofibroblasts behave differently than the subcutaneous-derived cells (Washington station). The physiological significance of these results awaits further analysis, but thus far, it seems that subcutaneous-derived adipocytes are programmed to follow a pathway different from perimuscular-derived adipocytes.

Impacts

1. Dr. S. Velleman (co-chair Dr. H. Chester-Jones) organized a symposium on growth and development at the 2007 Joint Annual Meeting of the American Dairy Science Association, the Poultry Science Association, the Asociación Mexicana de Producción Animal, and the American Society of Animal Science held in San Antonio, TX from 8-12 July. Three of the 4 speakers were NC-1131 members; Drs. M. Doumit, W. Dayton, and B. Johnson. The presentations were selected for publication in the Journal of Animal Science; one of only a few of the symposia held at the meeting that were selected.

2. Committee members were invited to give over 25 oral presentations at national scientific and livestock meetings during the past year. The presentations covered a wide range of topics from basic laboratory results to applying this basic knowledge to the animal industry. The number of invited presentations and variety of audiences testify to the quality of work and impact that this committee has on muscle growth in domestic animals.

3. Procedures have been developed for expressing two important extracellular matrix (ECM) proteins in muscle cells and then having the expressed proteins transported out of the cell and assemble in a functional manner in the extracellular milieu. Availability of these procedures will allow for the first time mechanistic studies of the role of the ECM in skeletal muscle development and growth, something that has been impossible up to now.

4. Microarray analysis has identified 51 genes that are overexpressed in transgenic mice that
express an inhibitor of myostatin. Because myostatin has important effects on skeletal muscle growth, verification and identification of the genes whose expression is affected by its inhibition has potentially very important implications and raises the possibility that new genes having important roles in postnatal muscle growth may be identified.

5. Research by the committee on the effects of growth promotants such as anabolic steroids has progressed beyond the administer and observe stage to determining the signaling pathways in muscle cells that are involved in the response to these steroids. This new effort will undoubtedly reveal a complex and initially bewildering array of signaling molecules, pathways, and interactions, but eventually could lead to an understanding that will permit rational approaches to significantly increasing the rate of skeletal muscle growth.

Publications

Refereed Publications


Dodson, M. V. 2007. In order to recruit animal sciences students into the university, you need to teach them about animal science jobs. NACTA J. 51:72-73.

Dodson, M. V. 2007. Codger and computers: to "unplug" or not to "unplug?" NACTA J. 51:72.


Fernyhough, M.E., J.L. Vierck and M.V. Dodson. 2006. Assessing a non-traditional view of adipogenesis: adipocyte dedifferentiation h mountains or molehills? Cells, Tissues, Organs (formerly ACTA Anatomica; 287 libraries worldwide) 182: 226-228


Physiol. 575: 241-250.


Velleman, S.G., Coy, C.S., and McFarland, D.C. 2007. Effect of syndecan-1, syndecan-4, and glypican-1 on turkey muscle satellite cell proliferation, differentiation, and responsiveness to


In Press


Han, B., Junfeng Tong, C. Ma, M. J. Zhu, and M. Du. 2007. Insulin-like growth factor-1 (IGF-1) and leucine stimulate mammalian target of rapamycin (mTOR) signaling in pig myogenic satellite cells. Mol. Reprod. Develop. (in press).


Abstracts


Li, J., S.A. Reed and S.E. Johnson. 2007. Hepatocyte growth factor induces cell cycle withdrawal in satellite cells in a MAPK-dependent manner. Mol. Biol. Cell 18:


Mares, S.W., V.F. Thompson, G. Beinbreck, and D.E. Goll. 2007. Do the calpains degrade actin, ±-actinin, or myosin? FASEB Summer Research Conference, Biology of the Calpains in Health and Disease. Abstract #10,


Book Chapters


Theses


Weaver, A.D. Sarcomere length influences proteolysis. Ph.D. Dissertation, Purdue University.

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http://ars.sdstate.edu/nc131/main.htm