ABSTRACT: Skeletal muscle development and growth is a complex process that involves the interaction of muscle cells with their extracellular environment. Because muscle development involves the interaction of the cell surface and extracellular matrix molecules, research focus has been placed on the proteoglycans. Proteoglycans are macromolecules containing a central core protein with attached carbohydrates, called glycosaminoglycans, that are located at both the cell surface and the extracellular matrix. Research focus has been placed on understanding the mechanisms of the membrane-associated heparan sulfate proteoglycans, syndecan-4 and glypican-1, which are both capable of regulating cellular responsiveness to fibroblast growth factor 2 (FGF2). Fibroblast growth factor 2 is a potent stimulator of muscle cell proliferation and a strong inhibitor of differentiation. Studies on syndecan-4 and glypican-1 show that these proteoglycans differentially regulate muscle cell proliferation, differentiation, and cellular responsiveness to FGF2 with syndecan-4 predominantly modulating muscle cell proliferation and glypican-1 modulating differentiation. Site-directed mutagenesis approaches were used to define the effect of the syndecan-4 and glypican-1 covalently attached side chains on their activity. In general, a functional association was found between the glycosaminoglycan and N-glycosylated chains attached to the central core proteins of syndecan-4 and glypican-1 affecting their regulation of muscle cell proliferation, differentiation, and FGF2 responsiveness. Current research efforts are directed at identifying the cellular signaling pathways modulated by syndecan-4 and glypican-1.

Key words: extracellular matrix, glypican-1, muscle, proteoglycan, satellite cell, syndecan-4

INTRODUCTION

Skeletal muscle growth and development are highly organized processes regulated by interactions between muscle cells and their extracellular environment. During early embryonic development, muscle cells are derived from mesodermal precursor cells originating from mesodermal somites (Asakura et al., 2002). Muscle precursor cells become committed myoblasts, then differentiate into myocytes, and eventually fuse to form multinucleated myofibers (for review, see Chargé and Rudnicki, 2004). These changes in the state of muscle cells are due to cell adhesion, cell migration, and cell-to-cell interactions, which are regulated by signals from the extracellular environment.

Communication between the extracellular matrix (ECM) and muscle cells has a pivotal role in the regulation of muscle cell proliferation and differentiation. Proliferation represents the replication of muscle cells available to fuse and differentiate into multinucleated myotubes, whereas differentiation refers to the development of muscle-specific structures including multinucleated myotubes and fibers. Increased proliferation will provide a larger pool of muscle cells available for differentiation. Changes in the level of differentiation will affect muscle fiber size and the number of muscle fibers.

The ECM is a dynamic network of molecules secreted by the cells and includes collagens, proteoglycans, and noncollagenous glycoproteins. Traditionally, the ECM was described as a ground substance that the cells were embedded in and functioned as a structural framework.
for the cells but did not biologically influence cellular behavior. However, more recently the ECM has been shown to be an integral component of cellular communication by regulating cell shape, cell migration, and gene expression. The ECM is tissue-specific and changes with tissue age (Scott, 1995; Rozario and DeSimone, 2010). There are 3 layers of connective tissue surrounding muscle: the endomysium, perimysium, and epimysium. The endomysium separates individual muscle fibers; the perimysium surrounds bundles of muscle fibers; and the epimysium forms a sheath around the entire muscle. In skeletal muscle, the ECM is a major component in the intramuscular connective tissue layers (Thorsteinsdóttir et al., 2011). Mature muscle should contain ample connective tissue spacing with distinct endomysial spacing between the fibers and well-defined perimysial spacing between the muscle fiber bundles. The predominant ECM protein in the connective tissue layers are the collagens. Types I, III, IV, V, and VI collagen have been identified in skeletal muscle (Nishimura et al., 1997). Although the collagens have important structural and functional roles in ECM regulation of muscle growth properties, this paper will focus on the skeletal muscle proteoglycans and novel roles they may play in regulating avian breast muscle development and growth.

PROTEOGLYCANS

Proteoglycans are a diverse family of macromolecules containing a central core protein and at least one covalently attached glycosaminoglycan (GAG) chain. The central core protein can vary greatly in size from approximately 40,000 to greater than 350,000 daltons (Iozzo and Murdoch, 1996; Iozzo, 1998). Because of this diverse nature, proteoglycans are involved in several different processes including tissue hydration, regulation of gene expression, cell proliferation and differentiation, migration, and adhesion, which are all essential for muscle development.

The GAG chains are polymers of disaccharide repeats that are highly sulfated and contain a high negative charge. The negative charge permits ionic interactions with molecules such as water or growth factors. Glycosaminoglycan chains attached to the core protein include chondroitin sulfate, dermanatan sulfate, keratan sulfate, and heparan sulfate. Chondroitin sulfate is composed of repeats of glucuronic acid and N-acetylgalcosamine with sulfate groups in the 4- or 6-position of the amino sugar. Heparan sulfate consists of repeats of glucuronic acid and N-acetylgalcosamine. Keratan sulfate contains disaccharide repeats of galactose and N-acetylgalcosamine with the sulfate at the 6-position of the amino sugar. In skeletal muscle, proteoglycan expression changes during muscle development and growth from one rich in large chondroitin sulfate proteoglycans to a mixture of chondroitin, dermanatan, and heparan sulfate proteoglycans (Young et al., 1990; Fernandez et al., 1991; Velleman et al., 1999). This expression pattern of proteoglycans indicates that different proteoglycans may have distinct developmental functions during the muscle growth process.

HEPARAN SULFATE PROTEOGLYCANS

Two major groups of membrane-associated heparan sulfate proteoglycans, the syndecans and glypicans, are found in skeletal muscle (Figure 1). The syndecans are a family of 4 transmembrane heparan sulfate proteoglycans whose structure consists of a core protein, an extracellular domain with several consensus sequences for GAG and N-glycosylated chain attachment, a hydrophobic transmembrane domain, and a short cytoplasmic tail. Syndecan-1 through syndecan-4 have all been identified in skeletal muscle (Larrain et al., 1997; Fuentealba et al., 1999; Liu et al., 2006). Six members of the glypic family have been identified in mammals, and only glypican-1 has been reported in skeletal muscle (Campos et al., 1993). The molecular structure of glypican-1 is distinct from that of the syndecans. The central core protein does not contain a transmembrane domain or cytoplasmic domain and is linked to the cell surface by a glycosylphosphatidylinositol anchor. The glypican core protein also contains a cysteine-rich globular domain. The attached GAG chains for turkey glypican-1 are near the cell surface and for the syndecans extend into the extracellular environment. This distribution of GAG chains may be associated with the functional properties of each of these proteoglycans. Both the syndecans and glypican-1 are capable of regulating fibroblast growth factor 2 (FGF2) signal transduction in muscle. Fibroblast growth factor 2 is a potent stimulator of muscle cell growth and a strong inhibitor of differentiation into muscle specific structures (Dollenmier et al., 1981). Understanding how the muscle cells respond to stimuli like FGF2 has importance in maximizing muscle growth and ultimately maintaining or improving meat quality.

EFFECTS OF FGF2 ON MUSCLE GROWTH

Growth factors are strong stimulators or inhibitors of myoblast and myogenic satellite cell proliferation and differentiation. A biological effect of FGF2 during myogenesis is to inhibit the transcription of myogenin, a muscle specific transcriptional factor required for the initiation of myotube formation (Brunetti and Goldfine, 1990). By suppressing myogenin expression, FGF2 maintains the skeletal muscle cells in a state of proliferation. A prolonged period of proliferation would result in an increased pool of muscle cells available for muscle fiber formation. For FGF2 to interact with its tyrosine kinase receptor, it must bind to the heparan sulfate chains attached to proteoglycans, such as the syndecans or glypicans. If the heparan sulfate chains...
are removed from the proteoglycans, FGF2 no longer functions as an inhibitor of muscle differentiation (Rapraeger et al., 1991). The function of different heparan sulfate proteoglycans in the regulation of FGF2 signal transduction is not well understood at the present time.

REGULATION OF FGF2 BY GLYPICAN-1 AND SYNDECAN-4

Although both glypican-1 and syndecans are coreceptors for FGF2, their expression has been shown to be differentially regulated during skeletal muscle proliferation and differentiation (Brandan et al., 1996; Larraín et al., 1997; Liu et al., 2006). In general, syndecan expression is increased during proliferation and decreased during differentiation, whereas the pattern of glypican-1 expression is the reverse (Brandan et al., 1996; Larraín et al., 1997; Liu et al., 2006). It is not clear why these 2 groups of heparan sulfate proteoglycans, which are both co-receptors for FGF2, are expressed in opposite manners. Based on the differential expression pattern, Brandan and Larraín (1998) hypothesized that the syndecans may function as a presenter of FGF2 to its receptor and glypican-1 sequesters FGF2 from its receptor during differentiation to prevent the inhibitory effects of FGF2 on differentiation when it is released from the cell surface and can still actively bind FGF2.

Recent research has been conducted to further define the biological functions of the syndecans and glypican-1 during the growth and development of the turkey breast muscle. Although all of the syndecans are present in skeletal muscle, syndecan-3 and syndecan-4 are expressed during myogenic satellite cell activation and subsequent fusion with existing myofibers (Cornelson et al., 2001, 2004). Satellite cells are responsible for postnatal muscle growth and the regeneration of muscle. Syndecan-4 may also play a role in muscle cell adhesion and migration because it has been shown to affect migration of Chinese hamster ovary K1 cells (Longley et al., 1999). Myofiber formation requires the migration of muscle cells and their subsequent alignment and fusion into myofibers.

FUNCTION OF SYNDECAN-4 AND GLYPICAN-1 ATTACHED GLYCOSAMINOGLYCAN CHAINS

To further define the functional contribution of the syndecan-4 and glypican-1 attached heparan sulfate GAG chains, a site-directed mutagenesis strategy was used to remove each individual GAG chain covalently attached to the central core proteins of syndecan-4 and glypican-1 (Zhang et al., 2007, 2008). Syndecan-4 has 3 GAG attachment sites in its core protein at serine residues 38, 65, and 67. Using site-directed mutagenesis, syndecan-4 was mutated, leaving only 1 GAG chain attached to serine residues 38, 65, or 67, or no GAG chains attached to the core protein. The activity of each of these attachment sites was studied during proliferation, differentiation, and the responsiveness to FGF2 in turkey breast muscle satellite cells. The wild-type syndecan-4 and the GAG chain mutants all delay proliferation and the initial stages of differentiation but did not affect the responsiveness of the satellite cells to FGF2 (Zhang et al., 2008). These data indicate that syndecan-4 can function in an FGF2-independent manner and the GAG chains attached to the syndecan-4 core protein are not required for syndecan-4 to affect turkey satellite cell proliferation and initial stages of differentiation.

A similar approach was used to study the functional contribution of the GAG chains attached to the glypican-1 core protein. Glypican-1 has 3 GAG attachment sites at serine residues 483, 485, and 487. Zhang et
al. (2007) cloned a full-length turkey glypican-1 cDNA (GenBank AY551002). The wild-type glypican-1 was mutated using site-directed mutagenesis, leaving either 1 GAG chain at serine residues 483, 485, or 487, or no GAG chains attached to the core protein to obtain 1-chain and no-chain mutants. The wild-type glypican-1, 1-chain, and no-chain mutants were overexpressed in turkey myogenic satellite cells. The overexpression of glypican-1 increased FGF2 responsiveness during proliferation compared with the 1-chain and no-chain mutants, but did not affect proliferation compared with the controls. In contrast to syndecan-4, which can function in an FGF2-independent manner, glypican-1 function requires the GAG chains to mediate myogenic satellite cell responsiveness to FGF2.

FUNCTION OFSYNDECAN-4
AND GLYPICAN-1
N-GLYCOSYLATION CHAINS

Proteoglycan research has historically focused on the biological impact of the attached GAG chains due to their high negative charge and resulting ionic interactions and, more recently, their biological activity mediated by the central core protein. Many proteoglycans also contain N-glycosylated chains attached to the core protein, but these glycosylation sites have largely been an area not focused on because they are a minor component of the entire proteoglycan. Both syndecan-4 and glypican-1 contain N-glycosylated chains, but their function is not understood. N-glycosylated chains have been reported to have an important role in protein function. These include protein folding (Parodi, 2000; Helenius and Aebi, 2001) and localization of membrane proteins to the cell surface (Martinez-Maza et al., 2001; Yan et al., 2002). Therefore, it was hypothesized that the N-glycosylated chains attached to syndecan-4 and glypican-1 core proteins likely played a role in their biological function.

Site-directed mutagenesis was used to generate syndecan-4 N-glycosylated chain mutants with the core protein containing the attached GAG chains or without GAG chains to measure the functional interaction between the N-glycosylation and GAG chains (Song et al., 2011). Syndecan-4 has 2 N-glycosylated chain attachment sites at asparagine residues 124 and 139. The overexpression of syndecan-4 N-glycosylated mutants with or without the GAG chains did not change cell proliferation, differentiation, and responsiveness to FGF2 compared with wild type syndecan-4 except that overexpression of syndecan-4 N-glycosylated mutants without the GAG chains increased cell proliferation at 48 and 72 h of proliferation. Taken together, these data indicate that both the N-glycosylated and GAG chains are required for syndecan-4 to regulate myogenic satellite cell proliferation, but not differentiation. In support of syndecan-4 having a role in proliferation and not differentiation, Liu et al. (2006) reported that syndecan-4 expression was greater during satellite cell proliferation compared with differentiation.

The syndecan-4 GAG and N-glycosylated chains may affect proliferation through the regulation of focal adhesion formation. Focal adhesions are points of attachment between the substrate and cell, and are essential for cell migration, which is a process that occurs during proliferation leading to the formation of multinucleated myotubes. Woods and Couchman (1994) showed that syndecan-4 is an important regulator of focal adhesion composition. Research overexpressing syndecan-4 in Chinese hamster ovary K1 cells increased focal adhesion formation and decreased cell migration (Longley et al., 1999). During muscle development, it is possible that the overexpression of syndecan-4 results in more focal adhesions and decreased cell migration that would inhibit proliferation.

Similar to syndecan-4, site-directed mutagenesis was used to generate glypican-1 N-glycosylated chain 1-chain and no-chain mutants with or without GAG chains (Song et al., 2010). Glypican-1 has 3 N-glycosylated core protein attachment sites at asparagine residues 76, 113, and 382. With the GAG chains attached, the glypican-1 N-glycosylated mutants did not affect myogenic satellite cell proliferation compared with wild-type glypican-1 when overexpressed in turkey myogenic satellite cells. These data are in accord with the findings of Zhang et al. (2007) in that the glypican-1 GAG chains have no effect on satellite cell proliferation. However, when both the GAG and N-glycosylation were deleted, satellite cell proliferation was increased. During proliferation, deletion of the GAG and N-glycosylated chains also increased cellular responsiveness to FGF2. Differentiation was also increased by the deletion of both the GAG and N-glycosylated chains, but not with the GAG chains attached. These data indicate an interaction between the glypican-1 GAG and N-glycosylated chains, which may affect the 3 dimensional structure of the core protein affecting glypican-1 function.

Glypican-1 can be released or shed from the cell membrane through the action of phospholipases (Mythreye and Blobel, 2009). The shedding of glypican-1 from the cell surface occurs by the cleaving of the glycosylphosphatidylinositol anchor. When glypican-1 is shed from the cell, it can still bind FGF2, thereby preventing the interaction of FGF2 with its tyrosine kinase receptor and result in an increase in differentiation with the sequestration of FGF2 from its receptor.

SUMMARY AND CONCLUSIONS

In summary, several factors are involved in the molecular regulation of muscle development and growth. Developing a comprehensive understanding of the mechanisms of hyperplasia and hypertrophy is critical to the improvement and maintenance of meat quality. For example, the poultry industry has largely selected animals based on phenotypic growth rate and muscling.
This type of phenotypic selection regimen may alter muscle fiber proportions and modify the available connective tissue spacing surrounding the muscle fibers and bundles (Dransfield and Sosnicki, 1999).

The ECM macromolecules, especially the proteoglycans, affect multiple cellular processes associated with muscle growth and meat quality, including water-holding capacity and growth factor regulation. Changes in heparan sulfate proteoglycan or FGF2 expression can either prolong or decrease the periods of muscle cell proliferation and differentiation occurring during hyperplasia and hypertrophy. For example, in turkeys selected for increased 16-wk BW, expression of both heparan sulfate proteoglycans and FGF2 is increased during the embryonic phase of growth (Liu et al., 2002, 2003). These changes in FGF2 and heparan sulfate proteoglycan expression would likely prolong the period of proliferation and result in more muscle fiber deposition during hyperplasia. Thus, changes in the expression of ECM molecules, such as the heparan sulfate proteoglycans, will affect muscle development and growth properties affecting muscle structure and ultimately influencing meat quality.

**LITERATURE CITED**


