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The role of syndecan-4 and attached glycosaminoglycan chains on myogenic satellite cell growth

Xiangfeng Zhang [a], Karl E. Nestor [a], Douglas C. McFarland [b], Sandra G. Velleman [a,*]

[a] *Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691, USA*
[b] *Department of Animal and Range Sciences, South Dakota State University, Brookings, SD 57007, USA*

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Abstract

The syndecans are a family of cell-surface heparan sulfate proteoglycans consisting of a core protein with covalently attached glycosaminoglycan (GAG) chains. Syndecan-4 expression in skeletal muscle is increased in growth-selected animals during proliferation. Previous studies have suggested that cell-surface heparan sulfate proteoglycans like syndecan-4 are involved in fibroblast growth factor 2 (FGF2) signaling by FGF2 binding to the heparan sulfate chains. Fibroblast growth factor 2 is a potent stimulator of muscle proliferation and an intense inhibitor of differentiation. To investigate the functional contribution of the attached syndecan-4 GAG chains, a turkey syndecan-4 full length cDNA was cloned and mutated at two or all three potential GAG attachment sites at Ser_{38}, Ser_{65}, and Ser_{67} resulting in all possible one-chain mutants and a no-chain mutant. Turkey satellite cells were transfected with the wild-type syndecan-4, one-chain mutants, no-chain mutant, or the empty vector and assayed for cell proliferation, differentiation, and FGF2 responsiveness. The wild-type syndecan-4, one-chain mutants, and no-chain mutant inhibited cell proliferation and delayed initial differentiation but did not alter FGF2 responsiveness or function through the mitogen-activated protein kinase pathway. There was no difference between the wild-type syndecan-4, one-chain, and no-chain mutants during these stages. These data suggest that syndecan-4 functions 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 the initial differentiation.

Keywords: Glycosaminoglycan; Fibroblast growth factor 2; Heparan sulfate; Muscle; Proteoglycan; Syndecan-4

1. Introduction

During early embryonic development, skeletal muscle is formed through the activation, migration, proliferation, differentiation, and fusion of mesodermal muscle precursor cells. Postnatal muscle growth occurs through the activation and fusion of myogenic satellite cells with existing muscle fibers. Satellite cells are located between the muscle fiber basement membrane and plasmalemma (Mauro, 1961). Satellite cells predominantly exist in a quiescent state but during the period of postnatal muscle growth and regeneration of skeletal muscle in response to injury, the satellite cells are activated to proliferate and differentiate (Schultz, 1978; Schultz and McCormick, 1994). Although the process of satellite cell activation, proliferation, and differentiation has been studied, the molecular mechanisms regulating these processes are still not well understood. The activation of satellite

Abbreviations: DMEM, Dulbecco's Modified Eagle Medium; EGFP, enhanced green fluorescent protein; ECM, extracellular matrix; ERK, extracellular signal-regulated kinase; FGF2, fibroblast growth factor2; FGFR, fibroblast growth factor receptor; GAPDH, glyceraldehyde phosphate dehydrogenase; GAG, glycosaminoglycans; HS, heparan sulfate; HSPG, HS proteoglycans; LB, Luria–Bertani; MAPK, mitogen-activated protein kinase; M-MLV, Moloney murine leukemia virus reverse transcriptase; PBS, phosphate buffered saline; PI3, phosphoinositide 3-kinase; PVDF, polyvinylidene difluoride; PKC, protein kinase C; RT, reverse transcriptase; RNAi, RNA interference; SDS, sodium dodecyl sulfate; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

* Corresponding author. Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, 1680 Madison Avenue, Wooster, OH 44691, USA. Tel.: +1 330 263 3905; fax: +1 330 263 3949.
E-mail address: velleman.1@osu.edu (S.G. Velleman).

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cells is marked by the rapid expression of MyoD (Fuchtbauer and Westphal, 1992; Grounds et al., 1992) in response to growth factors and changes in the extracellular matrix (ECM) environment. Growth factors modulating satellite cell activity include, but are not limited to, fibroblast growth factor 2 (FGF2; Dollenmeier et al., 1981), transforming growth factor β (Florini et al., 1986), epidermal growth factor (Ham et al., 1988), and insulin-like growth factor (Schmid et al., 1983). Fibroblast growth factor 2, the focus of the present study, is a strong stimulator of muscle cell proliferation and inhibitor of differentiation (Dollenmeier et al., 1981).

The ECM is a dynamic environment which is tissue-type specific, changes with the age of a tissue, and is modified based on the physiological status of the tissue. Macromolecules composing the ECM have been shown to interact with growth factors and regulate cellular signal transduction pathways involved in tissue growth including that of skeletal muscle (Velleman, 1999). The ECM is composed of collagenous proteins, noncollagenous glycoproteins, and proteoglycans. Proteoglycans are a group of macromolecules, which consist of a core protein with covalently attached glycosaminoglycan (GAG) chains. Typical GAG chains attached to the proteoglycan core protein include heparan sulfate (HS), chondroitin sulfate, dermatan sulfate, and keratan sulfate.

Heparan sulfate proteoglycans (HSPG) likely play an important role in the regulation of satellite cell activity through their interaction with growth factors and interaction with cellular receptors. Heparan sulfate is required for the stable binding of FGF2 to its tyrosine kinase receptor (Rapraeger et al., 1991). The participation of HS in the ternary fibroblast growth factor receptor complex is facilitated through HS-binding motifs on the FGF2 ligand and the tyrosine kinase receptor (Schlessinger et al., 2000). Two major groups of membrane-associated HSPG are found in skeletal muscle, the syndecans and glypicans. The glypicans are attached to the cell membrane through a glycosylphosphatidylinositol anchor. There are six members to this family, but only glypican-1 has been reported in skeletal muscle (Campos et al., 1993). The syndecans have a central core protein containing extracellular, transmembrane, and cytoplasmic domains. The syndecans have four members, syndecan-1 through -4, and all four have been identified in skeletal muscle (Brandan and Larraín, 1998; Cornelison et al., 2001; Liu et al., 2004, 2006). In addition to the structural differences between these two families of HSPG, each of these proteoglycans is differentially expressed during proliferation and differentiation (Brandan et al., 1996; Larraín et al., 1998; Cornelison et al., 2001; Liu et al., 2004, 2006). These differences in structure between the two families of proteoglycans may lead to different functional properties reflected in their unique expression patterns. Although the expression of these proteoglycans is known, their biological function with regard to muscle development is not well understood.

Recent studies with syndecan-4$^{-/-}$ mice have shown that the syndecan-4$^{-/-}$ mice are unable to regenerate damaged muscle and are deficient in satellite cell activation, proliferation, MyoD muscle transcriptional regulatory factor expression, myoblast fusion, and differentiation (Cornelison et al., 2004). These data suggest a regulatory role for syndecan-4 in satellite cell function and muscle growth. In support of syndecan-4 potentially affecting muscle growth, Liu et al. (2006) showed that syndecan-4 expression is higher in satellite cells isolated from turkeys selected for increased 16-week body weight compared to randombred control turkeys not selected for growth characteristics. This increase in syndecan-4 expression during proliferation could result in enhanced FGF2 signaling leading to an increase in satellite cell proliferation.

Fibroblast growth factor 2 signaling occurs through the binding of FGF2 to the HS chains attached to the proteoglycan core protein. The functional contribution of the syndecan-4 attached HS chains has not been well defined in regard to FGF2 signal transduction during skeletal muscle development. Turkey syndecan-4 core protein has three potential GAG attachment sites at serine residues Ser_{38}, Ser_{65}, and Ser_{67}. These sites contain the amino acid sequence SerGlySer or SerGlySerGly

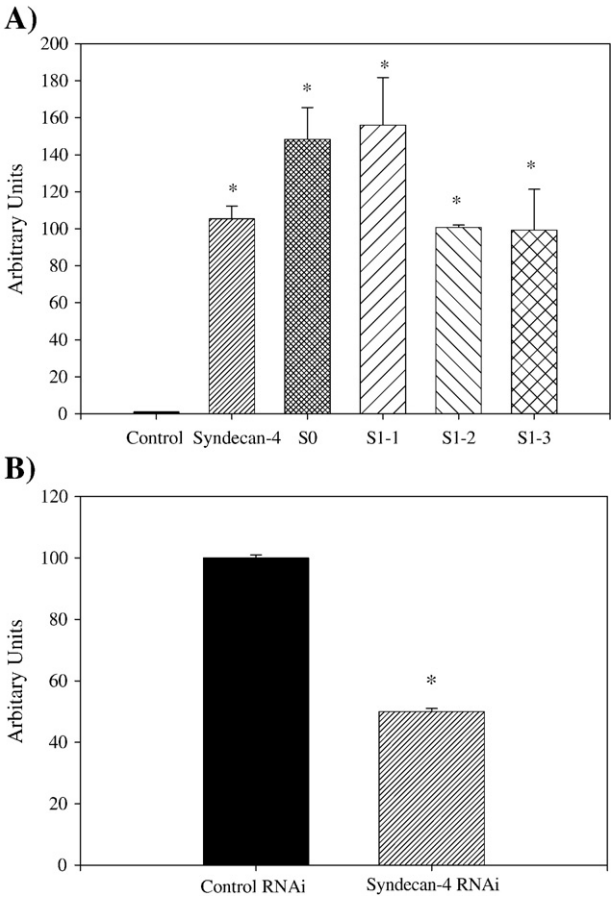

Fig. 1. Real-time quantitative PCR analysis of the expression of syndecan-4 mRNA. A) Turkey RBC2 line male satellite cells were transfected with the pCMS-EGFP vector without a gene insert as a control, syndecan-4, the no-chain mutant (S0), and one-chain mutants (S1-1, S1-2, and S1-3). The RNA expression was measured 48 h after the transfection. B) Turkey satellite cells were transfected with a RNA interference (RNAi) to syndecan-4 or a non-specific control RNAi. The mRNA expression of syndecan-4 was measured in cells 48 h post transfection. The relative syndecan-4 expression in each sample was normalized by the expression of glyceraldehyde phosphate dehydrogenase (GAPDH). The bars represent the standard error of the mean. An * indicates a significant difference from the control ($P<0.05$). The overexpression and siRNA assays were repeated three times.