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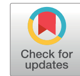
The effect of syndecan-4 and glypican-1 knockdown on the proliferation and differentiation of turkey satellite cells differing in age and growth rates



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ABSTRACT

Posthatch skeletal muscle growth requires myogenic satellite cells and the dynamic expression of cell membrane-associated proteins. The membrane associated heparan sulfate proteoglycans, syndecan-4 and glypican-1, link the satellite cell niche to the intracellular environment. Sydnecan-4 and glypican-1 are differentially expressed with age in turkey satellite cells and their over-expression impacts both satellite cell proliferation and differentiation, but their effect on satellite cells from lines with different growth potentials is not known. The objective of the current study was to determine if syndecan-4 and glypican-1 regulation of satellite cell proliferation and differentiation is affected by age and growth selection. Pectoralis major satellite cells isolated at 1 d, 7 and 16-wk of age from a Randombred Control 2 (RBC2) line and a 16-wk body weight (F) line selected from the RBC2 line turkeys were studied. Syndecan-4 and glypican-1 expression was knocked down in both lines. The F-line cells proliferated faster than RBC2 line cells regardless of age, while differentiation tended to be greater in RBC2 line cells than F-line cells at each age. Syndecan-4 knockdown decreased proliferation at 7- and 16-wk but not 1 d cells, and increased differentiation at 1 d and 7 wk but not 16 wk cells. Glypican-1 knockdown differentially affected proliferation depending on cell age, whereas differentiation was decreased for 7- and 16-wk but not 1 d cells. These data suggest syndecan-4 and glypican-1 differentially affected satellite cell function in an age-dependent manner, but had little impact on differences in proliferation and differentiation due to growth selection.

1. Introduction

Skeletal muscle development and growth occurs in two distinct phases during embryonic and posthatch development. Embryonic muscle fiber development occurs through the process of hyperplasia. During hyperplasia mononucleated myoblast cells proliferate, migrate, align, and fuse to form multinucleated myotubes that differentiate into nascent myofibers (Biressi et al., 2007). After the myoblasts fuse, they withdraw from the cell cycle (Stockdale and Holtzer, 1961) and myofiber formation is complete at hatch (Smith, 1963). Subsequent posthatch growth is dependent on the hypertrophy of exisiting muscle fibers (Moss, 1968; Mozdziak et al., 1997). Posthatch muscle fiber hypertrophy is mediated by a myogenic stem cell population termed adult myoblasts or satellite cells, which are located between the myofiber basal lamina and sarcolemma (Mauro, 1961). Satellite cells fuse with existing muscle fibers by donating their nuclei, which increases the protein synthesis potential of muscle fibers (Stockdale and Holtzer, 1961; Moss and Leblond, 1971).

Satellite cells are a dynamic population of cells with mitotic activity being the highest immediately after hatch (Mozdziak et al., 1994; Halevy et al., 2003; Velleman et al., 2010) and declining to < 5% of total muscle nuclei with the vast majority of those cells becoming quiescient (Hawke and Garry, 2001). Satellite cells are a heterogenous population of cells with variable proliferation, differentiation, and responsiveness to the innibitory or stimulatory effects of growth factors. With age (Velleman et al., 2010; Harthan et al., 2013a) and selection for growth in turkeys (McFarland et al., 1993; Merly et al., 1998; Velleman et al., 2000), satellite cell activity has been shown to be variable.

Satellite cell activity is regulated by the proximate extrinsic extracellular matrix environment, basal lamina and vascular system surrounding the cells, collectively termed the satellite cell niche (Kuang et al., 2008). Satellite cell proliferation and differentiation in turkeys has been shown to be differentially affected by the membrane-associated heparan sulfate proteoglycans syndecan-4 and glypican-1 (Velleman et al., 2006, 2007, 2013; Zhang et al., 2007, 2008; Song

Abbreviations: Dulbecco's Modified Eagle's Medium, DMEM; 16 wk body weight line, F; Fibroblast growth factor 2, FGF2; Glyceraldehyde-3-phosphate dehydrogenase, GAPDH; Glycosylphosphatidylinositol, GPI; Glypican-1, G1; Least square means, lsmeans; Negative control, Ctrl; Pectoralis major, p. major; Phosphate buffered saline, PBS; Randombred control 2, RBC2; Real-time quantitative PCR, RT qPCR; Small interfering RNA, siRNA; Standard error of the mean, SEM; Syndecan-4, S4

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et al., 2010, 2012a, b; Harthan et al., 2013b). Syndecan-4 and glypican-1 act as a bridge between the extrinsic extracellular matrix and intracellular environment communicating signal transduction events that affect both satellite cell proliferation and differentiation.

Syndecan-4 is a transmembrane heparan sulfate proteoglycan with a central core protein containing an extracellular domain with 3 attached heparan sulfate chains (Zhang et al., 2008),a transmembrane domain, and a cytoplasmic domain (Carey, 1997; Couchman, 2003). In turkeys, syndecan-4 affects satellite cell proliferation and differentiation in a fibroblast growth factor 2 (FGF2)-independent manner (Velleman et al., 2007; Zhang et al., 2008; Song et al., 2011). Syndecan-4 plays a primary role in satellite cell migration through RhoA signal transduction (Shin et al., 2013). Satellite cell migration is crucial in the formation of multinucleated myotubes and subsequent fiber formation.

Glypican-1 is an extracellular heparan sulfate proteoglycan linked to the satellite cell membrane by a glycosylphosphatidylinositol (GPI) anchor. The glypican-1 core protein contains a globular-cysteine rich domain and three heparan sulfate chains (Fransson, 2003; Zhang et al., 2007; Filmus et al., 2008). Unlike syndecan-4 which functions in an FGF2-independent manner, glypican-1 sequesters FGF2 during differentiation from its tyrosine kinase receptor (Brandan and Larraín, 1998; Gutiérrez and Brandan, 2010). Fibroblast growth factor 2 is a potent inhibitor of differentiation (Dollenmeier et al., 1981; Brunetti and Goldfine, 1990). Glypican-1 sequesters FGF2 by being shed into the extracellular matrix after cleavage of its GPI anchor (Brandan et al., 1996; Velleman et al., 2013) or by being localized to lipid raft domains lacking FGF2 receptors (Gutiérrez and Brandan, 2010). Glypican-1 expression is also necessary for the expression of the myogenic transcriptional regulatory factor myogenin (Gutiérrez and Brandan, 2010). Myogenin expression is required for the formation of multinucleated myotubes.

Through measuring syndecan-4 and glypican-1 expression (Harthan et al., 2013a) and over-expressing syndecan-4 and glypican-1 in non-growth selected turkey satellite cells in 1 d, 7- and 16-wk old pectoralis major (p. major) satellite cells, Harthan et al. (2013b), showed that syndecan-4 and glypican-1 were differentially expressed and affected proliferation, differentiation, and FGF2 responsiveness in an age-specific manner. However, there are no published reports to date on the interaction of age and growth selection on syndecan-4 and glypican-1 regulation of p. major muscle development. Both syndecan-4 and glypican-1 play integral roles in the assembly and differentiation of the p. major muscle. Thus, understanding how these heparan sulfate proteoglycans mechanistically affect satellite cell activity with age and growth selection will lead to the development of new strategies maximizing their function to improve p. major muscle development and growth.

2. Materials and methods

2.1. Syndecan-4 and glypican-1 small interfering RNA

Small interfering RNA (siRNA) sequences were designed using BLOCK-iT RNAi Designer (https://rnaidesigner.thermofisher.com/rnaiexpress/) for the gene sequences for turkey syndecan-4 (GenBank AY852251) and turkey glypican-1 (GenBank AY551002). The primer sequences for the syndecan-4 siRNA were the following: sense: 5′-CAC CAU GCU GGA CAA UGA AAU CAU U-3′; anti-sense: 5′-AAU GAU UUC AUU GUC CAG CAU GGU G-3′, which target the coding regions from 332 to 356 of the turkey syndecan-4 gene. The primer sequences for the glypican-1 siRNA were the following: sense: 5′-UCU UUG CCU UCU GCC UUC CUC CUU U-3′; anti-sense: 5′-AAA GGA GGA AGG CAG AAG GCA AAG A-3′, which target the coding regions from 1614 to 1638 of the turkey glypican-1 gene. Syndecan-4 and glypican-1 siRNA were chemically synthesized Stealth RNAi (Thermo Fisher Scientific, Pittsburgh, PA) in the desalted, pre-annealed duplex form. A Stealth RNAi negative control siRNA duplex with 40% GC content (Thermo Fisher Scientific) was used as a control for syndecan-4 and glypican-1 siRNA.

2.2. Turkey myogenic satellite cells

Satellite cells were isolated from the p. major muscle of 1 d, 7 wk, and 16 wk posthatch male randombred control 2 (RBC2) line and F-line turkeys as described in Velleman et al. (2000). Isolated cells were expanded and stored in liquid nitrogen until use for experiments. The RBC2 line represents a 1967 commercial turkey that has been maintained at The Ohio State University, Ohio Agricultural Research and Development Center Poultry Research Unit without conscious selection for any traits. The F-line turkeys were derived from the RBC2 line by selecting for only increased 16-wk body weight (Nestor, 1977) and has been continually selected base upon this single trait for over 40 generations. Thus, gene expression differences are only due to selection for 16-wk body weight and not genetic background differences. The F-line turkeys have a significantly larger body weight and p. major muscle weight at 16 wk of age than the RBC2 line (Lilburn and Nestor, 1991).

2.3. Cell culture and small interfering RNA transfection

Satellite cells were plated in 24 well or 48 well cell culture plates (Greiner Bio-One, Monroe, NC) coated with 0.1% gelatin (Sigma-Aldrich, St. Louis, MO). For 7 wk and 16 wk cells, 24-well plates were seeded with 15,000 cells per well and 48-well plates with 9000 cells per well. Because 1 d cells proliferated at a quicker rate than the other ages, 24-well plates were seeded with 12,000 cells per well and 48-well plates with 7800 cells per well. Cells were plated in medium composed of Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich) with 10% chicken serum (Sigma-Aldrich), 5% horse serum (Sigma-Aldrich), 1% antibiotic antimycotic solution (Sigma-Aldrich), and 0.1% gentamicin (Omega Scientific, Tarzana, CA), and cultured at 38 °C in a 5% CO2 incubator (Thermo Fisher Scientific). For syndecan-4 and glypican-1 knockdown during proliferation, cells were transfected, after 24 h of cell attachment, with 20 pmol of syndecan-4, glypican-1, or negative control siRNA per 1 μl of Lipofectamine 3000 (Thermo Fisher Scientific) per well following the manufacturer's protocol. After 4 to 6 h, the medium was changed to a growth medium consisting of McCoy's 5A medium (Sigma-Aldrich) with 10% chicken serum, 5% horse serum, 1% antibiotic antimycotic solution, and 0.1% gentamicin, which was replaced every 24 h. For syndecan-4 and glypican-1 knockdown during differentiation, the plating medium was changed, after 24 h of cell attachment, to the growth medium, which was replaced every 24 h. After 72 h of proliferation in growth media, cells were transfected with syndecan-4, glypican-1, or negative control siRNA as described above. After 4 to 6 h of transfection, differentiation was induced by changing to a low-serum, differentiation medium composed of DMEM with 3% horse serum, 1% antibiotic antimycotic solution, 0.1% gentamicin, 0.1% porcine gelatin (Sigma-Aldrich), 1.0 mg/ml bovine serum albumin (Sigma-Aldrich), which was replaced every 24 h.

2.4. RNA isolation and real-time quantitative PCR

Cells were cultured in 24-well plates as described above. For verification of gene expression knockdown during proliferation, cells were transfected, after 24 h of cell attachment in plating media, for 4 to 6 h, and then the media was changed to growth media. Plates were collected at 72 h of proliferation in growth media. For verification of the gene expression knockdown during differentiation, cells were transfected, after 72 h of proliferation in growth media, for 4 to 6 h, and then the media was changed to low serum, differentiation media. Plates were collected at 48 h of differentiation in low serum media. After removing the media, the plates were stored at −70 °C until analysis. Total RNA was extracted using RNAzol (Molecular Research Center, Cincinnati, OH) following the manufacturer's protocol. A spectrophotometer

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