Effect of Syndecan-1, Syndecan-4, and Glypican-1 on Turkey Muscle Satellite Cell Proliferation, Differentiation, and Responsiveness to Fibroblast Growth Factor 2[1]

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ABSTRACT The membrane-associated heparan sulfate proteoglycan families consisting of the syndecans and glypicans are low-affinity receptors for fibroblast growth factor 2 (FGF2). Fibroblast growth factor 2 is a potent stimulator of skeletal muscle cell proliferation and a strong inhibitor of differentiation. Because syndecan-1, syndecan-4, and glypican-1 potentially play unique, but pivotal, roles in muscle cell proliferation and differentiation, these proteoglycans were examined for their effect on muscle cell proliferation and differentiation and FGF2 responsiveness. In the present study, turkey Randombred Control 2 line myogenic satellite cells were transfected with expression vector constructions of syndecan-1, syndecan-4, or glypican-1 to assay their role during muscle development and the effect on FGF2 responsiveness. During proliferation, only syndecan-1 increased proliferation. Both syndecan-4 and glypican-1 decreased proliferation at 72 h but generally did not affect the proliferation process. There was no interaction between the transfected gene and cell proliferation response to FGF2. Glypican-1 increased differentiation early in the process (24 h), and at later times differentiation was decreased by glypican-1. Both syndecan-1 and syndecan-4 overexpression decreased differentiation. During differentiation, except for glypican-1 at 48 h of differentiation, there was no interaction between gene treatment and FGF2 responsiveness. This result indicates that FGF2 responsiveness was not affected by the overexpression of syndecan-1, syndecan-4, and glypican-1 during differentiation. These data demonstrate that syndecan-1, syndecan-4, or glypican-1 differentially affect the processes of turkey muscle cell proliferation and differentiation, and can regulate these developmental stages in an FGF2-independent manner.

Key words: glypican-1, muscle, syndecan-1, syndecan-4, turkey

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INTRODUCTION

During early embryonic development, presumptive myoblasts migrate to the appropriate sites for skeletal muscle formation and give rise to myoblasts. The myoblasts proliferate, align with each other, and subsequently fuse to form multinucleated myotubes that develop into mature muscle fibers (Swartz et al., 1994). The satellite cells, another group of myogenic cells that reside between the basement membrane and the plasma membrane of the muscle fibers, are the primary source of mononucleated cells that contribute to support postnatal muscle growth by hypertrophy and muscle regeneration (Mauro, 1961; Moss and LeBlond, 1971). In almost all mammals and birds, muscle fiber formation is complete—and the muscle fiber number is determined—around the time of birth or hatching (Smith, 1963). The satellite cells are myogenic stem cells that fuse with existing muscle fibers, donating their nuclei to the existing muscle fiber and increasing protein synthesis.

Satellite cells are normally quiescent and must be activated to fuse with existing muscle fibers. The activation, proliferation, and differentiation of satellite cells are precisely regulated, in part, through cellular interactions with extrinsic factors. These extrinsic ligands include growth factors such as transforming growth factor-β, epidermal growth factor, platelet-derived growth factor, and fibroblast growth factor 2 (**FGF2**; Dodson et al., 1996). For many of these extrinsic ligands to assert their effects on cellular gene expression, proliferation, differentiation, and migration, they must interact with a cellular receptor system.

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Cell-associated proteoglycans are a highly complex group of macromolecules that are emerging as key regulators of the interaction of cells with the extracellular matrix and extrinsic ligands. Proteoglycans represent a diverse family of glycosylated proteins that contain a core protein with covalently attached glycosaminoglycans (Hardingham and Fosang, 1992). Glycosaminoglycans attached to the core protein include chondroitin sulfate, dermatan sulfate, keratan sulfate, and heparan sulfate. The heparan sulfate containing proteoglycans are low-affinity receptors for FGF2. Heparan sulfate proteoglycans have been shown to regulate FGF2 binding to its high-affinity tyrosine kinase receptors (Rapraeger et al., 1991; Yayon et al., 1991). Fibroblast growth factor 2 is a potent stimulator of muscle cell proliferation and a strong inhibitor of differentiation (Dollenmeier et al., 1981). Therefore, the expression of heparan sulfate proteoglycans and mechanism for regulating FGF2 responsiveness during muscle cell proliferation and differentiation may play a pivotal role in the development and growth of muscle.

Two major groups of membrane-associated heparan sulfate proteoglycans, the syndecans and glypicans, have been identified in skeletal muscle. The syndecans are a family of 4 transmembrane heparan sulfate proteoglycans. Their structure consists of a signal peptide, an extracellular domain with glycosaminoglycan attachment sites, a hydrophobic transmembrane domain, and a cytoplasmic tail. Syndecan-1 through syndecan-4 have been identified in skeletal muscle (Larraín et al., 1997; Brandan and Larraín, 1998; Fuentealba et al., 1999) and are differentially expressed (Cornelison et al., 2001; Liu et al., 2004, 2006). In contrast, the glypican family has 6 members attached to the cell surface by a glycosylphosphatidylinositol anchor with a Cys-rich globular domain. Only glypican-1 has been found in skeletal muscle (Campos et al., 1993).

Although the syndecans and glypican have all been implicated in regulating muscle cell responsiveness to FGF2, whether these proteoglycans can also function in an FGF2-independent manner has not been well investigated. The objective of the present study was to determine whether syndecan-1, syndecan-4, and glypican-1 were able to affect the proliferation and differentiation characteristics of turkey muscle cells in an FGF2-independent fashion. These membrane-associated heparan sulfate proteoglycans were chosen because of the potentially different roles they may play in the proliferation and differentiation processes. Syndecan-1 was selected because of its high expression during muscle cell proliferation (Brandan and Larraín, 1998; Liu et al., 2004) and because in epithelial tissue, syndecan-1 expression has been shown to be regulated by a fibroblast growth factor-inducible response element (Rautava et al., 2003). Syndecan-4 has been implicated as playing a role in myogenic satellite cell maintenance or activation, which is important for the process of postnatal muscle growth by hypertrophy (Cornelison et al., 2001). Glypican-1, based on its high expression during muscle cell differentiation, has been proposed to sequester FGF2 from its tyrosine kinase receptor to permit differentiation to take place (Brandan et al., 1996; Liu et al., 2006). The data from the current study will provide initial information with regard to the relationship between FGF2, syndecan-1, syndecan-4, and glypican-1 during muscle cell proliferation and differentiation.

MATERIALS AND METHODS

Turkey Myogenic Satellite Cells

Myogenic satellite cells were isolated from the pectoralis major muscle of 7-wk-old male randombred control 2 line (**RBC2**) turkeys as described in Velleman et al. (2000). The RBC2 line has not been selected for growth traits (Nestor, 1977) and results in the present study will thus not reflect the effect of changes in myogenic satellite cell proliferation or differentiation due to growth selection. To avoid sex effects, only satellite cells isolated from male RBC2 turkeys were used in the current study.

Proliferation Assay

The RBC2 line turkey satellite cells were plated at a density of 15,000 cells per well in gelatin-coated 24-well plates (Greiner BioOne, Monroe, NC) and grown for 24 h in Dulbecco's Modified Eagle's Medium (**DMEM**; Atlanta Biologicals, Lawrenceville, GA) containing 10% chicken serum (Invitrogen, Carlsbad, CA) and 5% horse serum (Invitrogen), with 1% antibiotics-antimycotics (Invitrogen) at 37°C in a 95% air, 5% CO_2 incubator. After 24 h, the cultures were transiently transfected with a full-length mouse syndecan-1 cDNA (Saunders et al., 1989), full-length turkey cDNA for syndecan-4 (GenBank accession number AY852251), full-length turkey cDNA for glypican-1 (GenBank accession number AY551002) expression vector constructs in pCMS-EGFP (Invitrogen), or the vector pCMS-EGFP (Invitrogen) using Invitrogen's Optifect reagent with 1 μg of plasmid according to the manufacturer's protocol. Plasmid DNA used in the transfections was purified with Promega's (Madison, WI) PureYield Plasmid Midiprep System. Transfection efficiency ranged between 60 and 65%. Beginning after the transfection (5 h), plates were removed at 24-h intervals for 72 h, rinsed with PBS, air-dried, and stored at –70°C until analysis. The DNA concentration was analyzed using Hoechst 33258 fluorochrome by the method of McFarland et al. (1995) adapted from the fluorometric procedure described by Rago et al. (1990) using double-stranded calf thymus DNA as the standard. The DNA concentration was measured on a Fluoroskan Ascent FL (Thermo-Electron Co., Waltham, MA). The proliferation assay was repeated 7 times for syndecan-1 and syndecan-4, and 8 times for glypican-1.

Responsiveness to FGF2 During Satellite Cell Proliferation

The RBC2 line turkey satellite cells were plated at a density of 15,000 cells per well in gelatin-coated 24-well

plates (Greiner BioOne) and grown for 24 h in DMEM containing 10% chicken serum (Invitrogen) and 5% horse serum (Invitrogen), with 1% antibiotics-antimycotics in a 37°C 95% air, 5% CO_2 environment. After 24 h, the cultures were transfected with the syndecan-1, syndecan-4, glypican-1 expression vectors, or pCMS-EGFP vector using Invitrogen's Optifect Reagent with 1 μg of plasmid DNA per culture well according to the manufacturer's protocol. Plasmid DNA used in the transfections was purified with Promega's PureYield Plasmid Midiprep System. After the transfection, serum-free defined medium (McFarland et al., 2006) was added to the cell cultures containing 0, 2.5, and 10 ng/mL of FGF2 (Pepro Tech, Rocky Hill, NJ). The medium was changed daily for 72 h. At 72 h posttransfection, the plates were rinsed with PBS, air-dried, and stored at –70°C until analysis. Fibroblast growth factor 2 responsiveness was measured by the DNA content of the wells in cultures with a 60 to 65% transfection efficiency. The DNA concentration was analyzed using Hoechst 33258 fluorochrome by the method of McFarland et al. (1995) adapted from the fluorometric procedure described by Rago et al. (1990) using double-stranded calf thymus DNA as the standard. The DNA concentration was measured as described above. The responsiveness assay was repeated 6 times for each expression vector construct.

Differentiation Assay

The creatine kinase assay for differentiation was adapted from the method of Florini (1989). The RBC2 line turkey satellite cells were plated at a density of 15,000 cells per well in gelatin-coated 24-well plates (Greiner BioOne) and grown for 24 h in DMEM containing 10% chicken serum (Invitrogen) and 5% horse serum (Invitrogen), with 1% antibiotics-antimycotics (Invitrogen) in a 37°C 95% air, 5% CO_2 environment. After 24 h, the cultures were transfected with the syndecan-1, syndecan-4, glypican-1 expression vectors, or pCMS-EGFP vector using Invitrogen's Optifect Reagent with 1.0 μg of plasmid DNA per culture well and 2.7 μL of Optifect reagent (Invitrogen) in 100 μL of Opti-MEM (Invitrogen) according to the manufacturer's protocol. Plasmid DNA used in the transfections was purified with Promega's PureYield Plasmid Midiprep System. The medium was changed daily until 72 h posttransfection or until the cells reached 65% confluency. Differentiation was initiated by changing the medium to DMEM, 3% horse serum, 1% antibiotics-antimycotics, 0.1% gentamicin, 1% gelatin, and 1% BSA. Beginning at 0 h differentiation, plates were removed at 24-h intervals for 96 h, rinsed with PBS, air-dried for 10 min, and stored at –70°C until being assayed. Plates were removed from the freezer and allowed to thaw for 5 to 10 min. To each well, 1.2 mL of creatine kinase assay buffer [4 m*M* glucose (Fisher Scientific, Pittsburgh, PA), 2 m*M* Mg acetate (Fisher Scientific), 0.2 m*M* adenosine diphosphate (Sigma-Aldrich, St. Louis, MO), 2 m*M* adenosine monophosphate (Sigma-Aldrich), 2 m*M* phosphocreatine (Calbiochem, LaJolla, CA), 0.5 U/mL of hexokinase (Worthington Biochemical, Lakewood, NJ), 1 U/mL of glyceraldehyde-6-phosphodihydrogenase (Worthington Biochemical), 0.4 m*M* thionicotinamide adenine dinucleotide (Sigma-Aldrich), and 1 mg/mL of BSA prepared in 0.1 *M* glycylglycine (Sigma-Aldrich), pH 7.5] was added to each well, mixed by gentle pipetting, and the plates were wrapped in aluminum foil and incubated at room temperature for 10 min. The samples were transferred to 1.5-mL cuvettes and read at an absorbancy of 405 nm every 5 min for 20 min on a Spectronic Genesys 5 spectrophotometer (ThermoElectron Co.) The cell differentiation assay was repeated 3 times for each gene expression vector construct.

Responsiveness to FGF2 During Satellite Cell Differentiation

The RBC2 line turkey satellite cells were plated at a density of 2,500 cells per well in gelatin-coated 96-well plates (Greiner BioOne) and grown for 24 h in DMEM containing 10% chicken serum (Invitrogen), and 5% horse serum (Invitrogen) with 1% antibiotics-antimycotics (Invitrogen) in a 37°C 95% air, 5% CO_2 environment. After 24 h, the cultures were transfected with the syndecan-1, syndecan-4, glypican-1 expression vectors, or pCMS-EGFP vector using Invitrogen's Optifect Reagent with 0.3 μg of plasmid DNA per culture well and 0.7 μL of Optifect reagent (Invitrogen) in 50 μL of Opti-MEM (Invitrogen) according to the manufacturer's protocol. Plasmid DNA used in the transfections was purified with Promega's PureYield Plasmid Midiprep System. After the 5-h transfection incubation at 37°C in a 95% air, 5% CO_2 incubator, the medium was changed to McCoy's 5A (Sigma-Aldrich), 10% chicken serum (Invitrogen), 5% horse serum (Invitrogen), 1% antibiotics-antimycotics (Invitrogen), and 0.1% genetamicin (Invitrogen). The medium was changed every 24 h until 72 h following the transfection or until the cell reached 65% confluency in the wells. Differentiation was initiated by changing the medium to DMEM containing 3% horse serum, 1% antibiotics-antimycotics, 0.1% gentamicin, 1% gelatin, and 1 mg/mL of BSA containing 0, 2.5, or 10.0 ng/mL of FGF2 (Pepro Tech Inc.). The medium was changed daily for 96 h. Beginning at 0 h differentiation, plates were removed at 24 h, rinsed with PBS, air-dried, and stored at –70°C until analysis. At the time of assay, all plates were thawed at room temperature for 10 min. During this time, a standard curve of creatine phosphokinase (Sigma-Aldrich) was prepared in a 96-well plate to use as a standard, with a range of 0 to 20 mU brought to a final volume of 200 μL in creatine kinase assay buffer. To the experimental plates, 200 μL of creatine kinase assay buffer was added to each sample well, and the plated were wrapped in aluminum foil and incubated at room temperature for 10 min. The optical density at 405 nm was then read every 5 min for 25 min with a Dynex Revelation microplate reader (Dynex Technologies, Chantilly, VA). The FGF2 responsiveness assay during differentiation was repeated 4 times for each expression vector construct.

Statistical Analysis

Differences in proliferation rates were evaluated using a Student's *t*-test. Differences were considered significant if $P < 0.05$. The FGF2 responsiveness data were analyzed by gene with a 2-way ANOVA. The main effects were gene treatment and fibroblast growth factor concentration. The 2-way interaction between the main effects was obtained.

RESULTS

The syndecan-1, syndecan-4, and glypican-1 transfected RBC2 line satellite cells and control cells were cultured and assayed for DNA accretion, an indication of proliferation. In general, overexpression of syndecan-1 increased the proliferation of the satellite cells through 48 h (Figure 1, panel A). Although not significantly different, at 72 h of proliferation the syndecan-1-transfected RBC2 satellite cells exhibited less growth than the control. Syndecan-4 overexpression had no significant effect on proliferation until 72 h posttransfection. At that time, proliferation was decreased in the syndecan-4-transfected RBC2 cells (Figure 1, panel B). The glypican-1-transfected cells showed increased proliferation at 5 h and then had no effect on proliferation until 72 h, when proliferation was reduced (Figure 1, panel C).

Fibroblast growth factor 2 responsiveness was measured at 72 h in the transfected syndecan-1, syndecan-4, and glypican-1 cultures (Figure 2, panels A to C). Except at 0 ng/mL of FGF2, RBC2 cells overexpressing syndecan-1 were not more responsive to FGF2. At 0 ng/mL, the syndecan-1-transfected cells were reduced in their proliferation and this trend remained with increased FGF2 treatment (Figure 2, panel A). Syndecan-4-transfected cells were less responsive to FGF2 treatment at 0- and 2.5-ng/mL concentrations (Figure 2, panel B). Although not significantly different at 10 ng/mL, the syndecan-4-transfected cells were similar in their responsiveness to FGF2, suggesting that syndecan-4 is responsive to the cell growth-stimulating effects of FGF2. In contrast, glypican-1-treated cell cultures with 0 and 2.5 ng/mL of FGF2 had increased cell growth in response to FGF2 (Figure 2, panel C). However, at 10 ng/mL of FGF2, there was no significant difference between the glypican-1-treated cells and the control. Although the transfections with syndecan-1, syndecan-4, and glypican-1 resulted in treatment effects, there was no significant interaction with FGF2 responsiveness.

During differentiation, syndecan-1 in general decreased differentiation beginning at 24 h through the 96 h of sample collection, except at 72 h of differentiation (Figure 3, panel A). Beginning at 48 h, syndecan-4 transfection reduced differentiation in the RBC2 cell cultures (Figure 3, panel B). Glypican-1 transfection initially resulted in an elevation in differentiation at 24 h (Figure 3, panel C). However at 48 and 72 h the differentiation process was reduced in the glypican-1 treated cells.

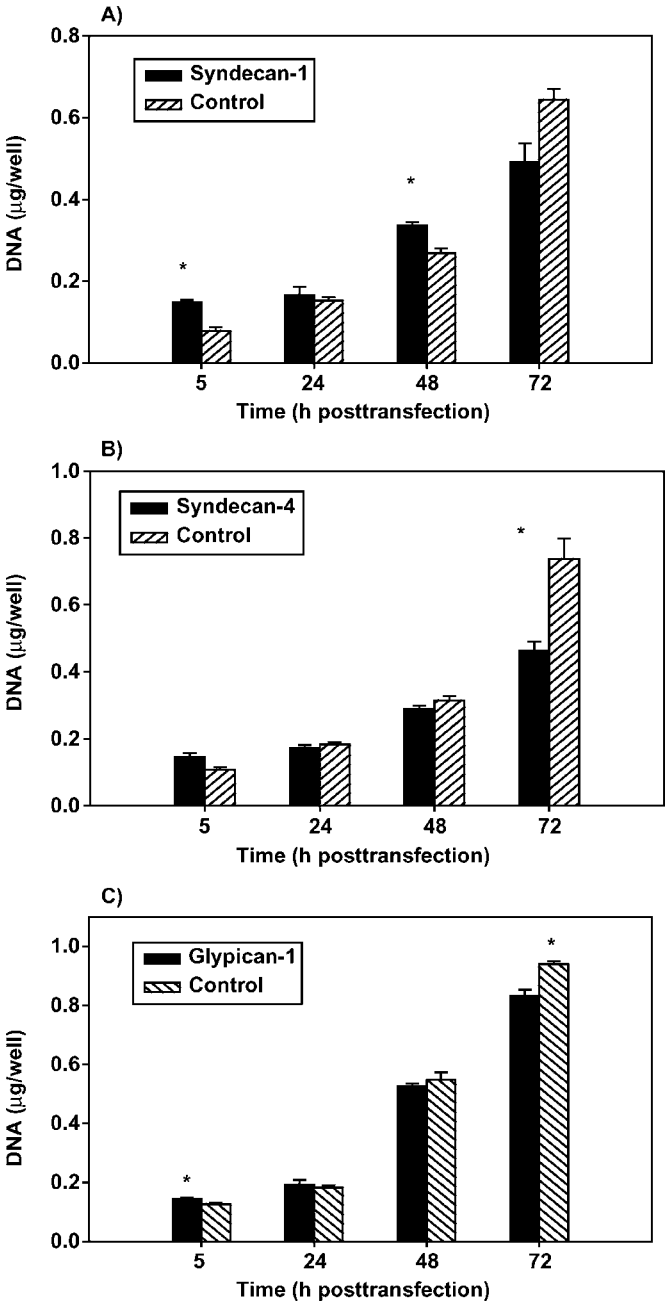

Figure 1. Proliferation of the randombred control 2 line turkey satellite cells transfected with A) syndecan-1, B) syndecan-4, and C) glypican-1. Samples were collected for 72 h during the proliferation process. The control used in these experiments was the expression vector pCMS-EGFP without a gene insert. The error bar represents the SE. *Indicates a difference ($P < 0.05$).

Fibroblast growth factor 2 responsiveness was measured during differentiation. Although there was a significant effect of the increasing concentration of FGF2 and of syndecan-1 transfection, there was no significant interaction between the FGF2 and syndecan-1 (Figure 4, panel A). At 48 h of differentiation, the interaction of FGF2 and syndecan-1 was approaching significance ($P = 0.15$). Similar to syndecan-1, there was no interaction of FGF2 and syndecan-4 treatments (Figure 4, panel B). The glypican-1 treatment resulted in a significant interaction with FGF2 at 48 h of differentiation (Figure 4, panel C).

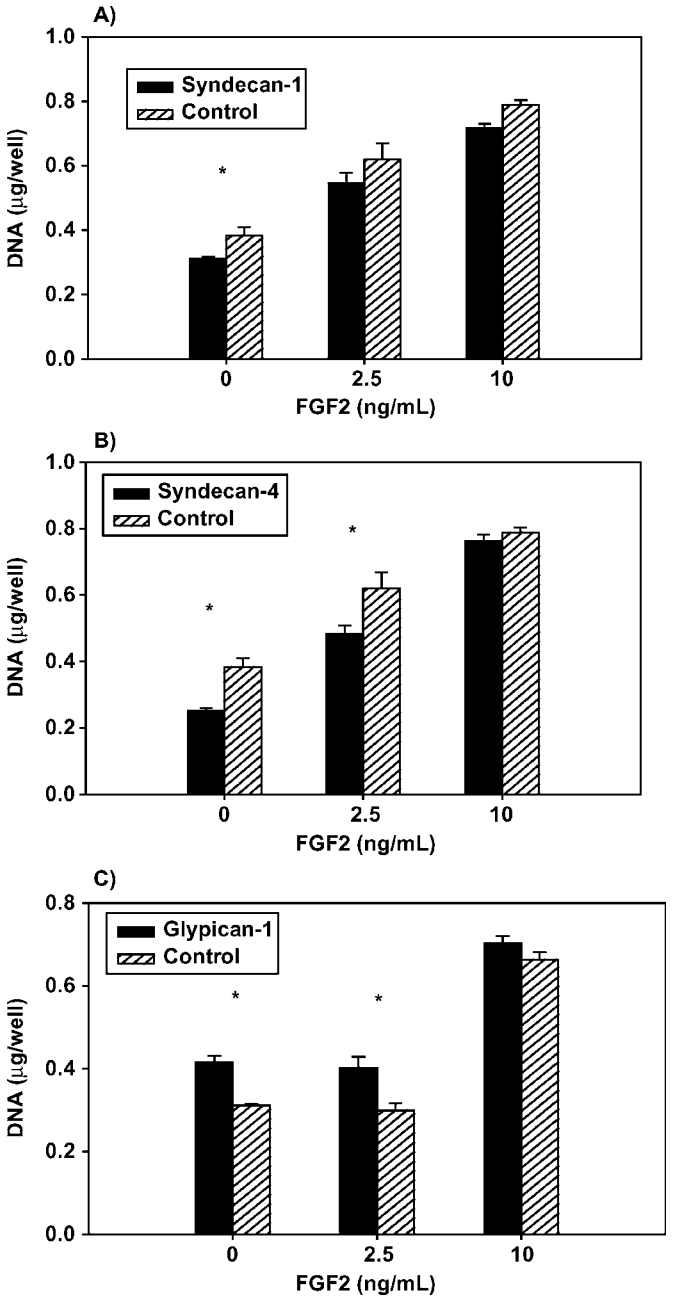

Figure 2. Responsiveness of randombred control 2 line turkey satellite cells during proliferation: The satellite cells were transfected with A) syndecan-1, B) syndecan-4, and C) glypican-1 to increasing concentrations of fibroblast growth factor 2 (FGF2). The control used in these experiments was the expression vector pCMS-EGFP without a gene insert. The error bar represents the SEM. *Indicates a difference ($P < 0.05$).

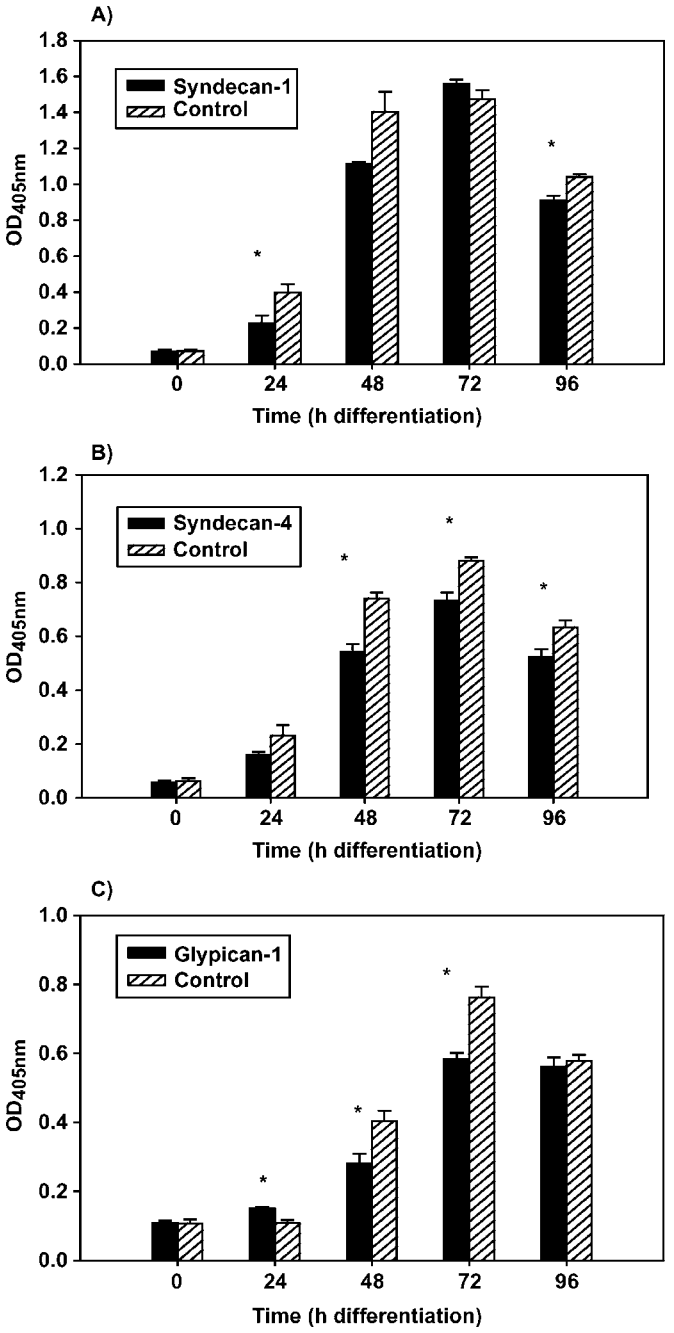

Figure 3. Differentiation of randombred control 2 line turkey satellite cells transfected with A) syndecan-1, B) syndecan-4, and C) glypican-1. Samples were collected for 96 h after the initiation of differentiation. The control used in the differentiation experiments was the expression vector pCMS-EGFP without a gene insert. The error bar represents the SEM. *Indicates a difference ($P < 0.05$). OD = optical density.

DISCUSSION

Fibroblast growth factor 2 is a potent stimulator of muscle cell proliferation and a strong inhibitor of differentiation (Dollenmeier et al., 1981). The cellular response to FGF2 is elicited through FGF2 interacting with a family of tyrosine kinase fibroblast growth factor receptors. Heparan sulfate proteoglycans act as low-affinity receptors for FGF2, permitting the formation of an affinity interaction with its receptors. Although it is well documented that FGF2 interacts with heparan sulfate proteoglycans, how this interaction functions in regulating cellular responsiveness to FGF2 is not completely understood. Heparan sulfate proteoglycans can act as both positive and negative regulators of FGF2 (Ornitz, 2000; Schlessinger et al., 2000). However, it is unclear whether the syndecans and glypicans, the focus of the present study, always require FGF2 to affect muscle cell growth properties or whether they can function in an FGF2-independent manner.

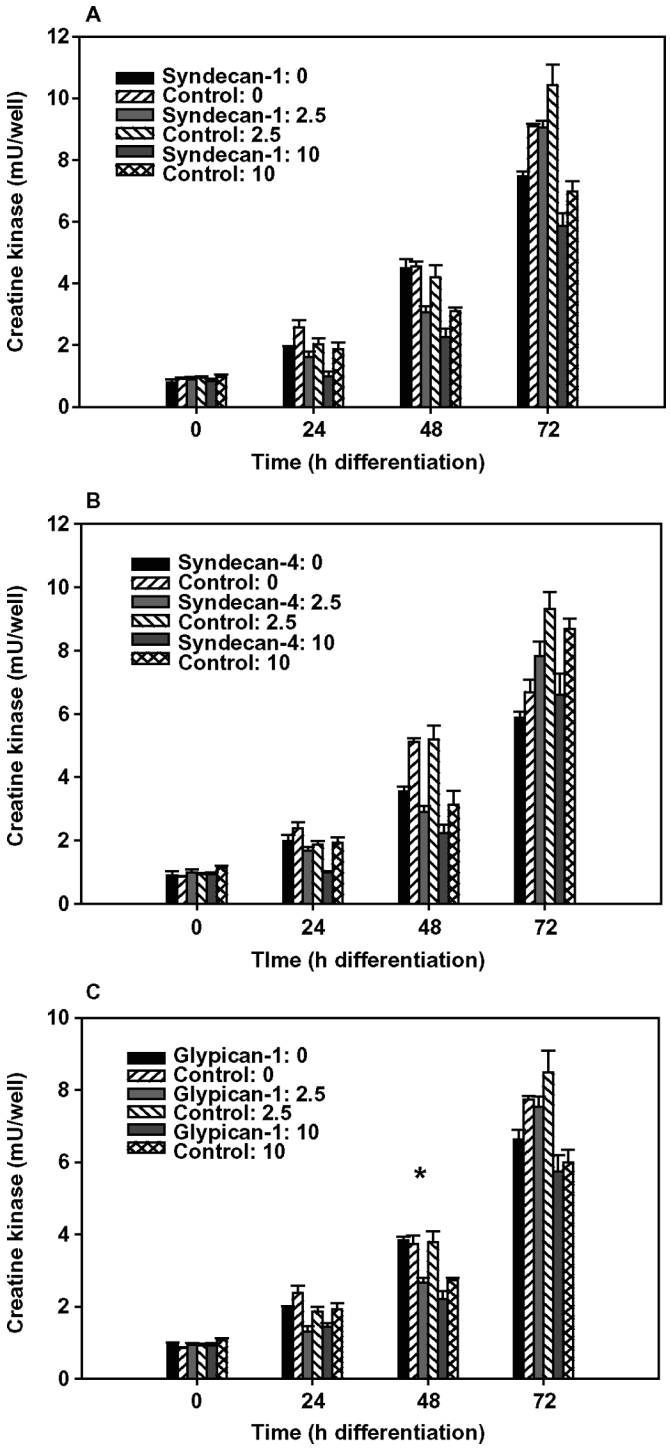

Figure 4. Responsiveness of randombred control 2 line turkey satellite cells during differentiation. The satellite cell cultures were transfected with A) syndecan-1, B) syndecan-4, and C) glypican-1. The control used in these experiments was the expression vector pCMS-EGFP without a gene insert. Cell cultures were administered 0, 2.5, and 10 ng/mL of fibroblast growth factor 2 (FGF2), corresponding to 0, 2.5, or 10 in the figure. Cultures plates were collected every 24 h beginning at 0 h of differentiation for 72 h. The error bar represents the SEM. *Indicates a significant difference ($P < 0.05$).

In skeletal muscle, the syndecans and glypican-1 are differentially expressed (Liu et al., 2004, 2006) during the proliferation and differentiation of myogenic satellite cells in vitro. Satellite cells are the major contributors to postnatal muscle growth (Moss and LeBlond, 1971). Therefore, the regulation of satellite cell proliferation and differentiation is critical in achieving maximal muscle growth. Satellite cells are extremely responsive to the mitogenic activity of growth factors, which either stimulate or inhibit satellite cell proliferation and differentiation. Because syndecan-1, syndecan-4, and glypican-1 expression occurs differentially during in vitro muscle development, it is likely that each of these heparan sulfate proteoglycans plays a unique role in the muscle development process. Syndecan-1, based on its elevated expression during muscle cell proliferation, has been hypothesized to play a role in proliferation, whereas glypican-1, because of its increased expression during differentiation, is thought to be involved in the differentiation process (Brandan and Larraín, 1998). In a study by Cornelison et al. (2004), satellite cells isolated from syndecan-4 homozygous deletion mice had impaired proliferation and differentiation, suggesting that syndecan-4 may be important in both proliferation and differentiation.

In the current study, expression constructs of syndecan-1, syndecan-4, and glypican-1 were overexpressed in turkey myogenic satellite cells to measure their effect on proliferation, differentiation, and FGF2 responsiveness during proliferation and differentiation. None of the changes in gene expression caused by the overexpression of syndecan-1, syndecan-4, or glypican-1 resulted in an interaction with FGF2 during proliferation or differentiation except for glypican-1 at 48 h of differentiation. These data strongly suggest that syndecan-1, syndecan-4, and glypican-1 can affect the proliferation and differentiation of myogenic turkey satellite cells independent of FGF2. However, these data do not mean that a cellular response to FGF2 can occur in a manner that is independent of the presence of heparan sulfate proteoglycans.

In C_2C_{12} stable transfected myogenic cells with constitutive expression of syndecan-1, it was concluded that syndecan-1 decreased differentiation and the cells were more sensitive to FGF during differentiation, and that syndecan-1 functioned through a basic fibroblast growth factor-dependent mechanism during differentiation (Larraín et al., 1998). Similar to this finding, Velleman et al. (2004) reported that F-line turkey satellite cells transiently transfected with syndecan-1 were not able to differentiate into multinucleated myotubes with G418 selection. The difference in conclusions between the current study and the report of Larraín et al. (1998) is largely based on assay and analysis differences. Although the experimental approach was different, response to FGF treatment was measured in both studies. The statistical analysis in the current study measured the significance of treatment effects as well as the interaction between the FGF2 treatment and transfection of syndecan-1. The report of Larraín et al. (1998) measured concentration of FGF2 in the differentiation medium with only an SD analysis. Based on the treatment of either the transfection of syndecan-1 or FGF2 concentration, both independently affected the cellular response during differentiation. However, there was no significant interaction between the syndecan-1 transfec-

tion and the FGF2 treatment. The lack of an interaction between the syndecan-1 transfection and the FGF2 treatment has led to the conclusion that syndecan-1 can function to affect muscle differentiation independent of FGF2.

The pectoralis major muscle satellite cells derived from the RBC2 line of turkeys were used in this study to eliminate any effects from growth selection on proliferation, differentiation, or satellite cell responsiveness. Growth selection for increased 16-wk BW in the F line turkeys has been shown in a previous study to affect the rate of myogenic satellite cell proliferation and differentiation compared with the RBC2 line (Velleman et al., 2000). The F-line male satellite cells have a faster rate of proliferation and differentiation than the RBC2 line male satellite cells. The differences in the growth characteristics between the F and RBC2 line male satellite cells may account for some differences observed in the proliferation and differentiation curves obtained in the current study compared with the results reported for the growth-selected F-line in a previous study (Velleman et al., 2006), but the overall effect of transfecting glypican-1 was similar.

The mechanism by which syndecan-1, syndecan-4, and glypican-1 modulate signaling in the myogenic cells affecting cell growth properties needs to be elucidated to further understand their roles in skeletal muscle proliferation and differentiation. It is likely that each of these proteoglycans functions in a different manner in signaling cellular changes. Although syndecan-1 and syndecan-4 are similar in structure, numerous differences have been reported with regard to these 2 molecules. For example, the cytoplasmic domain of the syndecans contains 3 regions, conserved domains 1 and 2, and a variable (**V**) region. The V region is heterogeneous among syndecan-1 through syndecan-4. The heterogeneity of the V region is likely to play a large role in the functional differences between the syndecans. For example, in syndecan-4 the V region plays a central role in the binding and activation of protein kinase C α. Protein kinase C α may be involved with the formation of focal adhesions associated with cell adhesion and cytoskeleton reorganization (Oh et al., 1997). Unlike the syndecans, glypican-1 is attached to the cell surface by a glycosylphosphatidylinositol anchor. In skeletal muscle differentiation in vitro, glypican-1 expression increases (Brandan et al., 1996; Liu et al., 2006) and is also released or shed from the cell surface during this time (Brandan et al., 1996). The shed form of glypican-1 has been hypothesized to sequester FGF2, preventing FGF2 from binding to its cell surface receptors (Brandan and Larraín, 1998). This sequestering of FGF2 would permit differentiation to proceed, because FGF2 is a potent inhibitor of the differentiation process. Although the mechanisms of these proteoglycans in the regulation of skeletal muscle growth properties are not fully understood, the results from the current study do support the view that each of these proteoglycans can affect skeletal muscle development independent of FGF2.

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