Heparan Sulfate Proteoglycans during Terminal Skeletal Muscle Cell Differentiation: Possible Functions and Regulation of their Expression

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Abstract
Heparan sulfate proteoglycans are key molecules found associated with the cell surface and extracellular matrix (ECM). These macromolecules seem to be essential to achieve terminal skeletal muscle differentiation. In this review, we present data about the types of beparan suifate proteoglycans present in skeletal muscle cells, how their expression changes during differentiation and we propose some mechanisms that might be controlling and/or affecting their expression. Finally, we discuss some possible Functions for these heparan sulfate proteoglycans during skeletal muscle differentiation.
Key words: myogenesis, proieoglyeans, growth factors, cell differentiation, heparan.

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Proteoglycans are produced by most eukaryotic cells and are versatile components of peri cellular and extracellular matrices (ECM). Proteoglycans are composed of proteins that have one or more chains of sulfated carbohydrates covalently linked, called glycosarainoglycans. Depending on the carbohydrate sequence and sulfation modifications, the glycosaminoglyeans can be classified as heparin, heparan sulfate, derma tan sulfate, chondroitjn sulfate and keratan sulfate. An increasingly growing list of work focusing on the understanding of their basic structure, biosynthctic pathways and functions is available (see reviews, [6, 18,46,55]).

Heparan sulfate protcoglycans are present on the cell surface and interact with a great variety of compounds, including ECM constituents [29, 49], adhesion molecules [15] and growth factors [6,46]. Some of the heparan sulfate proteoglycans present on the surface can also interact with cytoskeletal components [6, 12] making these molecules key elements in the interaction of the cell with the extracellular environment. These proteoglycans can be associated with the basal lamina, i.e. perlecan or with the plasma membrane via different mechanisms. Those present in the plasma membrane can be anchored by either a transmem-braue core protein, for example members of the syndecan family [35, 50] or by a glycosylphosphatidylinositol anchor (GPI), for example glypican [13, 16]. This suggests that the presence of heparan sulfate proteoglycans on the cell surface or ECM may influence the response of a particular cell type to changes in the environment.

This idea is particularly attractive during skeletal muscle cell differentiation. The fusion of m on enucleated cells is a central event in terminal differentiation of skeletal muscle. Mononucleated rayoblasts undergo a series of events in-cluding cell fusion to form multinucleated myotubes together with the synthesis and assembly of myofibrillar proteins to form muscle fibers [19, 61]. *In vitro* studies using skeletal muscle derived cell lines, have shown that inhibition of sulfation of proteoglycans [36] and the presence of basic fibroblast growth factor (bFGF) [9] have a strong inhibitory effect on skeletal muscle differentiation. The signaling pathways that are activated by the binding of bFGF to its receptor have been shown to be heparan sulfate proteoglycan dependent [3]. Thus, skeletal muscle cells treated with heparitinase can undergo normal terminal differentiation, even in the presence of bFGF [44]. Several interesting questions arise from these observations; How many heparan sulfate proteoglycans are present on the surface of myoblasts, and is their expression regulated during skeletal muscle differentiation? What is the specific function of heparan sulfate proteoglycans on the cell surface of skeleta 1-muscle cells?

Expression of Heparan Sulfate Proteoglycans during Skeletal Muscle Differentiation

Skeletal muscle cells are a useful model for studying cell differentiation. The fusion of mononucleatcd cells to form multinucleated myotubes is a central event in skeletal muscle development. Controlling the onset and progression of this process is a complex set of interactions between

myoblasts and their environment. Some of the regulatory proteins that control this process have been identified. Thus, when myogenesis begins, myogenic regulatory genes belonging to the MyoD family (myogenin, mrfS, mrf4) are activated [19]. These regulatory factors can bind to specific DNA consensus sites called E-boxes, which function as transcriptional enhancers of muscle-differentiation genes (for review see [34]}. Recent data suggests that another set of factors the MEF2 family are also required to specify muscle fate or to direct muscle differentiation [37], The expression and activity of these master genes are regulated by polypeptide growth factors including bFGF [9], transforming growth factor |3 (TGF-J3) [8], insulin-like growth factor (IGF) [56] and hepatocyte growth factor/scatter factor (HGF/SC) [1] as well as by retinoic acid (RA) [22]. One or more of these growth factors, when present as a result of autocrine or paracrine signaling hold myoblasts in the undifferentiated state whereas RA induces myoblast differentiation [22] (see Figure 1).

With respect to changes in the expression of heparan sulfate proteoglycans in skeletal muscle cells, we have studied Ihe expression of glypican, a proteoglycan present in several cell types such fibroblasts [16], Schwann cells [13] and skeletal muscle cells [11]. Glypican has the ability to bind bFGF [52]. Glypican expression is up-regulated during differentiation of a mouse skeletal muscle cell line (C2Ci2) (Table 1, Fig. 1). Differentiated skeletal muscle cells express glypican on their surface, which can be released by phosphatidylinositol specific phospholipase C (Pi-PLC) [11]. Analysis of hydrophobicity revealed that only purified glypican obtained from detergent extracts was able to be incorporated into liposomes, whereas the PI-PLC released form was not, suggesting that the glycosylinositol moiety present in this proteoglycan was essential for association with the plasma membrane [11]. We also found that glypican co-localizes with laminin in adult rat skeletal muscles sections, indicating a possible role for this proteoglycan as a cell receptor for ECM components [11]. This observation is supported by the fact that glypican can bind to immobilized laminin [13].

Perlecan, a multifunctional heparan sulfate proteoglycan, is an intrinsic constituent of basement membranes and

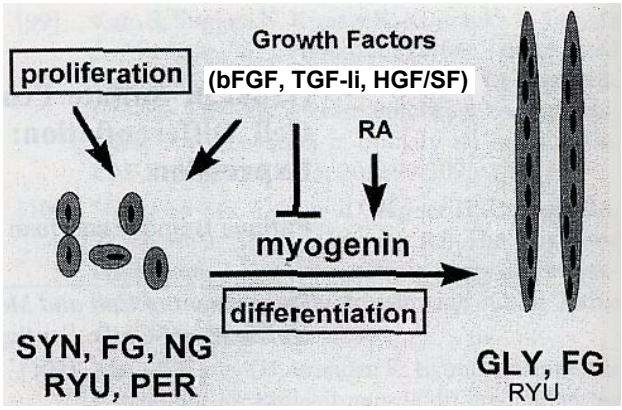

Figure 1. Different heparan sulfate proteoglycans are expressed during skeletal muscle differentiation. Some heparan sulfate proteoglycans are expressed in myoblasts (on the left): SYN, syndecan-1; FG, fibroglycan or syndecan-2; NG, neuroglycan or syndecan-3; RYU, ryoducan or syndecan-4; PER, perlecan. Other are expressed after triggering differentiation (on the right) GLY, glypican. The inhibitory effect of growth factors (BFGF, TGF-$ and HGF/SF) and stimulatory effect of retinoic acid (RA) on skeletal muscle differentiation is shown.

ECMs [25,55]. It consists of at least five separate domains which include a putative heparan sulfate binding region, a domain homologous to the low density lipoprotein-receptor, a domain similar to the neural cell adhesion molecule, and two domains homologous to regions in laminin [40, 41]. This proteoglycan has the ability TO bind bFGF [3]. In contrast to glypican the expression of perlecan during skeletal muscle differentiation is downregulated (Table 1, Fig. 1) [30, 31]. Perlecan is present on the myoblasts surface, associated with an incipient ECM [30, 31]. Interestingly it was been shown by *in situ* hybridization studies, using a probe encoding domain 111 of perlecan, thai perlecan mRNA was absent in human adult skeletal muscle, consistent with our findings [40].

The best characterized cell surface proteoglycans are the syndecans, a family of at least four transmembrane heparan sulfate proteoglycans that are implicated in a

Table I. Changes in the expression of heparan sulfate proteogtycans during skeletal muscle differentiation.

PROTEOGLYCAN	INCREASE	DECREASE	UNCHANGE
SYNDECAN-1 [33] FIBROGLYCAN		XXX	
(unp. observation) NEUROGLYCAN			X
(unp. observation) RYODUCAN (unp.		XXX	
observation) PERLECAN [29]		X	
GLYPICAN [47]		XXX	
XXX - strong change; X = weak change.	XXX		

variety of interactions with the pericellular microenvironment, (for reviews see [6, 18]). Syndecans bind a variety of ECM constituents, such as fibronectin [49], thrombospondin [54], tcnascin f48] and collagen types I, 111 and V [29] as well as bFGF [28]. We have evaluated the expression of syndecan-I [32], neuroglycan (syndecan-3) and ryoducan (syndecan-4) (Brandan et al,, manuscript in preparation) during skeletal muscle differentiation. m this case we have found thai the expression of syndecan-1. -3 and -4 is down regulated (Table i, Fig. 1}. In contrast the expression of fibroglycan (syndecan-2) {Brandan et al., manuscript in preparation) seems to be constitutive throughout differentiation (Table I, Fig. 1).

Possible Mechanisms Involved in the Regulated Expression of Heparan Sulfate Proteoglycans during Differentiation

Analysis of published promoter regions for heparan sulfate protcoglycans [2, 14, 23, 64] have revealed several interesting putative binding sites for transcription factors. These are presented in Table II,

Glypican, the only heparan sulfate proteoglycans which increases its expression during skeletal muscle differentiation, contains several E-boxes, putative target sites for the action of inyogenic regulators such MyoD and myogenin [42], as well as sites for NF-KB and SP1 in the promoter region [2]. Because E-boxes function as transcriptional enhancers of muscle differentiating genes, it is templing to speculate that myogenin or a related skeletal muscle transcriptional factors might be directly involved in the up-regulated expression of glypican observed during differentiation. Alternatively, it is possible to speculate that the expression of glypican increases during differentiation as a consequence of the removal of growth factors. Consistent with this idea is [he fact that the synthesis of glypican by human lung fibroblasts is down regulated by the presence of BFGF and TGF-p [45].

It has been suggested that the mcchanism(s) responsible for the downregulation of syndecan-1 expression during muscle differentiation is the presence of E-boxes in the promoter region of syndecan-1 [5]. We have evaluated the participation of two E-boxes in the promoter region of the syndecan-1 gene, by transiently transfecting myobiasts with CAT reporter vectors containing a portion of the rat syndecan-1 promoter that contain or lack the E-boxes. Our results clearly demonstrate that in this case the presence of the E-boxes is not required for the decrease in expression of syndecan-1 that is observed after differentiation is triggered. These results are also supported by the finding that treatment of differentiating myobiasts with sodium butyrate, an agent known to inhibit myogenin expression [20, 26], did not affect the pattern of syndecan-1 expression during differentiation. These results are not surprising in the sense that the expression of genes regulated by myogenin are usually up-regulated and as discussed, the expression of syndecan-1 was down-regulated. We have also found that promoter activity of syndecan-1 was not significantly affected by BFGF or TGF-p. However exposure to both growth factors resulted in a significant increase in syndecan-1 gene activity. Treatment of the cells with RA, an inducer of skeletal muscle differentiation [22], had an inhibitory effect on the expression of syndecan-I reporter. To date only TNF-ct has been shown to decrease expression of syndecan-1 in cndothelial cells [27]. Furthermore, RA was able to abolish the stimulatory effect observed after treatment of myobiasts with BFGF and TGF-P and fetal calf serum [32]. This Is particularly interesting because both growth factors and RA are known to be present in the vicinity of condensing mesenchymal cells [57] and limb buds during early developmental stages [38].

Table 11. Presence of different regulatory elements in the promoter region of heparan sulfate proteoglycans.

PROTEOGLYCAN	REGULATORY ELEMENT
SYNDECAN-I	E-Box. Aimtenapedia, SP1, NF-KB, RARE [34]
PERLECAN	SPI, TATA box absent [35]
GLYPICAN	E-Box, SP1, NF-KB, TATA box absent [37]
SYNDECAN-4	SPI, TATA box, NF-KB [36]

These results indicate that the main regulatory elements responsible for the transcriptional regulation of syndecan-1 expression during myoblast differentiation arc contained in the proximal 277 bp segment of the syndecan-1 promoter. The inhibitory cffeci of RA on syndecan-1 expression could be explained by the presence of putative RA responsive elements (RARES) in the promoter region. These are located 55,80 and 100 bp upstream of the TATA box [2_i].This sequence is sufficient to cause the inhibitory effect of RA on the murine Oct4 promoter [51J.

As seen in Table II perlecan lacks canonical TATA box and CAAT boxes, but several SPI transcription factor binding sites are present within the promoter region of perlecan [14] which resembles that of mouse syndecan-1. These observations suggest that similar regulatory mechanisms (e.g. growth factors and retinoic acid) may be involved in the regulation of expression of these cell surface maeromolecules during differentiation ofskeletal muscle cells. These observations are attractive because it may imply that common mechanisms are participating in the regulation of these two different proteoglycans, and that the coordinate regulation indicate a similar function. If this is the case, then it is appropriate to inquire about the specific function that these two proteoglycans may play during differentiation ofskeletal muscle (see below).

Therefore, the presence or availability of growth factors to interact with skeletal muscle cells during differentiation, and the growth factors that are removed in order to trigger differenliation [7], could influence the level of expression of different proteoglycans. The expression of perlecan and syndecan-1 would be dependent on BFGF and TGF-p, whereas glypican expression would be inhibited by the

factors. Alternatively, downregulation of perlecan and syndecan-1, could be explain by the presence of SP1 binding sites in both promoters. Recently it has been shown that SP1 is downregulated by MyoD during differentiation [59].

Potential Functions of Heparan Sulfate Proteoglycans during Skeletal Muscle Differentiation

The presence of heparan sulfate proteoglycans are necessary for the modulation of terminal myogenesis [44], probably by acting as low affinity receptors for bFGF [3, 63] or HGF/SF [65], potents inhibitors of myogenesis [1, 9]. The specific action of both growth factors is to inhibit the expression of myogenin, a master gene required for the expression of several skeletal muscle specific genes [42, 53, 61]. The function of syndecan-1 and perlecan in myoblasts and the consequences of their down regulation during differentiation are not known. It is well known that proliferation of myoblasts is strongly stimulated by bFGF and HGF/SF. At the same time these growth factors are strong inhibitor of skeletal muscle differentiation [1,9], On the other hand, it has been shown that heparan sulfate proteoglycans act as co-receptors for bFGF [63], and HGF/SF [65, 47] allowing the binding of the growth factors to its signaling receptor on the plasma membrane and stimulating its biologiea! effects. Therefore, the presence of syndecan-1 and/or perlecan in the membrane may facilitate bFGF and HGF/SF binding to its receptors. When differentiation is triggered, regulatory mechanisms as mentioned above, could inhibit the expression of syndecan-1 and/or perlecan, together with the expression of bFGF receptor [39] and c-met receptor [1], resulting in a decrease or loss of the capability of bFGF and HGF/SF to bind to their receptors activating differentiation of skeletal muscle cells thorough a myogenin dependent pathway.

It has been shown that ncrlecan, but not syndecan-1, -2, -3 or glypican, when added to culture medium is able to bind bFGF and promote biological activity [3]. On the other hand, overexpression of reeombinant membrane associated heparan sulfate proteoglycans (syndecan-1, -2, -4 and glypican) can act as low affinity receptors for bFGF in a hematopoietic cell line that expresses low levels of heparan sulfate proteoglycan [52]. Experiments designed to maintain constitutive syndecan-1 andlor perlecan expression and to abolish their expression in myoblasts are in progress. This experimental approach will specifically evaluate the participation of this proteoglycan as a growth factor co-receptor.

The expression of glypican is enhanced during differentiation. This proteoglycan is present in the surface of skeletal muscle cells and is processed to a soluble form that is incorporated in the ECM [7]. In transverse sections of skeletal muscle, it co-localized with laminin in the basal lamina surrounding individual muscle fibers [11], It has been postulated that this proteoglycan sequesters bFGF after differentiation has been initiated, preventing the inhibitory effect of bFGF on myogenin expression. Immobilized bFGF then may be released from the ECM by specific proteases [62] when proliferation of satellite skeletal muscle cells is necessary; e.g. in the case of skeletal muscle regeneration following injury. We have demonstrated that glypican is released from the plasma membrane into the incubation medium by an endogenous mechanism, likely to be a phospholipase mediated [7]. Interestingly, it has been shown that treatment of endothelial cells, smooth muscle cells and bone marrow cultures with glycosylphosphatidylinositol-specific phospholipase C, released a cell surface associated bFGF bound to heparan sulfale proteoglycan [4, 10]. Therefore, it is possible that one of the functions of glypican in skeletal muscle cells is to sequester bFGF and HGF/SF after differentiation has been triggered, and to liberate it after muscle damage. Figure 2, indicates a model whereas heparan sulfate proteoglycans may be acting as presenters of growth factors to bFGF and HGF/SF to their transducing receptors (FGFR-1 and c-met) in myoblasts but sequesters for them after triggering differentiation.

Another potential role for the presence of heparan sulfate proteoglycans on the surface of myoblasts is to bind ECM adhesive proteins. We have demonstrated that treatment of skeletal muscle cells induced to differentiate with chlorate has a strong inhibitory effect on the organization of the ECM, and an inhibition of differentiation [36]. The inhibitory effect on differentiation can be abolished by adding exogenous ECM even in the presence of chlorate [36]. These results suggest that proteoglycans are required for an appropriate organization of the ECM which is needed to achieve the complete differentiation of skeletal muscle cells. Syndecan-1 has been shown to bind several ECM

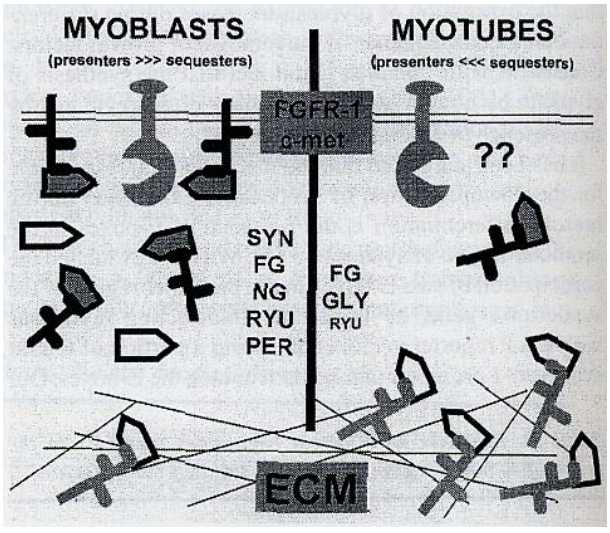

Figure 2. Heparan sulfate proteoglycans are modulators of myogenesis. Heparan sulfale. proteoglycans can be acting as growth factors presenters to their transducing receptors (FGFR-1, c-met) (on the left) or as growth factor sequesters by concentrating them in the extracellular matrix (ECM) (on the right). Heparan sulfate proteoglycans are indicated accordingly to the legend of Figure 1.

adhesive molecules, including fibronectin [49], thrombospondin [54], tenascin [48] and collagen types !, III and V [29]. Cell adhesion to these matrix proteins might not be required following differentiation when individual muscle fibers are in direct contact with basement membrane ECM. Furthermore, it has been shown that syndecan-1 can influence cell invasion [33]. Myoblasts are able to migrate through basal lamina during early stages of differentiation [24]. It is tempting to speculate that the presence of syndecan-1 on the surface of myoblasts may influence their migratory pathway to give rise slow or fast primary myotubes [58]. One attractive ECM candidate directly involved in skeletal muscle differentiation is laminin. This glycoprotein promotes myogenesis in rat skeletal muscte cultures [21, 60], an its expression [43] as well as one of its cellular receptor, a 1 integrin, is up-regulated during differentiation [17]. Interestingly, glypican, is able to interact with laminin [1 3]. Perhaps the interaction between laminin and glypican or another heparan sulfate proteoglycan may be required to achieve terminal myogenesis.

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