SMALL  DERMITAN SULFATE/CHONDROITIN SULFATE PROTEOGLYCANS AND HEPATOCYTE SPHEROIDS

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Abstract: Various proteoglycan (PG) molecules have been identified in the liver ECM. Large part of the hepatic PGs belongs to a group of heparan sulfate PGs, which mainly exist in association with plasma membrane of the hepatic cells. While a family of chondroitin sulfate (CS) PGs takes only a small part of the hepatic PGs and were mainly found in association with fibril collagens of insoluble ECM. Decorin and biglycan, both of which belong to small CS/DS-PGs, were recently isolated from hepatic insoluble ECM. These small CS/DS PGs inhibited the formation of monolayer of hepatocytes when immobilized on the culture dish, resulted in inducing the formation of multicellular spheroids in the primary culture. The multicellular spheroid appeared to be a tridimensional assembly of biologically active hepatocytes which retained various differentiated morphological features and functions. While HS-PG derived from EHS sarcoma did not show the spheroid forming ability, Similar difference in spheroid forming ability between CS and HS was found also with proteoglycan analogs of synthetic GAG-phospholipid. In this article we described about the difference in a biological aspect of CS- and HS-PGs proteoglycans with a special interest on the spheroid formation.

Key words: ECM, proteoglycans, multicellular spheroid, GAG-phospholipid

Introduction

Extracellular matrix (ECM) in the liver parenchyma is predominantly located in the portal triad. Only a small portion of ECM is present in the Disse’s space along the sinusoid and consists of reticulin fibers. Various ECM molecules have been identified in these ECM (1) as shown in Figure 1. Collagens, structural glycoproteins, proteoglycans and elastin are the major categories of ECM molecules. These ECM molecules are present as insoluble ECM complexes, which provide a substrate for cell anchoring in vivo. Interactions of liver cells with such ECM molecules are essential for organogenesis as well as the wound healing after tissue injury. The interactions with ECM molecules are also important in maintaining differentiated functions of hepatic cells. This article briefly reviews the known proteoglycans in hepatic ECM, with special attention to the interactions of hepatocytes with dermanan/chondroitin sulfate proteoglycans (DS/CS PGs).

Proteoglycans in hepatic ECM

Proteoglycans (PGs) are complex molecules that contain a core protein with one or more covalently bound glycosaminoglycan (GAG) chains. GAGs are linear polymers of disaccharides that contain one hexosamine and either a carbohydrate or sulfate ester, or both. There are four classifications according to the structures of the repeating disaccharides. The four main types of GAGs are hyaluronic acid (HA), chondroitin sulfate/dermatan sulfate (DS/CS), keratan sulfate (KS), and heparan sulfate (HS)/heparin; all of which have been found in liver tissue by chemical analyses. PGs are generally referred to by the GAG type, such as heparan sulfate proteoglycan (HS-PG), chon-
droitin sulfate proteoglycan (CS-PG) and dermatan sulfate proteoglycan (DS-PG). An individual PG whose core protein has been sequenced is sometimes referred to by the name of core protein; i.e., decorin and biglycan. These are categorized into a group of small DS/CS-PG. A hybrid type proteoglycan, such as syndecan, contains a core protein with two types of GAG chains, HS and CS. Only hyaluronic acid consists of a sugar chain without a core protein in its structure. Heparan sulfate is the most abundant GAG type present in liver tissue, though the values appearing in previous reports vary; 47–85% of total GAGs are HS, 5–48% DS, and 2–12% CS (2). PGs are not only found in the extracellular space as a constituent of complex ECM, but also in the cytoplasm and cell membrane. The major portion of HS-PGs in the liver is found in the membrane compartment in a membrane-bound form, and only a small portion is in the extracellular and intracellular compartments, whereas DS-PGs or CS-PGs are more abundantly present as a constituent of fibers in the extracellular compartment. PGs found in hepatic ECM are listed in Figure 1.

**Figure 1. Liver extracellular matrix**

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Glycoconjugates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>Structural Glycoproteins</td>
</tr>
<tr>
<td>type I</td>
<td>elastin</td>
</tr>
<tr>
<td>III</td>
<td>laminin</td>
</tr>
<tr>
<td>IV</td>
<td>undulin</td>
</tr>
<tr>
<td>V</td>
<td>entactin</td>
</tr>
<tr>
<td>VI</td>
<td>tenascin</td>
</tr>
</tbody>
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HS: heparan sulfate, CS: chondroitin sulfate, DS: dermatan sulfate, PG: proteoglycan

DS/CS proteoglycans of hepatic reticulin fibers

Two types of fibers are histologically distinguished in the liver tissue. Thick fibers are found in the portal triad and reticular fibers are found in the Disse’s space along the liver sinusoid. Recently, we have isolated the reticular fibers from human liver that was obtained at autopsy from a patient who died of cerebral bleeding. PGs of the reticulin were extracted in 4 M guanidine-HCl and purified on DEAE sephacel chromatography in 8 M urea (3). The PGs appeared to be in double bands at 200 kD and 108 kD by Alcian Blue staining, and 108 kD and 45 kD after the digestion with chondroitinase ABC by both Coomassie Brilliant Blue staining and immune blotting with anti PG40 antiserum. The 45 kD band could not be generated by the digestion of PGs with chondroitin ACI (Manuscript in preparation). These results indicated that the major part of PGs in the isolated reticulin fibers were biglycan (DSPG I) and decorin (DSPG II), and were compatible with the previous reports that decorin was immunohistochemically identified along the hepatic sinusoid (4) and biglycan as well as decorin are synthesized by hepatic lipocytes (5) present in the Disse’s space.

As schematically shown in Figure 2, biglycan and decorin have a homologous core proteins of similar size and have a DS/CS chain near their N terminus (6). However, they are different molecules; the core proteins are immunologically distinct and the number of linked DS/CS chains are usually two for biglycan and one for decorin. Although little is known about the functions, the following has been reported. Small DS/CS proteoglycans regulate fibril formation of collagen by binding to type I collagen (7), and decorin reduce the various effects
Interaction of hepatocytes with DS/CS proteoglycans

Rat hepatocytes are of epithelial origin and have anchorage dependent characteristics. They exhibit monolayer assembly in the standard primary culture in which serum-containing medium as well as adherent culture substrate are employed. However, when either a less adherent culture substrate or serum-free medium were employed, not only the cell attachment but also the subsequent monolayer formation are suppressed. In such environments, hepatocytes assemble to form multicellular spheroids as shown in Figure 3. Culture substrates that form of multicellular spheroids, in combination with serum free medium, are listed in Figure 4. Polystyrene with a positive charged surface (Primaria, Becton Dickinson) (9), poly 2-hydroxyethyl methacrylate (Poly-HEMA) (10), polyurethane foam (PUF) (11), and P-N-P-vinylbenzyl-D-lactonamide (PVLA) (12) were substances found from in the non-biological environment, and liver ECM-derived DS/CS proteoglycans and albumin were substances found in the biological environment. As to DS/CS proteoglycans, those in immobilized form were more effective in spheroid formation than those in soluble form, and GAG chains of DS/CS without core protein only had a poor effect. The spheroid forming ability of DS/CS proteoglycans in a solid state appeared to be lost when treated with chondroitinase ABC which could liberate the DS/CS chain from the core protein. It may indicate that the core protein act as an anchor for DS/CS PGs on the surface of the insoluble material, while a free GAG chain present a less adherent environment to the cells. As to the substances from a non-biological environment, no structural similarity was found, but their physical characteristics are likely less adherent to the cell in common.

Synthetic proteoglycan analogs

Do only the DS/CS proteoglycans among various proteoglycans have the spheroid forming ability? PG-M, a large proteoglycan carrying
chondroitin sulfate GAG chains, are reported to be effective in the spheroid formation of chondrocytes (13). Since it is difficult to prepare native proteoglycans in a sufficient amount, proteoglycan analogs carrying various types of GAG were synthesized and tested for their ability to generate spheroids. Core protein portions of the proteoglycan analogs were replaced with ethanolamine or bovine serum albumin. Proteoglycan analogs carrying a CS chain, as shown in Figure 5, were effective for the spheroid formation of hepatocytes (manuscript in preparation), while those of HS, KS, and HA were less effective. Since HS/KS are as highly sulfated as CS/DS, and there was a difference even between CS and DS, the spheroid forming ability of CS/DS is not simply due to the net of negative charge. Structural specificity of CS and DS must be involved in the efficiency of spheroid formation.

Hepatocyte multicellular spheroids

Recent topics in hepatic PGs, with special interest in the ability of generating multicellular spheroids, have been described. Multicellular spheroids can be formed on various less adherent substrates in the presence of serum free medium. The spheroid is a tridimensional, spherical assembly of hepatocytes, with an average diameter of 110 m. Numerous microvilli and deep holes that form the open end of a bile canalicular-like structure were observed on the free surface. Each hepatocyte in the structure was biologically active, even in the central part, as judged by its ultrastructure. Bile canalicular-like structures and junctional complexes were also observed between hepatocytes (9, 14). These morphological features may suggest that the spheroid is not a simple cell aggregate, but is tissue-organized at a primitive level.

Some liver specific functions were reported to be well preserved by the spheroid. Albumin production (3, 11), glucokinase (15), P450 (10), UDP-glucronyl transferase (10), ureagenesis (11) are likely well preserved in the spheroid. Another important aspect of the spheroid's feature is that growth ability of hepatocytes in the spheroid are strongly suppressed even in the presence of growth factor sufficient to induce the growth in monolayer hepatocytes. Since hepatocytes in the spheroid are in extremely high density, contact inhibition might be strongly exerted. However, hepatoma cells were able to continue growing even when assembled to spheroids. The reciprocal relation-
ship between low growth and highly differentiated functions in high density culture of normal cells seems to be more apparent in spheroid culture.

Although spheroids seem to resemble to those of organized tissue in vivo, only a few features have little has been clarified. Nevertheless, spheroid culture may provide another good experimental model in addition to monolayer culture for the investigations on cell/matrix and cell/cell interactions, tissue organization, and growth regulation in high density.

References


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