AGE-ASSOCIATED CHANGES OF GLYCOSAMINOGLYCAN COMPONENTS IN RABBIT TIBIAL PERIOSTEUM

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Introduction: The formation of specialized cartilaginous tissues (chondrogenesis) during development, growth and maturation of the vertebrate skeleton, takes place in multiple steps and involves activation of both intra- and extracellular signaling pathways, cell-cell and cell-matrix interactions and expression of differentiated metabolic pathways in chondrocytes. The extracellular matrix, which includes proteoglycans (PGs), their constituent glycosaminoglycan variants (GAG) and hyaluronan (HA) surrounds cells before and during chondrogenesis, providing a scaffold for hydodynamic properties to regulate molecular transport of nutrients and signaling factors, and material properties to facilitate and support cell migration, attachment, proliferation and tissue growth. Indeed, recent in vivo transgenic and knockout studies of PG core proteins (perlecan, glypican-1[1-3]) and heparan sulfate (HS) synthesizing enzymes (EXT-1 and EXT-2) have provided data in support of pivotal regulatory roles of PGs in skeletal differentiation and organogenesis. Despite the growing interest in cell biological and genetic mechanisms of embryonic and postnatal cartilage formation, there is only limited information on the molecular mechanism by which PG components influence chondrogenesis. To investigate this further, we have analyzed the GAG composition of rabbit tibial periosteeum. This tissue has been of great interest for its in vivo role during long bone growth and in fracture healing and also for its clinical uses in surgical reconstruction of cartilage and bone. Moreover, periosteum from young animals can be induced to undergo chondrogenesis in vitro in explant culture, making it a well suited experimental system to examine interactions between cells and extracellular matrix during chondrogenesis and cartilage formation [4].

Methods: Periosteum and the attached cambium layer (2x2 mm squares) was harvested after removal of the surrounding muscular tissues, from the right and left proximal tibiae of 5-7 wk and 1-2 yr old male rabbits as described [4]. Tissues were weighed, digested with proteinase K and total GAGs recovered by ethanol precipitation. 60 µg portions of GAG (based on DMMB assay) were sequentially digested with the following GAG lyases: chondroitinases ABC/ACH, 50 mU each in 0.1 M NH4Ac, pH 7.3, keratanase II/endo-b-galactosidase, 5 µU each in 0.1 M NH4Ac, pH 6.0 and finally heparatinase II, 5 mU in 50 mM NH4Ac pH 7.0, 100 µg/ml BSA). Saccharide products released after each glycosidase step were collected into the filtrate of Microcon 3 units, and intact GAGs recovered from the filter which were then subjected to the next digest. The glycosidase products were separated and identified by Fluorophore Assisted Carbohydrate Electrophoresis (FACE®) as described [5, 6], using Mono®gels (AdiCS, AdiHA and di-koligo KS) or Oligo®gels (AdiHS) purchased from Glyko (Fig. 2). All products were quantitated using Scion Image analysis software.

Results: The most abundant sulfated GAGs in periosteum of both immature and mature animals were 4-sulfated chondroitin/dermatan sulfate (CS/DS, Fig. 1 and Table 1) and they are likely substrituents of collagen fibril associated PGs such as decorin. The periosteum from the young animals also contained low level of unsulfated and 6-sulfated CS, which are commonly on cell-surface ligands of the transforming growth factor β (TGF-β) superfamily members. A small amount of 4-sulfated and 6-sulfated keratan sulfate (KS) chains (Table 1 and Fig. 2) were detected in the tissues at both ages, possibly extended on O- or N-linked PGs such as decorin. Small amounts of moderately sulfated and short keratan sulfate (KS) chains (Table 1 and Fig. 2) were detected in the tissues at both ages, possibly extended on O- or N-linked oligosaccharides of matrix glycoproteins such as BSP or fibromodulin. Heparan sulfate (HS) was also present at both ages (Table 1). These chains were composed of ~50% unsulfated disaccharides (Fig. 2), and the majority of the remaining disaccharides contained N-sulfated hexosamine residues (Fig. 2- HS-NS). Moreover, additional sulfation of the uronic acids (Fig. 2, HS-NS,2S) or the N-sulfated hexaminores (Fig. 2, HS-NS,6S) were detected, and both these modifications are essential for HS mediated activation of the signaling cascade for growth factors such as FGF. Another notable finding (Fig. 1 and Table 1) was the significant change in the tissue contents of CS which decreased from ~0.48 µg in 5-7 wk old animals to ~0.21 µg per wet weight in 1-2 yr old animals, and the concomitant increase in HA from ~0.14 µg at 5-7 wks of age to 0.24 µg per wet weight tissue at 1-2 yr of age.

Discussion: The data reported here represent the first detailed analyses of the GAG components in the extracellular matrix of tibial periosteeum. The detection and delineation of fine structural composition of minor GAGs such as KS and HS, should reveal their role in cell differentiation processes such as selectin mediated cell adhesion and growth factor signaling events, and studies are underway to identify the PG core proteins that carry these GAG components. Moreover, the remarkable increase in HA content of the periosteeum from skeletally mature animals may suggest a role of this matrix component in modulating the age-related decrease in chondrogenic or osteogenic potential of the tissue [7].

Table 1: GAG Content of Tibial Periosteeum (µg/mg wet weight of tissue)

<table>
<thead>
<tr>
<th>Age</th>
<th>µg HA (n=5)</th>
<th>µg CS (n=5)</th>
<th>µg KS/PL (n=1)</th>
<th>µg HS (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-7 weeks</td>
<td>0.14±0.01</td>
<td>0.46±0.04</td>
<td>0.08</td>
<td>0.04</td>
</tr>
<tr>
<td>1-2 yr.</td>
<td>0.24±0.03</td>
<td>0.20±0.01</td>
<td>0.05</td>
<td>0.02</td>
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