Heparan sulfate 6-*O*-sulfation -What we have learnt from the knockout mice

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Educational Training
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Positions
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Honors
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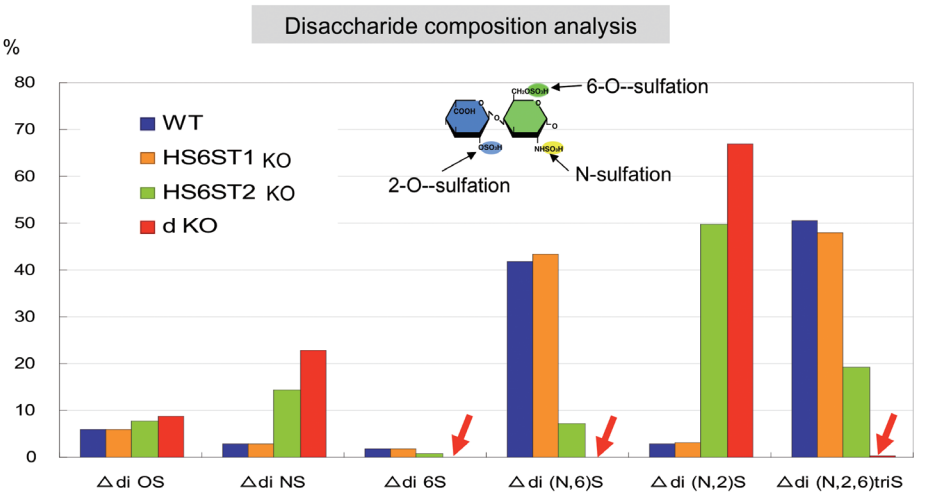
Heparan sulfate (HS) functions in various cellular processes such as proliferation, differentiation and migration, and consequently HS plays important roles in various physiological phenomena such as morphogenesis, inflammation, and tumor cell malignancy. 6-*O*-sulfate residues in HS are involved in those various biological and pathological processes primarily by modulating various signaling pathways such as FGFs-, VEGFs-, and Wnts-signalings. HS 6-*O*-sulfotransferases (HS6STs) transfer sulfate from PAPS to position 6 of the *N*-sulfoglucosamine/*N*-acetylglucosamine residue in heparin/HS. Three isoforms (HS6ST1, 2, 3) were identified in vertebrate animals. Each has distinct substrate preference, albeit overlapping each other, and is expressed in a spatiotemporally regulated manner in most organs. However, how three isoforms function differently is largely unknown. To address those questions we raised HS6ST1- and HS6ST2-KO mice.

Most of HS6ST1-KO mice died between E15.5 and the perinatal stage, and a few mice that survived were grown up to adults, and exhibited development abnormalities. Histochemical and molecular analyses of these mice revealed some reduction in the number of fetal microvessels in the labyrinthine zone of the placenta. Alveolar enlargement was also seen in the KO mice, which is characteristically seen in emphysema. Compositional analyses of the HS chains isolated from various organs of the KO mice revealed significant reduction of GlcNAc($6SO_4$) and Hex-GlcNSO_3($6SO_4$) levels, suggesting that HS6ST1 is the pri-



mary one involved in HS biosynthesis in most tissues. The HS6ST1-KO placenta exhibited a significant reduction in VEGF-A mRNA and protein and HS from their lung had a reduced ability to bind Wnt2. Therefore, those cytokine signaling defects likely contribute to increased embryonic lethality and impaired tissue growth and differentiation.

Heparin, a highly sulfated form of HS, resides in connective tissue mast cells and participates in the storage of mast cell proteases (MCPs). We prepared fetal skin-derived mast cells (FSMCs) from wild-type, HS6ST1-KO, HS6ST2-KO, and HS6ST1/HS6ST2-dKO-mice and determined the structure of heparin, the protease activity, and the mRNA expression of each MCP in cultured FSMCs. The activities of tryptase and carboxypeptidase-A (CPA) were decreased in HS6ST2-KO-FSMCs in which 6-*O*-sulfation of heparin was decreased at 50%, and were almost lost in HS6ST1/HS6ST2-dKO-FSMCs which lacked the 6-*O*-sulfation in heparin nearly completely. In contrast, chymase activity was retained even in the dKO-FSMCs. Each MCP mRNA was not decreased in any of those FSMCs. Western blot analysis showed that MCP proteins were almost absent from dKO-FSMCs indicating degradation/secretion of MCPs. These observations suggest that both the HS6STs and especially HS6ST2 are involved in 6-*O*-sulfation of heparin, and that the proper packaging/storage of MCPs may be regulated differently by the different 6-*O*-sulfation of heparin.

In summary we have learnt from the KO mice that 6-*O*-sulfation conducted differently by HS6ST isoforms may distinctively regulate various cytokine signaling pathways and affect interactions with ligands and receptor proteins.

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