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Grant Title: Regulation of cytokinesis and exosome formation by chondroitin sulfate

Abstract

Chondroitin sulfate, a long linear polysaccharide that is covalently bound to specific core proteins to form chondroitin sulfate proteoglycans, is distributed on the cell surfaces and in the extracellular matrix. To date, chondroitin sulfate proteoglycans have been reported to play important roles in physiological processes, such as cell-cell interactions, cell proliferation, differentiation, and morphogenesis. Many of the physiological roles are attributed to the chondroitin sulfate side chains. However, multiple glycosyltransferases involved in chondroitin sulfate biosynthesis in mammals are highly redundant, and functional analysis of chondroitin sulfate chains is difficult in mammals, in part because of this redundancy. In fact, each of four single-gene knockouts *chondroitin synthase-1 (Chsy1*), *chondroitin polymerizing factor* (*Chpf*), *chondroitin GalNAc transferase-1* (*Csgalnact1*), or *Csgalnact2* result in viable, fertile mice, although each knockout genotype results in reduced chondroitin sulfate production and/or a sulfation imbalance in chondroitin sulfate chains. Thus, to clarify the functions of chondroitin sulfate chains in early mammalian embryogenesis, we focused on glucuronyltransferae-I (GlcAT-I). Because GlcAT-I is a single enzyme that transfers a glucuronic acid residue to the trisaccharide-serine, Galβ1-3Galβ1-4Xylβ1-*O*-Ser, finalizing the formation of the common linkage region, *GlcAT-I* knockout would result in mutant mice completely lacking chondroitin sulfate as well as heparan sulfate chains. Gene knockout of *GlcAT-I* (*B3gat3*) results in embryonic lethality before the 8-cell stage due to cytokinetic failure. A complementary analysis in which the bacterial chondroitin sulfate-degrading enzyme chondroitinase ABC was used to remove chondroitin sulfate selectively from wild-type, 2-cell embryos indicated that the failure of cytokinesis is causally linked to the loss of chondroitin sulfate. These findings show that chondroitin sulfate chains are indispensable for embryonic cell division. To examine which step of cytokinesis might be impaired due to the lack of chondroitin sulfate, expression of chondroitin sulfate was determined by immunostaining. Chondroitin sulfate was enriched at the intercellular bridge and midbody formed between two daughter cells at the last step of cell division. To further examine the molecular mechanism, localization of various molecules involved in abscission, the last step of cytokinesis, was examined in *GlcAT-I* knock out embryos. These results suggest that chondroitin sulfate chains are required for efficient scission during cytokinesis and exosome formation.