1W-10

Essential role of β3GnT7 for efficient KS-GAG production in cultured cells

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Objective: Keratan sulfate (KS) glycosaminoglycan (GAG) is one of the major carbohydrates in the corneal tissue and is suggested to have important role for biological function in the cornea. To study biosynthesis of KS-GAG in cultured mammalian cells, we constructed lentiviral vectors that express carbohydrate sulfotransferases. Using these vectors, we generated stably transfected cultured cells and analyzed production of highly sulfated KS-GAG by additional expression of glycosyltransferases.

Methods: We demonstrated that two carbohydrate sulfotransferases, KS galactose 6-O sulfotransferase (KSG6ST) and corneal GlcNAc 6-O sulfotransferase (CGn6ST, also known as GlcNAc6ST-5/GST-4β) are required for highly sulfated KS-GAG production. In this study, we constructed a lentiviral vector, which expresses both KSG6ST and CGn6ST, and infected the virus to two different human cell lines, HeLa and SV40-transformed human corneal epithelial (hCE) cells. Using 5D4 monoclonal antibody we analyzed highly sulfated KS-GAG in lentivirus-infected cells.

Results: On the infected HeLa cells by KSG6ST/CGn6ST-expression lentivirus, we found significant amount of 5D4-positive highly sulfated KS-GAG production, indicating that the lentivirus can induce production of highly sulfated KS-GAG in the infected cells. On the other hand, we observed much stronger 5D4-positive signal on hCE cells infected by the same virus than that on the infected HeLa cells, suggesting missing factor(s) for efficient production of highly sulfated KS-GAG in HeLa cells. Next we tested additional effect of β1,3 N-acetylgalactosaminyltransferase-7 (β3GnT7) activity in the infected HeLa cells and found enhanced production of 5D4-positive KS-GAG in the lentivirus-infected HeLa cells that are transfected with β3GnT7-expression vector.

Conclusions: From these results we concluded that β3GnT7 expression is essential for efficient production of highly sulfated KS-GAG in cultured cells.

1W-11

Fractones: specialized extracellular matrix structures governing the stem cell niches

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Objective: Throughout life, stem cells and their immediate progeny proliferate and differentiate in restricted zones termed niches. However, the structural and functional characteristics that are specific of the stem cell niches are unknown. We have previously characterized fractones, specialized ECM structures that directly contact stem cells, in mammalian embryonic tissues and in the adult brain neurogenic niche. Fractones resemble basement membranes by their composition, laminins, collagen IV and heparan sulfate proteoglycans (HSPG), but differ by their localization and morphology. Our current objective was to determine whether fractones are the specific stem cell niches structures controlling stem cell proliferation in the adult brain. Our mechanistic hypothesis was that fractone-HSPG are responsible for growth factor capture and activation at the stem cell surface. Our preliminary results indicated that FGF-2 binds fractones via HSPG.

Methods: To investigate the binding capabilities of fractones in vivo, we ICV injected adult mice with fluorescent-tagged FGF-2 (neurogenic stimulator), BMP-4 and BMP-7 (neurogenic inhibitors). To determine whether growth factor binding to fractones is responsible for growth factor activation at the stem cell surface, we injected heparitinase-I (cutting growth factor binding via heparan-sulfate chains) prior to growth factors and analyzed the effect on neural stem cell proliferation in vivo.

Results: Fractones specifically bound FGF-2, BMP-4 and BMP-7 via HSPG in the primary adult neurogenic zone, the subventricular zone (SVZ) of the lateral ventricles. Binding of FGF-2 and BMP-7 to fractones was required to respectively stimulate or inhibit neural stem cell proliferation in the SVZ.

Conclusions: Our results demonstrate that fractones promote stimulatory and inhibitory growth factors at the stem cell surface. We anticipate that fractones are the stem cell niche structures responsible for most ECM/growth factor interactions that ultimately govern stem cell fate and the production of new specialized cells throughout life.