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Principal Investigator: Dr. Marco Maccarana
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Abstract

Chondrotin/dermatan sulfate (CS/DS) is a polysaccharide which is present, sometimes as predominant component, virtually in all types of extracellular matrices and on the surface of all cells. The polysaccharide CS/DS contains oligosaccharide domains with exquisite structural and biological properties. CS is converted into DS by epimerization of glucuronic acid into iduronic acid (IdoA). Three enzymes are specifically involved in dermatan sulfate formation: dermatan sulfate epimerase 1 and 2 (DS-epi1 and 2) and dermatan sulfate 4-O-sulfotransferase 1 (D4ST1). Our group has cloned DS-epi1 and 2, established the deficient mouse models and is in the position to investigate these models to elucidate in vivo functions of dermatan sulfate.

Objectives

The objective was to increase the understanding of the functions of the DS-epimerases by characterizing the DS-epi2$^{-/-}$ mice (5), to identify the role of DS-epi1 in esophagus squamous cell carcinoma (3) and its role in migration of aortic smooth muscle cells (1).

Methods used

Iduronic acid analysis. Brain extracellular matrix investigation by IHC (5). Mass spectrometric structural analysis of CS/DS on human biopsies. Migration and invasion cellular assays. Biochemical analysis of cellular signaling events (3). Cellular migration recorded in living cells. Confocal and total internal reflection fluorescence microscopy (1).

Results

DS-epi2 deficient mice were generated (5). While DS-epi1 is the major epimerase in most tissues, in kidney and brain DS-epi2 is responsible for approximately 50% and 90% of the total epimerase activity, respectively. The last two tissues were examined: despite its high expression, the structure of CS/DS in DS-epi2-/- kidney is unchanged and in DS-epi2-/- brain there is a mere 30% reduction of iduronic acid. The content and distribution of CS/DS in brain was not affected, neither the general organization of the extracellular matrix. DS-epi2-ablated mice with the mixed C57BL/6-129/SvJ genetic background were born at the expected Mendelian frequency, were vital and fertile, and no obvious anatomical and histological abnormalities were observed.

The functions of DS-epi1 were investigated in two contexts: one was cancer, and more precisely human esophageal squamous cell carcinoma (3), and the second was the effect on cellular motility, in particular in aortic smooth muscle cells (1).

DS-epi1 is highly expressed in squamous cell carcinoma, both in the stroma and in the cancer cells. The fine structure of CS/DS in human biopsies was examined and revealed differences, also in iduronic acid content, between cancer and normal tissue. In cells derived from human

esophageal squamous cell carcinoma downregulation of DS-epi1 allowed us to recognize the importance of IdoA in hepatocyte growth factor-mediated cellular motility and invasion.
Aortic smooth muscle cells from wild type and DS-epi1-/- mouse differed in their ability to repopulate wounded areas due to loss of directional persistence of migration. DS-epi1-/- cells, however, had not lost the general property of migration showing even increased speed of movement compared to wild type cells. This could be explained by a decrease of focal adhesion sites and expression of focal adhesion kinase (FAK) and phospho-FAK (pFAK).