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Glycosaminoglycans FACE
the Future



profile

Vincent C. Hascall

Vincent C. Hascall is currently in the Department of Biomedical Engineering of the Lerner Research Institute in the Cleveland Clinic; and Co-Chairs the new Orthopaedic Research Center. He obtained his degrees from the California Institute of Technology (B.S., 1962) and the Rockefeller University (Ph.D., 1969). His thesis research with his fellow student, Stanley Sajdera, discovered the aggregation properties of cartilage proteoglycans, and subsequent research at the University of Michigan and the University of Lund, Sweden, with Dick Heinegard, defined the role of hyaluronan in the mechanism of aggregation. In 1975, he established a Proteoglycan Research Section at the National Institute of Dental Research, NIH, which he directed until taking his present position in Cleveland in 1994. His current research obsession concerns anything that involves hyaluronan (see: www.glycoforum.gr.jp). His honors include: the Karl Meyer Award for Glycoconjugate Research from the Society for Complex Carbohydrates (1992); honorary degrees from the University of Lund, Sweden (1986) and the University of Kuopio, Finland (2000); Chairman of the Proteoglycan Gordon Conference (1986); President of the Society for Complex Carbohydrates (1987); and his current tenure as Associate Editor of the Journal of Biological Chemistry.

Key words: hyaluronan, chondroitin sulfate, keratan sulfate, heparan sulfate, proteoglycans

With support from the Mizutani Foundation, we applied fluorophore-assisted carbohydrate electrophoresis (FACE) methods to quantitate hyaluronan and chondroitin/dermatan sulfate, and to determine the fine structure of the latter (1). The method utilizes selective enzymes, either lyases or hydrolases, to depolymerize the target molecules. The reducing termini of the resulting products are then tagged by reductive amination with a fluorescent reporter and displayed by electrophoresis on gels.

Several interesting applications of this methodology will be discussed. These include:

- 1) the demonstration that embryos from the hyaluronan synthase 2 (has2) knockout mouse do not contain hyaluronan (2);
- 2) the demonstration that subclasses of patients with corneal macular dystrophy can be defined by the fine structures of their keratan sulfate and chondroitin sulfate chains (3);
- 3) the demonstration that bone marrow stromal cells from myeloma patients utilize hyaluronan synthase 1 and synthesize ~6 times as much hyaluronan as bone marrow stromal cells from normal individuals, which utilize hyaluronan synthase 2 (4); and
- 4) that heparan sulfate oligosaccharides and fine structure undergo distinct changes during the development of diabetic nephropathy in rats (5). Each of these projects relies on the high sensitivity and specificity of the FACE methodology.

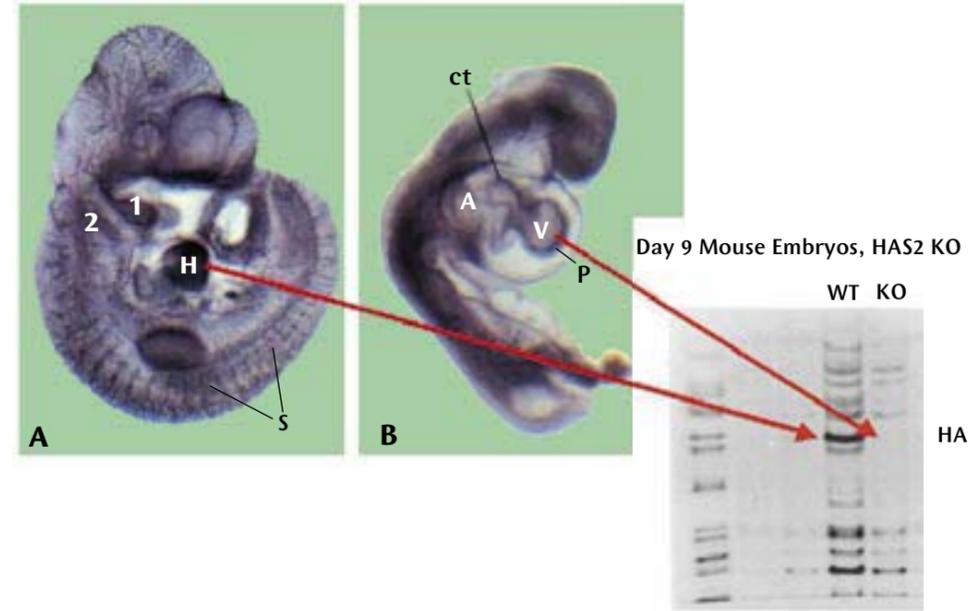


Figure 1. Micrographs of day 9 embryos from a wild type mouse (A) and a hyaluronan synthase 2 (HAS2) knockout mouse (B) are shown along with FACE analyses (reference 2). The already deformed knockout embryo dies at this stage because of the failure to form a functional heart. FACE analyses reveal a prominent disaccharide band derived from hyaluronan in the wild type and its complete absence in the knockout embryo (arrows). The FACE analysis represents the equivalent of 1/60th of a wild type embryo and 1/10th of a knockout embryo on the gel.

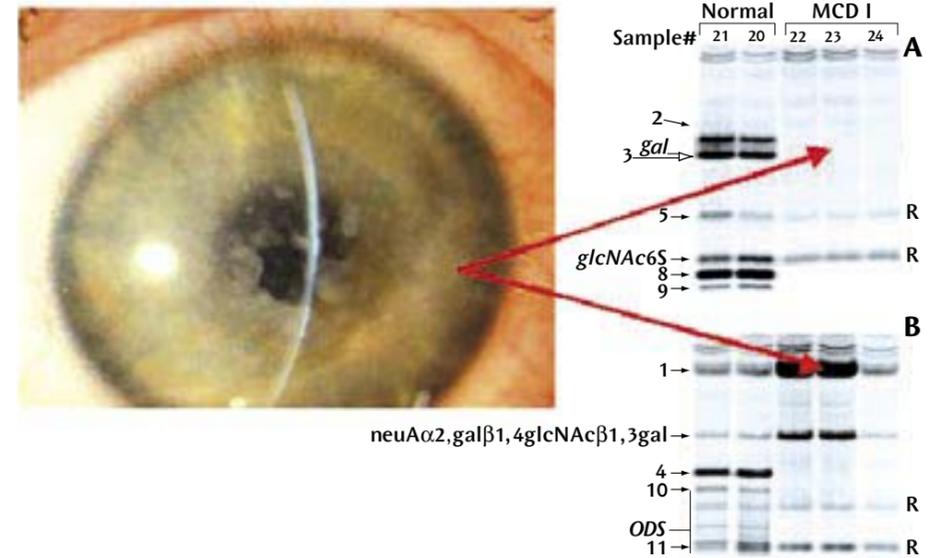


Figure 2. The photograph shows the macules in the cornea of a patient with type 1 macular density. They cloud the cornea and scatter light as is apparent from the image of the slit. At this stage of the pathology, corneal transplants are necessary to restore vision. The defect in the type 1 patients is a complete absence of the ability to sulfate keratan sulfate. This is apparent in the FACE analyses comparing the keratan sulfate on proteoglycans isolated from ear cartilages of normal individuals and of type 1 patients (reference 3). The analyses from the three patients do not have the sulfated disaccharide bands (2, 3 and 8 in panel A) that dominate the analyses from the two normal individuals. The presence of keratan sulfate on proteoglycans in the samples from the patients is readily apparent from the dominant unsulfated disaccharide band (1 in panel B).

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