



Date : June 15(Thu), 2006
11:00-11:25

Session 1 "Biosynthesis, Structure, and Degradation-1"

HARE/Stab-2 binds and mediates the uptake and degradation of multiple glycosaminoglycans.

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Paul H. Weigel, Ph.D., obtained his B.S. degree in Chemistry from Cornell University and his doctoral degree from The Johns Hopkins University School of Medicine (1975). He joined the University of Texas Medical Branch at Galveston as an Assistant Professor in 1978 and became Professor of Biochemistry & Cell Biology in 1987. Since 1994, he has been Professor and Chairman in the Department of Biochemistry & Molecular Biology, College of Medicine, at the University of Oklahoma Health Sciences Center in Oklahoma City. Dr. Weigel's group has made research contributions

in several fields, including the discoveries of multiple coated pit pathways for receptor mediated endocytosis. He has had a long-standing interest in the biochemistry and the biology of hyaluronan. His group was the first to identify and isolate a gene for hyaluronan synthase, from *Streptococcus*. Dr. Weigel's laboratory also developed the use of structurally defined iodinated-hyaluronan of high specific radioactivity to detect and to study specific hyaluronan receptors and binding proteins. This approach enabled his laboratory to purify, and later clone, the liver sinusoidal endothelial cell receptor that removes circulating hyaluronan from the blood by receptor mediated endocytosis (now called HARE or Stablin-2).

The full length 315 kD hHARE is 2551 amino acids

190 kD hHARE cleavage site

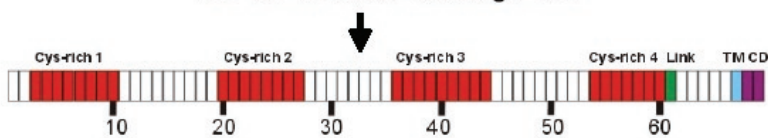


Figure 1: Exon organization of the human HARE (Stab-2) gene.

The gene has 69 exons (rectangles). From the 5' end (left) the four large blocks of exons encode four Cys-rich domains (red), which contain multiple fascilin and EGF-like domains. A LINK domain (green) is encoded entirely within exon 61. Exons 67 and exons 68/69 encode the transmembrane (TM; blue) and cytoplasmic domains (CD; purple), respectively.

The cleavage site that produces the smaller 190 kDa HARE isoform is indicated by the arrow. Many trillions of different possible mRNA transcripts could be created by alternate splicing of the 69 exons encoding the full-length protein. Initial studies to identify possible splice variants of human HARE indicate that multiple types of mRNA variants are present in a tissue-specific manner. Splice variant transcripts have been identified that could create soluble, membrane-bound, or frame-shifted variants of HARE.

Glycosaminoglycans (GAGs) are continuously synthesized and degraded in the extracellular matrices of tissues throughout the body. Although some of this GAG turnover can occur locally in the tissue of origin, a major turnover mechanism is mediated by the transfer of partially degraded GAGs to lymph nodes and liver where they are then degraded completely. The systemic clearance of

hyaluronan (HA) and chondroitin sulfate (CS) from the circulatory and lymphatic systems is mediated by HARE, the HA Receptor for Endocytosis (also designated Stablin-2). Fulllength human HARE is a glycoprotein of 2551 amino acids (~315 kDa in SDS-PAGE on a 5% gel). However, immunopurified HARE from human or rat spleen and liver is present as two major membrane-bound iso-receptors,

e.g. in humans there is the full-length ~315 kDa form and a truncated~190 kDa form (Fig. 1). Both HARE species are highly expressed in the sinusoidal endothelial cells of liver, lymph node, and spleen. There is no detectable mRNA for the 190 kDa isoform, which is derived from the larger protein by specific proteolysis (the 190 kDa N-terminus is Ser-1136 of the 315 kDa HARE and this region does not contain recognition sequences for any known proteases).

Formation and function of the 190 kDa HARE isoform. Stable 293-Flp-In cell lines expressing only the 190 kDa human HARE isoform mediate the specific endocytosis of HA and multiple CS types by a coated pit pathway (*J. Biol. Chem.* 279: 36201, 2004). The smaller hHARE isoform does not bind and mediate the internalization of heparin, heparan sulfate, or keratan sulfate. To study the 315 kDa HARE protein for the first time, we created Flp-In 293 cell lines expressing the full-length human HARE cDNA. In support of the above cleavage mechanism, we observed the same proteolytic processing to create the 190 kDa isoform in 293 cell lines stably expressing only the full-length cDNA. Based on pulse-chase experiments using 35S-Cys/Met, the full-length 315 kDa HARE is synthesized, glycosylated, and then presented on the cell surface before the appearance of the 190 kDa HARE. An N-terminal GFP-190 HARE fusion protein expressed in 293 cell lines was also cleaved to produce free GFP and free 190 kDa HARE. Conversely, full-length hHARE lacking the cytoplasmic and transmembrane domains (a secreted ecto-domain) was not proteolytically cleaved, indicating that membrane anchorage is required for receptor processing to create the 190 kDa isoform. We conclude that the proteolytic cleavage of full-length HARE to create the second smaller isoform is a natural, regulated, and non-artifactual process and that both isoforms are actively engaged in GAG clearance.

Formation and function of the 315 kDa HARE isoform. Stably transfected 293 cells expressing the recombinant human 315 kDa HARE endocytose and degrade HA, chondroitin, and a variety of CS types, but not heparin, heparan sulfate, or keratan sulfate. All 315HARE 293 cell lines expressed both receptor isoforms (315 kDa and 190 kDa), although individual clones differed in total receptor expression levels and in their HA binding, endocytosis and degradation activities. Three monoclonal antibodies, raised against rat HARE, cross-reacted with both human receptor isoforms. The antibodies also partially blocked specific endocytosis of HA mediated by hHARE in stable cell lines. We also developed cell lines that secrete the 315 kDa HARE ecto-domain, which was purified from media via Ni-chelate chromatography. In an ELISA format, the ecto-domain binds to HA with a higher affinity than to chondroitin sulfates A-E. Unlabeled chondroitin sulfates A-E also blocked HA endocytosis by 315 kDa HARE stable cell lines, to different degrees. We conclude that both the 315 kDa and 190 kDa hHARE receptor isoforms can bind to and mediate the endocytic clearance of multiple types of GAGs and therefore, both isoforms likely contribute to the normal homeostasis of HA and CS.

Splice variants of human HARE. Since the human *Stab-2* gene contains 69 coding exons, a huge number of potential splice variants could be generated (Fig. 1). Preliminary results show the presence of tissue-specific splice variants in spleen (six) and lymph node (three). Since the full-length hHARE protein may be modular and contain multiple GAG-binding domains of differing specificity, it is possible that some splice variants could have different GAG-binding patterns compared to the two wild-type isoforms. Also, some variants are predicted to be soluble proteins, and therefore, some HARE variants could have novel functions as matrix modifying or cell signaling factors. (This research is supported by NIH grant GM69961 and the Mizutani Foundation).

Keywords : hyaluronan receptor, chondroitin sulfate receptor, endocytosis, turnover, GAG-binding.