Ligand-binding Characterization of Non-human Liver Scavenger Receptors

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Abstract

Stabilin-2 is an important receptor for many ligands, including hyaluronic acid. Rat Stabilin-2 has never previously been cloned, therefore the exact properties of the receptor have not been evaluated. The focus of the project was to characterize rat Stabilin-2 receptors in comparison to human Stabilin-2. Stable cell lines expressing the receptor were used in determining how this receptor binds ligands and facilitates endocytosis. Our initial characterization found the rat Stabilin-2 receptor to be comparable to the human Stabilin-2 receptor.

Introduction

Stabilin-1 and Stabilin-2/HARE (Hyaluronic Acid Receptor for Endocytosis) are scavenger transmembrane receptors expressed in the capillary system of the liver, spleen, lymph nodes, and bone marrow. HARE is expressed as two isoforms: the full length 315 kDa isoform and the proteolytic product 190 kDa isoform. HARE binds 15 known ligands, including hyaluronic acid, chondroitin sulfate, and heparin. However, it is not known how each ligand binds to the receptor. The rat Stabilin-2 receptor was cloned by the previous UCARE team in the Harris lab, and stable cell lines were created that express sufficient amounts of the receptor to begin characterization experiments. Several different cloned rat cell lines were produced, which will also be compared against each other to test which has the best expression of the receptor. The cell line with the best expression will be used for future experiments with rat Stabilin-2.

Hyaluronic acid (HA) is a polysaccharide prevalent in all body tissues and fluids. It is synthesized at a cell's plasma membrane, transported through the blood, and then catabolized by receptor-mediated endocytosis in endothelial cells of the liver, which remove HA from circulation via Stabilin-2. We used this ligand to compare the activity of the Stabilin-2 receptors.

Methods

Characterization of the HARE receptor was done by measuring its activity in binding and endocytosis assays. Binding measures how many receptors are present on the cell surface and was measured in two ways. The first was incubating mammalian cells expressing rat, mouse, and human HARE receptors with ligand in various conditions at 4°C to endure no internalization would take place. These conditions included . Radioactive ^{125}I-HA was then added to the cells at 4°C and, the amount of ligand bound to the cells after various time points was determined by measuring the amount of radioactivity of the cells. Second, radioactive ligand was incubated with immobilized HARE protein to test the amount of binding. Endocytosis assays measure how many receptors are active, and to measure the rates of endocytosis, the cells were incubated with radiolabeled ligand at 37°C. The amount of ligand still present in the cells was again determined by measuring the radioactivity of the cells.

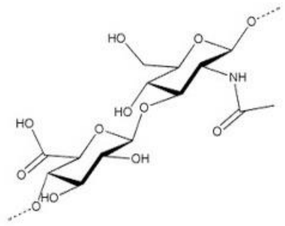


Figure 1. The structure of hyaluronic acid. HA is a polymer of disaccharides made of glucuronic acid and N-acetylglucosamine. HA polymers range in molecular weight from 5,000 to 20,000,000 kDa.

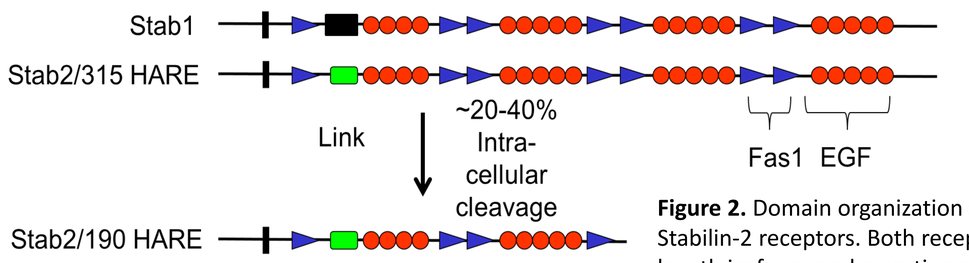
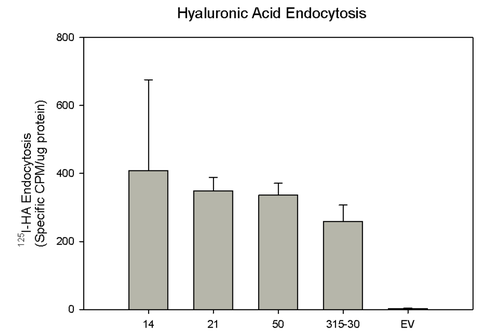


Figure 2. Domain organization of the Stabilin-1 and Stabilin-2 receptors. Both receptors are produced as a full length isoform, and a portion of the full length stabilin-2 produced is converted to a smaller isoform via proteolytic cleavage.



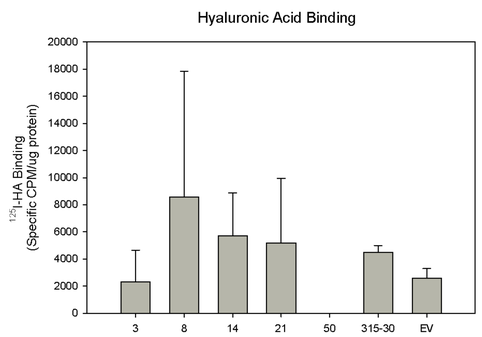


Figure 4. Binding measuring experiment with one time point using rat receptor cell lines made with different clones (3, 8, 14, 21, 50), a human receptor cell line (315-30), and an empty vector (EV) cell line that does not express any Stabilin receptor.

Figure 3. Endocytosis measuring experiment with one time point using rat receptor cell lines made with different clones (14, 21, 50), a human receptor cell line (315-30), and an empty vector (EV) cell line that does not express any Stabilin receptor.

Results

The full length rat HARE receptor has been shown to bind to and internalize hyaluronic acid. Each graph presented shows binding or endocytosis of most of the rat receptor clones to be comparable to or exceeding the activity of the human receptor. Some clones, such as clone 50 in the binding experiment (Fig. 4) produced lower results than expected, probably because of low expression of the receptor protein. There were also differences observed between the rat cell lines in how they grew in culture. It was observed that clone 14 was the most able to attach to the surface of the culture flask, and other clones, especially clone 21, detached more easily. Though it is not indicative of the expression of the Stabilin-2 receptor, this is an important observation as the cell line most easily grown in culture may be the best choice to continue to use in future experiments.

Conclusions

The rat Stabilin-2 receptor was found to produce levels of binding and endocytosis of hyaluronic acid that are comparable to human Stabilin-2. Each cell line expressing rat Stabilin-2 resulting from the cloning process has different levels of expression of the receptor.

References

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- Fraser, JR. "Hyaluronan: Its Nature, Distribution, Functions and Turnover." National Center for Biotechnology Information. U.S. National Library of Medicine, July 1997. Web. 22 July 2015.