Principal Investigator: Barbara Triggs-Raine
Grant Title: Determination of the role of hyaluronidase 2 in the uptake of hyaluronan

Abstract:

Hyaluronan (HA) is a large polysaccharide that is abundant in the extracellular matrix of vertebrate tissues. Every day, it is estimated that 5 of the 15 g of HA in the human body is turned over! HA is also used broadly in medical devices and procedures. Despite its abundance and wide medical application, the pathway of HA degradation is not well defined. There are three human hyaluronidase homologues that are believed to be involved in the degradation of HA in most tissues, HYAL1, HYAL2, and HYAL3. HYAL2 has broad expression and is shown to be expressed primarily on the surface of cells. The activity associated with this enzyme has been variably detected, and it is generally thought to have a weak activity toward high molecular mass HA. These findings support a model for HA degradation where HA within the matrix is initially cleaved by HYAL2 and then internalized by receptor-mediated endocytosis for further degradation through the action of HYAL1 and exoglycosidases. We examined the role of hyaluronidase (HYAL) 2 in the uptake of HA.

Our objectives were to 1) to assess the requirement of HYAL2 for endocytosis of HA in endothelial cells, 2) to determine if HYAL2 interacts with the HA receptor, HARE, and 3) to identify HYAL2-interacting partners.

The methods that were used included optical imaging of high and low molecular mass HA-conjugated to a near-infrared fluor. HYAL2 knockout and control mice were intravenously or subcutaneously injected and the fluorescence was visualized *in vivo* and in specific organs after dissection. HYAL2 and the HARE receptor, as well as other putative interacting proteins were co-localized with immunofluorescence. A change in the lot of commercial antibody toward HYAL2 led to problems pursuing the immunoprecipitation aspects of this study.

Results: Injection of high and low molecular mass HA into HYAL2 knockout and control mice resulted in similar levels of HA uptake. These results were unexpected and led to studies in mouse embryonic fibroblasts. Both high and low molecular mass rhodamine-conjugated HA was readily internalized into HYAL2 knockout and control mouse embryonic fibroblasts. In mouse tissues, HYAL2 appeared to co-localize in some tissues with the HARE, CD44, and LYVE-1 receptors.