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Opposite Roles of Zebrafish Galectins in In Vitro Attachment and Infection by the Infectious Hematopoietic Necrosis Virus (IHNV)

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IHNV is a rhabdovirus with a high mortality rate that has major economic impacts on the salmonid fisheries and aquaculture industry. The virus uses cell surface fibronectin as a receptor for attachment and infection, but the exact mechanism remains unknown. Previously published work in our lab and preliminary data revealed that β-galactoside-binding lectins, known as galectins, interact directly with the IHNV envelope glycoprotein to either promote or inhibit viral attachment and infection of fish epithelial cells. The zebrafish tandem-repeat galectin 9 isoform 1 (Drgal9-L1) displays two carbohydrate recognition domains (CRD) joined by a linker peptide that are similar but not identical in binding specificity. The goal of this study was to explore the mechanism of Drgal9-L1-mediated enhancement of IHNV attachment to epithelial cells (EPC cell line). We showed that Drgal9-L1 crosslinks IHNV to cell surface glycans in a carbohydrate-dependent and -specific manner. We determined that crosslinking was dependent on two functional CRDs through the development of a C-terminal mutant protein that did not enhance IHNV attachment or infection of EPC cells. Drgal9-L1 crosslinks IHNV to fibronectin on the cell surface, enhancing viral attachment, in a carbohydrate-dependent and -specific manner. In addition, Drgal9-L1 binds to alternative ligands, β1-integrin and CD147, to increase IHNV attachment. Double antibody inhibition and siRNA knockdown of fibronectin and β1-integrin in EPC significantly reduced Drgal9-L1 mediated attachment of IHNV. We also investigated the protective role of epidermal mucus glycans for preventing Drgal9-L1 mediated viral attachment to the epithelium. All three galectin classes were detected in the zebrafish epidermal mucus, and Drgal9-L1 as well as Drgal1-L2 were found to bind to mucus glycans in a carbohydrate-specific manner. In a plaque assay, mucus coating of the cell monolayer reduced the number of IHNV plaques on the EPC cells in a concentration and volume-dependent manner and annulled the Drgal9-L1 enhancement of viral attachment and infectivity. Finally, we identified an alternative mechanism of Drgal1-L2 antiviral protective activity, as binding of Drgal1-L2 to surface glycosylated receptors or mucus glycans significantly inhibits IHNV attachment. This research has wide ranging applications for aquaculture disease management, vaccine development, and a general model of galectin-modulated viral attachment.

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