O-GlcNAc modification of EGF-like domains by a novel O-GlcNAc transferase, EGOT

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Profile

Dr. Okajima received both his M.D. and Ph.D. degrees in 1996 and 2000 from Nagoya University School of Medicine. He did his doctoral work in the laboratory of Dr. Koichi Furukawa on the isolation and characterization of mammalian glycosyltransferase genes, specifically those involved in glycolipid biosynthesis. He went on to do post-doctoral work with Dr. Ken Irvine at Rutgers University. In Dr. Irvine's laboratory he worked on the *O*-linked fucose (*O*-fucose) modification. His major contribution was biological characterization of the enzyme responsible for addition of *O*-fucose to proteins, OFUT1. In 2005 he began his independent career as an assistant professor in the Department of Applied Molecular Biosciences, Nagoya University Graduate School of Bioagricultural Sciences. There he has continued to work in unusual forms of protein *O*-glycosylation, namely the *O*-GlcNAc modifications of extracellular protein domains. He was promoted to lecturer in 2008 and then to associate professor in 2009 in Biochemistry II, Nagoya University Graduate School of Medicine.

O-Linked-N-acetylglucosamine(O-GlcNAc) modification is a unique post-translational modification that cooperates with phosphorylation to regulate a variety of cellular processes, including intracellular signaling, cytokinesis, transcription, and protein stability, and serves to modulate nutrient sensing. O-GlcNAcylation is also involved in epigenetic control of cell differentiation and is implicated in the etiology of several human diseases, including type 2 diabetes and neurodegeneration. Although many reports have described biological roles of O-GlcNAcylation, they were limited to the intracellular protein functions, since the modification had been believed to be restricted to the cytoplasm and nucleus.

Our recent attempt to confirm the proposed structure for *O*-glycans on Notch epidermal growth factor (EGF) repeats led to the unexpected discovery of an novel extracellular *O*-GlcNAc modification in *Drosophila* S2 cells. Back in 1980's, it was reported that a small percentage of *O*-GlcNAc containing proteins was detected on the surface of lymphocytes. However, the reexamination using improved methods could not detect *O*-GlcNAc bearing protein on the cell surface of lymphocytes. A few reports have identified O-GlcNAc in the extracellular environment. Nevertheless, this could be attributed to a noncanonical secretion pathway for proteins that bypass the endoplasmic reticulum (ER) and Golgi compartments. Moreover, extracellularly *O*-GlcNAcylated proteins had not been molecularly identified, and all reported *O*-GlcNAcylated proteins were restricted to the cytoplasmic and nuclear compartment. Thus, the identification of O-GlcNAc on Notch receptors provided the first description of *O*-GlcNAcylated proteins in the animal extracellular environment.

Intracellular *O*-GlcNAc modification is catalyzed by the cytoplasmic enzyme *O*-GlcNAc transferase (OGT). This OGT cannot be responsible for Notch *O*-GlcNAcylation since the *O*-GlcNAc modifications on EGF domains should occur during secretion by the action of glycosyltransferases that are typically localized in the ER or Golgi apparatus. Indeed, we could detect *O*-GlcNAc transferase activity in the membrane fraction proteins prepared from S2 cells. To isolate enzymes responsible for Notch O-GlcNAcylation, we performed an RNAi-based search for genes that specifically affect O-GlcNAcylation. We identified one candidate gene, EOGT (EGF-domain O-GlcNAc transferase), whose downregulation decreased the signals corresponding to O-GlcNAcylated EGF20 peptides. EOGT is conserved from *Drosophila* to mammals, but shows no apparent homology to previously isolated GlcNAc-transferases, such as OGT or GlcNAcT enzymes, but rather is phylogenetically related to the plant xylosyltransferase. Although both OGT and EOGT are regulated by hexosamine flux, EOGT localizes to the lumen of the endoplasmic reticulum and transfers GlcNAc to EGF domains in an OGT-independent manner.

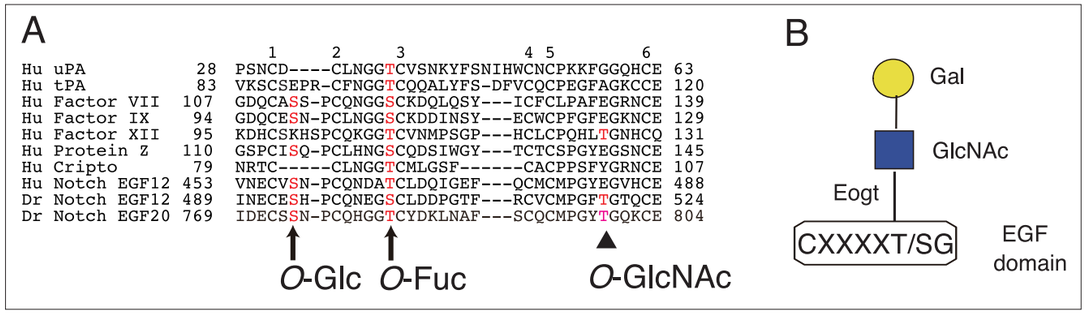

Figure 1. O-GlcNAcylation of EGF domains by EOGT.
A Amino acid sequence of EGF domains of human urinary plasminogen activator (uPA), tissue PA (tPA), blood clotting factors (VII, IX, and XII), Protein Z, Cripto, EGF domain 12 from *Drosophila* and human Notch1, and *Drosophila* Notch EGF20, are aligned. The sites for *O*-fucosylation or *O*-glucosylation are indicated by arrow. The site for *O*-GlcNAcylation found on EGF20 is indicated by arrowhead. Note that not all sites are experimentally confirmed to be modified. The conserved cysteine residues are numbered.
B The proposed structure for O-GlcNAc glycans in mammals.

Genetic analysis in *Drosophila* revealed that loss of *Eogt* gives phenotypes similar to those caused by defects in the apical extracellular matrix. Dumpy, a membrane-anchored extracellular protein, is O-GlcNAcylated, and EOGT is required for Dumpy-dependent epithelial cell-matrix interactions. These results indicated that O-GlcNAcylation of secreted and membrane glycoproteins is a novel mediator of cell-cell or cell-matrix interactions at the cell surface.

To explore whether O-GlcNAcylation of EGF repeats is present in higher vertebrates, we have investigated *Eogt1*, a mouse gene homologue of *Drosophila Eogt*. We showed that as with *Drosophila* EOGT, Eogt1 selectively utilizes UDP-GlcNAc as a donor substrate, specifically modifies the EGF domains, and the enzyme activity is enhanced in the presence of manganese. Importantly, except for the slight difference in temperature dependence, most of the enzymatic activity of Eogt1 is indistinguishable from that of the *Drosophila* enzyme. This observation is supported by the fact that the expression of *Eogt1* in *Drosophila* rescued the cell-adhesion defect caused by *Eogt* downregulation. In HEK293T cells, *Eogt1* expression promoted modification of Notch1 EGF repeats by O-GlcNAc, which was further modified, at least in part, by galactose to generate a novel O-linked-*N*-acetyllactosamine structure.

Although Dumpy is shown to be a functionally important substrate for *Drosophila Eogt*, the mammalian genome does not encode apparent Dumpy homologues or structurally related proteins, and thus the biological function of Eogt1 in mice, if any, would rely on other EGF domain-containing substrates. Future studies, including phenotypic analyses of *Eogt1*-deficient mice, will be necessary to address the roles of atypical O-GlcNAcylation in mammals. Nonetheless, these considerations raise the intriguing possibility that the O-GlcNAcylation reaction in the secretory pathway is evolutionarily conserved, whereas the significance of O-GlcNAcylated proteins in the biological processes had changed during the evolution.

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