Dual roles of gastric gland mucin-specific *O*-glycan in prevention of gastric cancer

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Profile

Jun Nakayama is a pathologist, who is interested in gastrointestinal pathology. He earned his M. D. at Shinshu University School of Medicine, Japan in 1983 and his Ph. D. in medicine at Shinshu University Graduate School of Medicine, Japan in 1987. In the graduate school, he studied the histochemical expression of blood group-related carbohydrate antigens in human colorectal mucosa supervised by Professor Emeritus (Associate Professor at that time) Tsutomu Katsuyama. He started his professional carrier on pathology at the Central Clinical Laboratories, Shinshu University Hospital, Japan from 1987. From 1993 to 1995, he received research training on glycobiology in the laboratory of Professor Minoru Fukuda at La Jolla Cancer Research Foundation (currently Sanford-Burnham Medical Research Institute) in San Diego, U.S.A. as a Visiting Scientist. Here, he worked on the expression cloning of α2,8-sialyltransfereases, and identified two distinct cDNAs encoding polysialyltransferase (ST8SiaIV) and GT3/GD3 synthase (ST8SiaII), respectively. After returning to Shinshu University, he succeeded in the expression cloning of α1,4-*N*-acetylglucosaminyltransferase (α4GnT). Currently, his research group is studying pathogenesis and prevention of gastric cancer focusing on α4GnT. He appointed Professor in Department of Molecular Pathology, Shinshu University Graduate School of Medicine in 2002, and from 2011 he is a Vice Dean of Shinshu Universty School of Medicine. Dr. Nakayama is a recipient of a Bergmeyer-Kawai Award (1999) from Japanese Society of Laboratory Medicine and serves as an Editorial Board Member for Journal of Histochemistry & Cytochemistry and Acta Histochemica et Cytochemica.

Gastric mucins are classified into two subtypes; i.e., surface mucin secreted from surface mucous cells lining the gastric mucosa, and gland mucin secreted from gland mucous cells such as pyloric gland cells and mucous neck cells located in lower layer of the mucosa[1]. The gland mucin characteristically contains *O*-glycans having terminal α1,4-linked GlcNAc residues (αGlcNAc) attached to its scaffold MUC6, and the expression of αGlcNAc is exclusively limited to the gland mucous cells and duodenal Brunner's glands[2]. Previously we isolated cDNA encoding α1,4-*N*-acetylglucosaminyltransferese (α4GnT), which is responsible for the biosynthesis of αGlcNAc by expression cloning[3], and showed that α4GnT is expressed in the gland mucous cells, where αGlcNAc is secreted[4].

Helicobacter pylori (*H. pylori*), a causative microbe for gastric cancer, largely colonizes the surface mucin, while this microbe is barely found in the gland mucin[5], suggesting that αGlcNAc plays a protective role in *H. pylori* infection. To test the hypothesis, we incubated *H. pylori* with recombinant soluble CD43 (sCD43) having αGlcNAc[6]. We found that the growth and motility of *H. pylori* were significantly suppressed. The abnormal morphology such as elongation and folding were also found. By contrast, the control sCD43 without αGlcNAc had no effects on the bacteria. Hirai *et al.* demonstrated that the cell wall of *H. pylori* characteristically contains a unique glycolipid, cholesteryl-α-D-glucopyranoside (CGL)[7]. We then demonstrated that αGlcNAc suppressed cholesterol α-glucosyltransferase (CHLαGcT) that forms CGL *in vitro*[8], and that the active form of CHLαGcT was present in the membrane fraction of the bacteria[9]. *H. pylori* requires exogenous cholesterol for the biosynthesis of CGL. Thus, we cultured *H. pylori* in the absence of cholesterol, and showed that *H. pylori* lacked CGL, exhibited reduced growth and motility, and died off completely upon prolonged incubation up to 21 days, indicating that CGL is indispensable for *H. pylori* survival[6]. These results show that αGlcNAc functions as a natural antibiotic against *H. pylori* by inhibiting the biosynthesis of CGL, thus protecting the gastric mucosa from the infection.

Recently, we generated α4GnT-deficient mice by disrupting the *A4gnt* gene that encodes α4GnT in mice[10]. Immunoistochemistry using HIK1083 antibody specific for αGlcNAc[2] and MALDI-TOF-MS analysis revealed that *A4gnt*$^{-/-}$ mice showed complete lack of αGlcNAc expression in gastric gland mucin, indicating that α4GnT is a sole enzyme responsible for the biosynthesis of the *O*-glycans *in vivo*. Surprisingly, all the mutant mice developed gastric differentiated-type adenocarcinoma through a hyperplasia-dysplasia-carcinoma sequence in the absence of *H. pylori* infection, indicating that αGlcNAc serves as a tumor suppressor for the gastric adenocarcinoma. In fact, significant reduction of αGlcNAc compared to MUC6 was found in human gastric tumors including early differentiated-type adenocarcinoma and its potentially premalignant lesion tubular adenoma. To elucidate pathways linking αGlcNAc to tumor suppression, microarray and quantitative RT-PCR analyses were carried out. We found that genes encoding inflammatory chemokine ligands such as Ccl2, Cxcl1, and Cxcl5, proinflammatory cytokines such as Il-11 and Il-1β, and growth factors such as Hgf and Fgf7 were upregulated in the gastric mucosa of *A4gnt*$^{-/-}$ mice. On the other hand, genes encoding Amh, Egf, and Pthlh were downregulated. In addition, inflammatory cell infiltrations such as mononuclear cells and neutrophils, and angiogenesis were progressively increased as they aged. These results demonstrate that the absence of αGlcNAc triggers gastric carcinogenesis through inflammation-associated pathways *in vivo*.

Taken together, the gastric gland mucin-specific αGlcNAc plays dual roles in preventing gastric cancer by inhibiting *H. pylori* infection and also suppressing tumor-promoting inflammation.

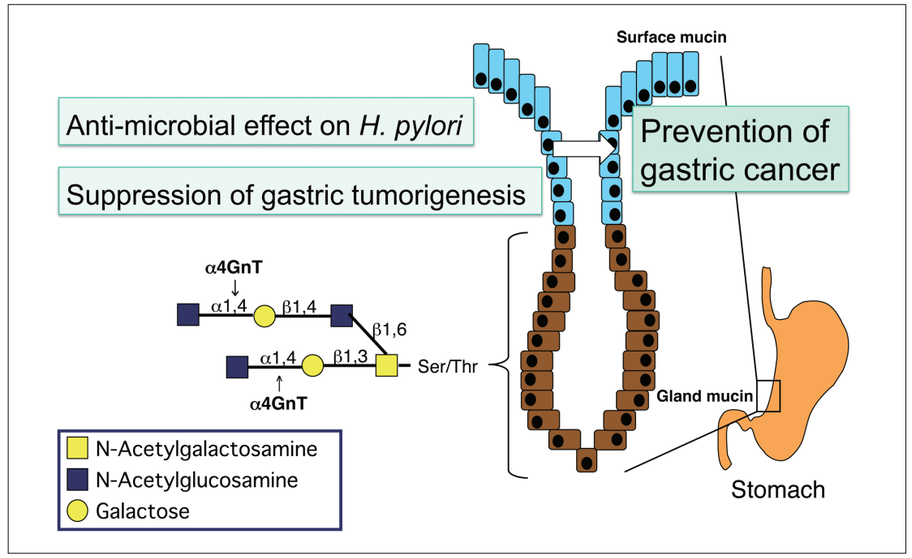

Figure 1. Dual roles of αGlcNAc in prevention of gastric cancer.
O-glycans having αGlcNAc are characteristically contained in the gland mucin secreted from lower layer of the gastric mucosa, and α4GnT is a sole enzyme that forms αGlcNAc *in vivo*. These particular *O*-glycans exert anti-microbial effect on *H. pylori*. In addition, they also suppress gastric tumorigenesis. Thus, αGlcNA in the gland mucin plays dual roles in prevention of gastric cancer.

Keywords adenocarcinoma, H. pylori, knockout mouse, mucin, stomach

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