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MINI REVIEW

Alzheimer's β-secretase cleaves a glycosyltransferase as a physiological substrate

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Alzheimer's beta-secretase (BACE1) is a membrane-bound protease that cleaves the amyloid precursor protein (APP) in the trans-Golgi network, an initial step in the pathogenesis of Alzheimer's disease. Although BACE1 is distributed among various tissues including brain, its physiological substrate other than APP have not been identified. We have recently found that when BACE1 was overexpressed in COS cells together with α2,6-sialyltransferase (ST6Gal I), the secretion of ST6Gal I markedly increased, suggesting that BACE1 cleaves ST6Gal I as a physiological substrate. Thus BACE1 is the first identified protease that is responsible for the cleavage and secretion of glycosyltransferases.
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Introduction

The expression of glyco-chains of complex carbohydrates is mainly controlled by the expression pattern of glycosyltransferases and glycosidases. The glycosyltransferases are localized in specific Golgi cisternae, or endoplasmic reticulum, in an ordered way to act sequentially on nascent glyco-chains. The majority of the glycosyltransferases are type II membrane proteins and some of them have a proteolysis-sensitive "stem region" that tethers the luminal active domain to a membrane anchor [1]. The stem region of glycosyltransferases is cleaved by an endogenous protease, or proteases, and the transferases are secreted out of the cell. Many glycosyltransferases have been found as extracellular, soluble forms in body fluids such as serum, colostrum, and milk [2–6]. It has been well documented that the proteolytic cleavage and secretion of glycosyltransferases into body fluids are affected by various pathological conditions such as malignant transformation and inflammation, but the endogenous protease responsible for the cleavage-secretion has not been identified [7].

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β-secretase plays a critical role for the pathogenesis of Alzheimer's disease

Alzheimer's disease is a progressive neurodegenerative disorder. Production of a pathogenic peptide, amyloid β-peptide (Aβ), from a transmembrane protein, amyloid precursor protein (APP), is a crucial process for the pathogenesis of Alzheimer's disease [8]. APP is first cleaved by β-secretase to generate a soluble NH_2-terminal fragment and a membrane-bound COOH-terminal fragment. The latter fragment is further cleaved by γ-secretase, resulting in production of pathogenic Aβ peptide (Figure 1). Thus β-secretase plays a critical role for initiation of Aβ formation and development of Alzheimer's disease. BACE1, beta amyloid converting enzyme 1, has been recently identified as a membrane-bound aspartic protease [9–11] and is now considered to carry the major β-secretase activity *in vivo* [12,13]. Northern blot analysis reveals that BACE1 mRNA was expressed in various cultured cell lines in addition to most organs including brain, but its physiological substrates other than APP have not been identified.

Cleavage of a α2,6-sialyltransferase (ST6Gal I) by β-secretase (BACE1)

ST6Gal I is a sialyltransferase that transfers sialic acid residue from CMP-sialic acid to acceptor glyco-chains. Like many

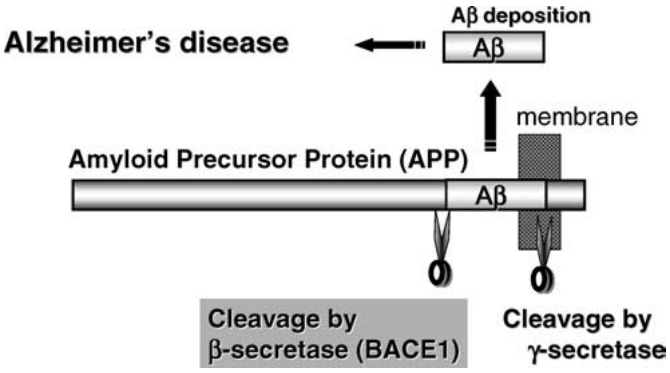

Figure 1. Generation of a pathogenic peptide (amyloid β-peptide). Amyloid precursor protein (APP) is initially cleaved by β-secretase (BACE1), and then further cleaved by γ-secretase to generate amyloid beta-peptide (Aβ). Deposition of Aβ results in amyloid plaque formation, which is the initial pathological change of Alzheimer's disease.

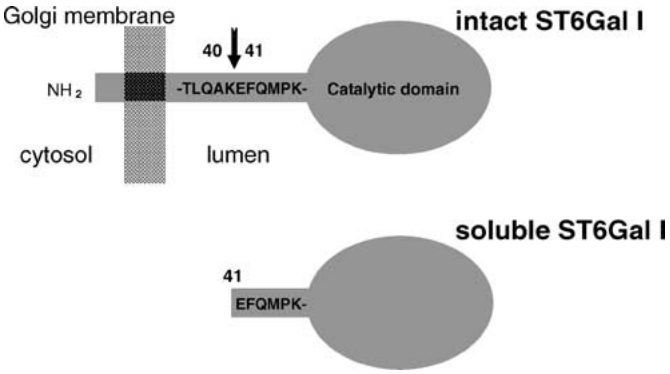

Figure 3. Schematic structure of rat ST6Gal I. The amino terminal sequence of solube ST6Gal I that is secreted from COS cells is identified to be Glu41-Phe42-Gln43-Met44-Pro45—(EFQMP—). ST6Gal I is cleaved between Lys40 and Glu41 at the stem region.

other glycosyltransferases, soluble forms of ST6Gal I have been isolated from body fluids (Figure 2). The soluble enzyme in rat serum increases with acute inflammation [1,2]. We have studied ST6Gal I as a model protein for understanding molecular mechanisms of the cleavage-secretion of Golgi glycosyltransferases. When rat ST6Gal I is overexpressed in COS cells, the enzyme is cleaved between Lys40 and Glu41, *i.e.,* fourteen amino acids distant from the transmembrane region (Figure 3). The cleavage significantly decreases when Leu37 or Lys40 is replaced with alanine (Figure 4), suggesting that a sequence specific protease is involved in the cleavage. We also prepared a mutant ST6Gal I that lacked whole stem and a part of adjacent luminal region [14]. The mutant ST6Gal I was hardly cleaved and, instead, its cell surface expression was significantly enhanced. These results suggest that the sequence specific protease could control the subcellular localization and cellular level

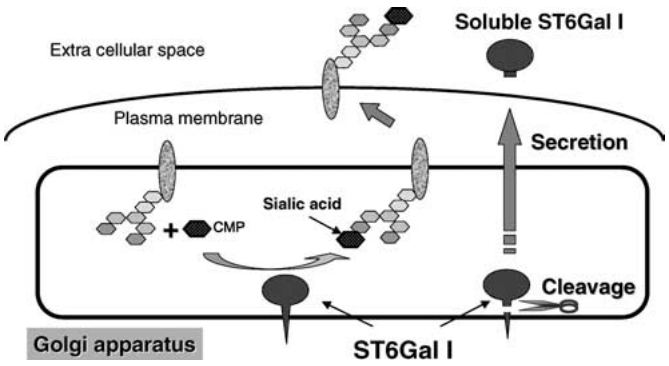

Figure 2. Cleavage and secretion of α2,6-sialyltransferase (ST6Gal I). ST6Gal I is a sialyltransferase that transfers the sialic acid residue from CMP-sialic acid to acceptor glycans of glycoproteins. ST6Gal I is cleaved by an endogenous protease(s) and then secreted out of the cell.

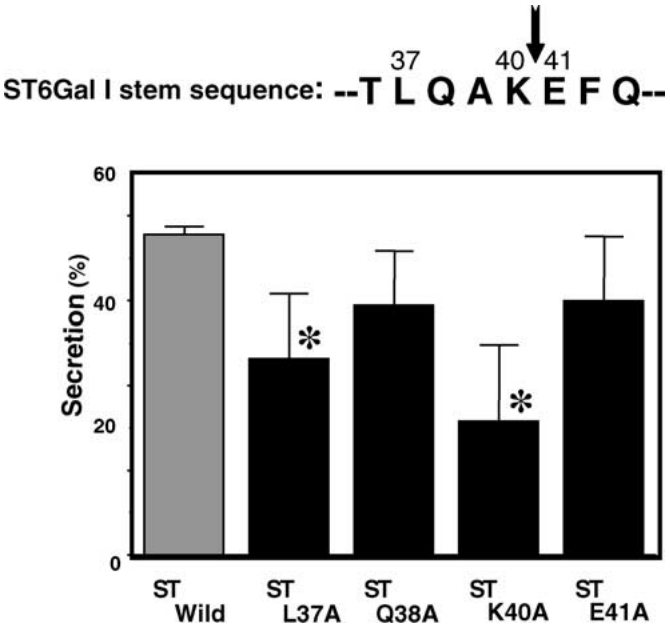

Figure 4. Cleavage and secretion of ST6Gal I is sequence specific. Site-directed mutagenesis around the cleavage site was performed. Note that the percentage of secretion of mutant ST6Gal I (L37A and K40A) is significantly lower than that of wild type. Significant difference is indicated with an asterisk ($P < 0.05$).

of ST6Gal I. BACE1 is a sequence-specific protease expressed in the Golgi apparatus of various tissues. We, therefore, examine to see whether BACE1 is responsible for the cleavage of ST6Gal I.

First of all, we analyzed the intracellular co-localization of BACE1 and ST6Gal I. BACE1 is co-localization with ST6Gal I in the Golgi apparatus, suggesting that BACE1 can act on the ST6Gal I within the same intracellular compartments [15]. We then analyzed the effect of co-transfection of BACE1 on

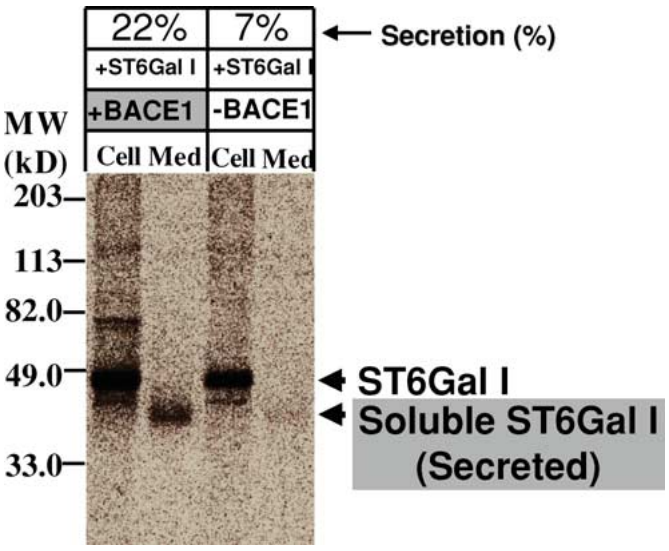

Figure 5. Co-transfection of BACE1 and ST6Gal I cDNA into COS cells. Transfection of BACE1 together with ST6Gal I cDNA increases the secretion of ST6Gal I protein (22%) into culture medium (Med) as compared with control experiment (7%).

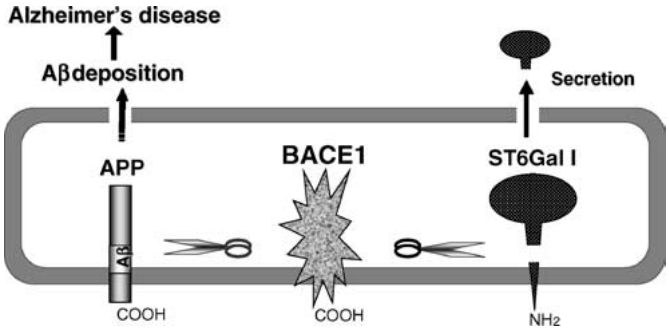

Figure 6. BACE1 cleaves ST6Gal I as well as APP.

secretion of ST6Gal I. When BACE1 is transiently expressed in COS cells, secretion of ST6Gal I is significantly increased (22%) as compared with control experiment (7%) (Figure 5). This result suggests that the BACE1 overexpression induces cleavage and secretion of ST6Gal I. We also demonstrated that the purified BACE1-Fc chimera cleaved protein A-ST6Gal I *in vitro*, to produce the soluble form, indicating that BACE1 directly cleaves ST6Gal I. To investigate the regulatory role of BACE1 for cellular sialylation, we prepared stable transformants which overexpressed BACE1. The transformants exhibited lower level of α2,6-sialylation than the control cells, suggesting that BACE1 cleaves ST6Gal I and controls cellular levels of α2,6-sialyltaion [15]. Thus we concluded that BACE1 is responsible for the cleavage and secretion of ST6Gal I *in vivo* as well as *in vitro* (Figure 6).

Glyco-chain dependent signaling in BACE1 deficient mice

It has been documented that the interaction of a sialylα2,6galactose residue with a B cell-specific lectin, CD22/Siglec-2, is important for B cell differentiation and antibody production [16–18]; mice deficient in ST6Gal I show a reduced level of serum IgM [19]. Our results herein raise the possibility that abnormality of BACE1 may cause dysmetabolism of the α2,6-sialyl residue and abnormal CD22/Siglec-2 signaling. Indeed, mice deficient in BACE1 appears to exhibit an abnormal level of serum globulin [20], although they grow normally up to the age of 3–4 months [12,13]. BACE1-deficient mice may have subtle abnormality in CD22/Siglec-2 signaling that an abnormality is not manifested under the "specific pathogen free" conditions. The amount of sialylα2,6galactose residue and CD22/Siglec-2 dependent signaling needs to be carefully examined in the BACE1-deficient mouse, with a special relation to the immune response and antibody production.

Because BACE1 is a crucial protease for Alzheimer's pathogenesis, inhibitors of BACE1 are expected to be promising therapeutics for Alzheimer's disease [21]. The inhibitors, however, may cause adverse side effects if BACE1 cleaves physiologically important substrates. Further search for BACE1 substrates is required for prediction and prevention of the possible side effects of the inhibitors.

Conclusion remarks

In 1999, BACE1 was identified as β-secretase and turned out to be a type I membrane protein. Researchers predicted that the possible substrate of BACE1, if any, would also be a type I membrane protein like APP. Contrary to the prediction, we found ST6Gal I, a type II membrane protein, to be a physiological substrate of BACE1. The stem region of ST6Gal I may be flexible enough to be accessed by catalytic site of BACE1. The majority of glycosyltransferases are type II membrane proteins, and some of them are actively cleaved at the stem region. These glycosyltransferases could be BACE1 substrates like ST6Gal I. We are currently undertaking the screening of other glycosyltransferases [1,22] as possible substrates of BACE1.

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