

Depletion of GGA3 Stabilizes BACE and Enhances β -Secretase Activity

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SUMMARY

β -site APP-cleaving enzyme (BACE) is required for production of the Alzheimer's disease (AD)-associated A β protein. BACE levels are elevated in AD brain, and increasing evidence reveals BACE as a stress-related protease that is up-regulated following cerebral ischemia. However, the molecular mechanism responsible is unknown. We show that increases in BACE and β -secretase activity are due to posttranslational stabilization following caspase activation. We also found that during cerebral ischemia, levels of GGA3, an adaptor protein involved in BACE trafficking, are reduced, while BACE levels are increased. RNAi silencing of GGA3 also elevated levels of BACE and A β . Finally, in AD brain samples, GGA3 protein levels were significantly decreased and inversely correlated with increased levels of BACE. In summary, we have elucidated a GGA3-dependent mechanism regulating BACE levels and β -secretase activity. This mechanism may explain increased cerebral levels of BACE and A β following cerebral ischemia and existing in AD.

INTRODUCTION

A key neuropathological event in Alzheimer's disease (AD) is the cerebral accumulation of an ~4 kDa peptide termed amyloid β -protein (A β), the principle component of senile plaques. The A β peptide is derived by serial proteolysis of β -amyloid precursor protein (APP) by β -secretase at the N terminus followed by γ -secretase at the C terminus (De Strooper and Annaert, 2000). β -secretase has been identified as a novel membrane-tethered member of the aspartyl proteases, termed BACE for β -site APP-cleaving enzyme (Sinha et al., 1999; Vassar et al., 1999). BACE is

an N-glycosylated type 1 transmembrane protein that undergoes constitutive N-terminal processing in the Golgi apparatus. The ectodomain contains four glycosylation sites and two signature sequences typically associated with aspartyl proteases (D T/S G T/S). BACE is targeted through the secretory pathway to the plasma membrane where it can be internalized to endosomes (Citron, 2004). The BACE C-terminal fragment (CTF) contains a specific di-leucine (DXXLL) sorting signal that is present in several transmembrane proteins (e.g., cation-dependent [CD] and cation-independent [CI] mannose 6-phosphate receptor [MRP]) and regulates endocytosis, and, ultimately, lysosomal degradation (Bonifacino and Traub, 2003). Mutagenesis of LL to AA results in retention of BACE at the plasma membrane (Huse et al., 2000; Pastorino et al., 2002). Furthermore, the di-leucine motif may play a role in BACE degradation since BACELL/AA mutations increase protein levels of BACE (Pastorino et al., 2002). More recently, we reported that BACE is normally degraded in lysosomes, and that mutagenesis of the di-leucine motif in the BACE CTF prevents accumulation of BACE in the lysosomes following inhibition of lysosomal hydrolases (Koh et al., 2005). The BACE acidic di-leucine motif has been shown to bind Golgi-localized γ -ear-containing ARF binding proteins (GGA)1, 2, and 3, and phosphorylation of BACE-S498 appears to increase their binding (He et al., 2002, 2003; Shiba et al., 2004; von Arnim et al., 2004; Wahle et al., 2005).

GGA1, 2, and 3 are monomeric adaptors that are recruited to the trans-Golgi network by the Arf1-GTPase. They consist of four distinct segments: a VHS (VPS27, Hrs, and STAM) domain that binds the acidic di-leucine sorting signal DXXLL; a GAT (GGA and Tom1) domain, which binds Arf:GTP; a hinge region which recruits clathrin; and a GAE (gamma-adaptin ear homology) domain, which exhibits sequence similarity to the ear region of γ -adaptin and recruits a number of accessory proteins. GGAs are necessary for the sorting of acid hydrolases to the lysosomes. Newly synthesized acid hydrolases modified with mannose 6-phosphate groups bind to MPRs. MPRs bind to the VHS domain of GGAs via the DXXLL