Insights & Perspectives

Golgi defects enhance APP amyloidogenic processing in Alzheimer's disease

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Increased amyloid beta (Aβ) production by sequential cleavage of the amyloid precursor protein (APP) by the β- and γ-secretases contributes to the etiological basis of Alzheimer's disease (AD). This process requires APP and the secretases to be in the same subcellular compartments, such as the endosomes. Since all membrane organelles in the endomembrane system are kinetically and functionally linked, any defects in the trafficking and sorting machinery would be expected to change the functional properties of the whole system. The Golgi is a primary organelle for protein trafficking, sorting and modifications, and Golgi defects have been reported in AD. Here we hypothesize that Golgi fragmentation in AD accelerates APP trafficking and Aβ production. Furthermore, Golgi defects may perturb the proper trafficking and processing of many essential neuronal proteins, resulting in compromised neuronal function. Therefore, molecular tools that can restore Golgi structure and function could prove useful as potential drugs for AD treatment.

Introduction

The Golgi apparatus is a highly dynamic cellular organelle with a unique stacked structure that functions in processing and sorting of membrane and luminal proteins during the transport from the endoplasmic reticulum (ER) to various destinations inside and outside of the cell. The Golgi is also actively involved in post-translational modifications of proteins and lipids, in particular glycosylation. Because of its central role in the secretory pathway, changes in the structure and function of the Golgi are expected to affect cellular protein homeostasis. Recently, a large number of human diseases have been linked to defects in Golgi structure and function [1]. Golgi structural defects have been reported in Smith-McCort dysplasia [2] and MACS (macrocephaly, alopecia, cutis laxa and scoliosis) syndrome [3, 4]. Golgi fragmentation has also been observed in neurodegenerative diseases, including Alzheimer's (AD) [5, 6], Parkinson's (PD) [7], and Huntington's (HD) [8] diseases and amyotrophic lateral sclerosis (ALS) [9–11]. Golgi trafficking defects have been reported in Pelizaeus-Merzbacher disease [12], proximal spinal muscular atrophy [13] and dyschromatosis universalis hereditaria [14]. Golgi glycosylation defects have been linked to Angelman syndrome [15] and Cutis Laxa type II and wrinkly skin syndrome [16, 17]. In some diseases, Golgi defects are caused by gene mutations. For example, the expression of a Golgi resident protein is lost in Gerodermia osteodysplastica disease [18, 19], "North Sea" progressive myoclonus epilepsy [20], Duchenne muscular dystrophy [21], Dyggve-Melchior-Clausen disease [22] and Smith-McCort Dysplasia [2]. However, in most other diseases the mechanisms of Golgi dysfunction remain unexplored.

We have recently found that Golgi fragmentation in AD is caused by phosphorylation of GRASP65, a Golgi stacking protein essential for Golgi structure formation [23]. GRASP65 is a peripheral protein on the cytoplasmic face of Golgi membranes that oligomerizes to stick Golgi cisternal membranes into multilayer stacks and to link Golgi stacks into a ribbon. Phosphorylation of GRASP65 changes the conformation of the protein and disrupts its oligomerization and stack formation [24–29]. In AD, GRASP65 is phosphorylated by Cdk5 that is activated by Aβ accumulation, resulting in GRASP65 dysfunction and Golgi fragmentation. Subsequently,

Golgi fragmentation accelerates APP trafficking and increases Aβ production [23]. Based on these results we hypothesize that Golgi fragmentation in AD enhances APP amyloidogenic processing, which contributes to AD development. Our study reveals that Golgi fragmentation and its biological consequences may underlie the hyper-accumulation of Aβ, the phenomenon responsible for the formation of toxic plaques. The mechanism involves a deleterious feedback loop: Aβ accumulation leads to phosphorylation of Golgi proteins (e.g. GRASP65) by activating Cdk5, resulting in Golgi fragmentation, which subsequently enhances Aβ production and hyper-accumulation by accelerating APP trafficking and amyloidogenic processing by the β-secretase BACE1 and the γ-secretase Presenilin 1 (PS1). A similar mechanism may apply to other diseases with Golgi defects. In this review article we summarize the molecular mechanisms underlying Golgi fragmentation in AD and discuss its impacts on the trafficking and processing of APP and APP processing enzymes as well as on Aβ production. Furthermore, we speculate that Golgi defects may perturb the proper trafficking and processing of many essential neuronal proteins, resulting in compromised neuronal function. The structural defects of the Golgi caused by Aβ accumulation and the resultant defects in protein trafficking and processing may underlie a so far unrecognized toxicity of the Aβ peptides.

Mechanism of Golgi fragmentation in AD

Using tissue culture cells and transgenic mice that express both the "Swedish" mutant of human APP (KM 593/594 NL, APPswe) and the exon 9 deletion mutant of PS1 (PS1△E9) [30], we first confirmed that the Golgi is fragmented in both AD cell culture and in mouse models [23], as previously reported in human AD patients [5]. To determine the cause of Golgi fragmentation in AD, we then analyzed Golgi structural proteins. As described above, the highly organized stacked structure of the Golgi is maintained by Golgi structural proteins, such as GRASP65 and its homo-

logue GRASP55 [31]. Both mitotic phosphorylation and apoptotic cleavage of these proteins can cause Golgi fragmentation [24, 32]. In mitosis, mitotic kinases such as Cdk1 phosphorylate GRASP65, leading to the disassembly of the GRASP65 oligomers and unstacking of the Golgi cisternae [26, 27, 32, 33]. In apoptosis, caspase-mediated cleavage of GRASP65 and other Golgi structural proteins also causes Golgi fragmentation [34]. To distinguish these two possibilities for Golgi fragmentation in AD, we determined whether GRASP65 is phosphorylated or cleaved. We did not observe any change in the protein levels of GRASP65 and other Golgi structural and membrane proteins nor their degradation products in these models compared to controls [23]. Thus, we concluded that apoptotic cleavage of Golgi structural proteins is not a major cause of Golgi fragmentation in AD. Instead, we found that GRASP65 is phosphorylated by a signaling cascade that is activated by Aβ accumulation, resulting in Golgi fragmentation. More specifically, Aβ accumulation leads to Ca^{2+} influx, which activates calpain, a

Ca^{2+}-dependent protease that cleaves p35 to generate the Cdk5 activator p25 [35]. Cdk5, a kinase known to phosphorylate tau, then phosphorylates GRASP65 and its interacting protein GM130 [36], essentially inactivating the GRASP proteins that stick the cisternae to each other, and hence leading to Golgi fragmentation [23, 35] (Fig. 1). This conclusion was supported by the fact that Golgi structure can be restored in the AD tissue culture models by the inhibition of Cdk5 or by the expression of a non-phosphorylatable GRASP65 mutant [23]. The direct cause of Golgi fragmentation is Aβ accumulation, as Aβ-treatment causes Golgi fragmentation in cultured neurons and other cell types; and this effect is reversible upon the removal of Aβ from the tissue culture medium.

Consequence of Golgi fragmentation on protein trafficking and processing

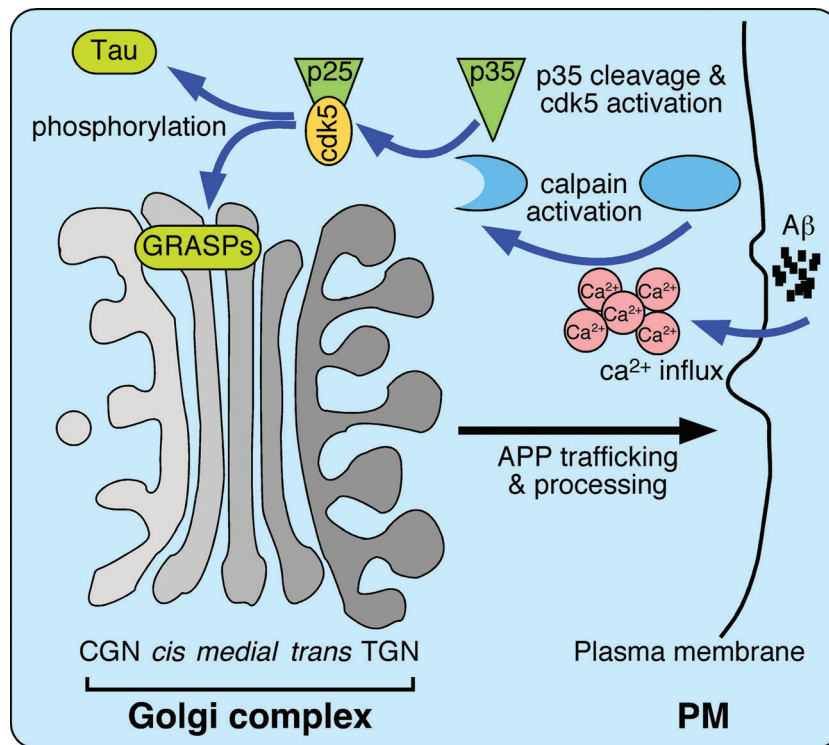In AD, the amyloid precursor protein (APP) and its processing enzymes, the

Figure 1. Mechanism of Golgi fragmentation in AD. Aβ accumulation leads to influx of Ca^{2+} ions; increased cytosolic Ca^{2+} activates calpain to cleave p35 to p25. p25 then activates Cdk5 for GRASP65 phosphorylation. GRASP65 phosphorylation results in Golgi fragmentation, which in turn accelerates APP trafficking and increases Aβ production [23].
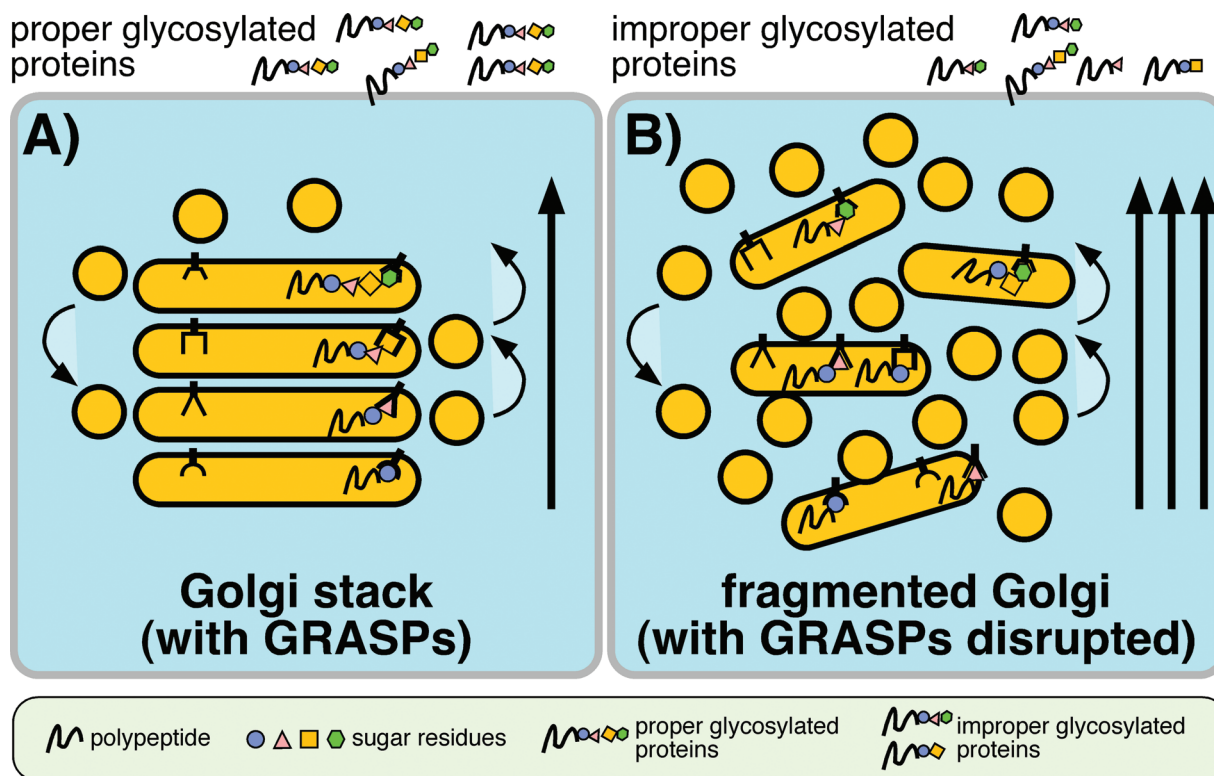
Hypotheses

proper glycosylated proteins

improper glycosylated proteins

A)

B)

Golgi stack (with GRASPs)

fragmented Golgi (with GRASPs disrupted)

〰 polypeptide   ● △ □ ◆ sugar residues   〰●◁◇ proper glycosylated proteins   〰●◁◇ improper glycosylated proteins

Figure 2. Golgi destruction accelerates protein trafficking and impairs accurate glycosylation. When Golgi cisternae are fully stacked (**A**) vesicles can only form and fuse at the rims. This slows down trafficking, but enforces accurate glycosylation. Once the cisternae are unstacked (**B**) more membrane area becomes accessible for vesicle budding and fusion, thereby increasing cargo transport. This, however, causes glycosylation and sorting defects (adapted and modified from [45]).

secretases, are synthesized in the ER and transported through the Golgi to the cell surface. Trafficking, maturation, sorting and processing of both APP and its cleaving enzymes require proper functioning of the Golgi apparatus [37, 38]. However, the structural-functional relationship of the Golgi is so far poorly understood. Golgi structure formation, especially stacking, is a pronounced feature of cellular organization in all metazoans and many unicellular eukaryotes, implying that stacking has important functional consequences. First, stacking may impact protein trafficking. The close spatial arrangement of cisternae in stacks minimizes the distance that molecules must travel. Local tethering proteins facilitate vesicle fusion with Golgi membranes [39], and therefore stacking is expected to enhance protein trafficking. However, stacking restricts the surface for vesicle budding and fusion to the rims of the cisternae, which may set a kinetic limit to

trafficking. Thus, the relationship between Golgi stack formation and trafficking is still not clear. Second, stacking may be required for accurate glycosylation. The Golgi harbors various glycosyltransferases and glycosidases in different sub-cellular compartments, and an ordered compartmentalization is likely required for precise, sequential modifications as cargo proteins pass from cisterna to cisterna [40–42]. In multi-cellular organisms, N-glycosylation of membrane and secretory proteins is complex and essential for their cellular functions, including cell adhesion and migration, cell-cell communication, signal transduction, endocytosis, and immunity [43]. Third, stacking may ensure that sorting occurs only when cargo molecules reach the trans-Golgi network (TGN), but not in earlier subcompartments. Therefore, we speculate that stacking controls the sequence and speed of protein transport through the Golgi mem-

branes, allowing a protein to remain in each compartment for a sufficient time period to ensure proper glycosylation and sorting.

We have performed systematic studies to test this hypothesis, and our results are summarized below. First, Golgi destruction accelerates protein trafficking determined using several markers. Inhibition of Golgi stack formation by microinjected GRASP65 antibodies accelerates CD8 intracellular transport [44]. Depletion of both GRASP55/65 destroys the Golgi structure and enhances trafficking of the cell adhesion protein integrin, the vesicular stomatitis virus G glycoprotein (VSVG), and the lysosomal enzyme cathepsin D [45]. The Golgi tethering protein GM130 remains unaffected [44–46], indicating that the observed effect is not caused by the disruption of membrane tethering [47]. Golgi destruction also increases the rate and efficiency of COPI vesicle formation in vitro [44] and membrane association of coat proteins in cells [45]. Second, Golgi destruction impairs accurate protein glycosylation. GRASP depletion does not impact the expression level and localization of Golgi enzymes, but decreases sialic acid levels on the cell surface [45].
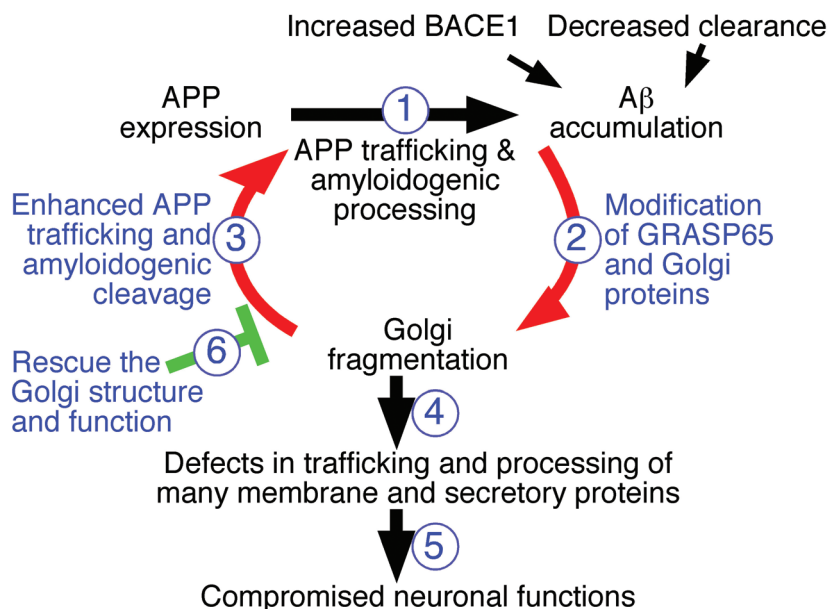
Figure 3. Golgi defects may contribute to AD disease development. APP expression and processing cause Aβ accumulation when BACE1 activity is increased or Aβ clearance is decreased (1), which induces Golgi fragmentation through modification of GRASP65 and other Golgi structural proteins (2), which in turn increases Aβ production by enhancing amyloidogenic cleavage (3). This deleterious feedback loop would impair the integrity of the secretory pathway for sorting, trafficking and modifications of many essential proteins (4), which may compromise neuronal function, activate inflammatory responses, or cause neuronal cell death (5). Therefore, rescue of Golgi structure may reduce Aβ production and delay AD development (6) (adapted and modified from [23]).

Third, Golgi destruction causes mis-sorting of proteins, e.g. cathepsin D precursor, to the extracellular space [45]. These results demonstrate that formation of a proper Golgi structure is required for Golgi functioning in trafficking, glycosylation, and sorting (Fig. 2) [45].

Golgi defects enhance APP trafficking and amyloidogenic processing in AD

Membrane transport pathways provide a connection between many sub-cellular compartments and the cell surface. Trafficking and sorting of membrane cargo is vital for normal cellular function; and defective protein trafficking and sorting have been linked to a variety of diseases. The Golgi, in particular the TGN, directs the protein cargo into distinct vesicles for sorting to various subcellular compartments, such as axons and dendrites in neurons, which

is crucial to maintain neuronal cell polarity and function. Perturbation of intracellular membrane trafficking is central to the molecular events that lead to AD [48]. APP is transported from the ER through the Golgi to the plasma membrane (PM) and undergoes post-translational modifications during its trafficking, including N- and O-glyco-sylation, phosphorylation, and tyrosine sulfation. Only a small fraction of APP reaches the PM and is acted upon by the α-secretase present there, whereas ma-jority of the APP remains in the Golgi [49]. The uncleaved APP at the PM is internalized with the help of the "YENPTY" internalization motif at its C-terminal end and is delivered to the endosomes, where it is cleaved by the resident β-and γ-secretases [50, 51]. Some of the APP molecules are traf-ficked to the lysosomes for degradation [52]. Membrane trafficking, including axonal vesicular transport, is severely disrupted in AD. Neurons surrounding amyloid plaques have axonal swellings and dystrophic neurites in early stages of AD [53, 54]. Similar to APP, other

proteins such as Alcadein-α, a type-1 transmembrane protein involved in axonal transport, accumulate around dystrophic neurites and axons [55].

By light and electron microscopy, we observed that the Golgi is severely fragmented in 12 month old APPswe/PS1ΔE9 transgenic mice [23] and that Golgi fragmentation occurs as early as five months of age. Interestingly, Aβ plaques are not seen in APPswe/PS1ΔE9 transgenic mice until they are six months old [56]. This indicates an intriguing possibility that Golgi defects precede the formation of Aβ plaques [5, 57], strength-ening the role of the defective Golgi in aggravating the disease. Golgi defects may enhance trafficking of APP and its processing enzymes, as well as many other proteins essential for neuronal function (Fig. 3). Our preliminary results demonstrated that rescue of the Golgi structure results in accumulation of full length APP in the Golgi membranes, indicating a delay in APP trafficking and processing. Furthermore, restoration of the Golgi structure by inhibiting Cdk5, or by expressing the N-terminal GRASP domain of GRASP65 that forms oligomers but lacks the regulatory phosphorylation sites at the C-terminus, significantly reduced Aβ production and increased sAPPα secretion [23]. Expression of the non-phosphorylatable GRASP domain rescues the Golgi from fragmentation in the presence of Aβ or Cdk5, which corrects APP trafficking and sorting and shifts the balance from amyloido-genic to non-amyloidogenic processing (Fig. 3). Future studies are required to understand the details on how Golgi fragmentation and restoration affect the trafficking and processing of APP and its processing enzymes.

Apart from GRASP65 phosphoryla-tion, Cdk5 also phosphorylates APP, which is known to regulate its metabo-lism, trafficking and processing [58–62]. Phosphorylation of APP by Cdk5 and GSK-3β (glycogen synthase kinase-3β) at T668 and S655 [62] affects its sorting into Golgi vesicles [50, 58, 63] and the balance between APP trafficking and cleavage [64]. Therefore, it is necessary to determine whether changes in APP post-translational modifications, in-cluding phosphorylation and glycosyla-tion, also contribute to the observed trafficking and processing defects of APP in AD.

Golgi defects may impact trafficking and sorting of APP processing enzymes

The trafficking pathways of APP and its processing enzymes, including the α-, β- and γ-secretases, play a central role in regulating the level of Aβ production and amyloid deposition. APP secretases follow the secretory and the endocytic pathways to reach their final destinations of action. Little is known about how their trafficking routes are affected by the defective Golgi. The α-secretases, ADAM10 and ADAM17, belong to the ADAM (a disintegrin and metalloprotease) family type-1 transmembrane proteins. After synthesis in the ER, ADAM is transported to the Golgi, where its pro-domain is cleaved by furin or a furin-like protease, thus activating its protease activity. After cleavage, ADAM is transported to the plasma membrane for its secretase functions [65]. The β-secretase, BACE1, is subjected to N-glycosylation at multiple sites in the catalytic domain. This domain is essential for its localization to the Golgi and endosomes [66], cleavage of the pro-domain by a calcium-dependent furin-like protease for activation [67], and phosphorylation at the cytoplasmic domain [66]. All of these modifications occur in the Golgi [68], indicating that malfunction of the Golgi in AD may impact BACE1 trafficking, maturation and activity. BACE1 is transported to the cell surface where it is internalized into the endosomes, which provide an optimal acidic environment for the activity of BACE1 [69]. BACE1 trafficking is also regulated by GGA family proteins (Golgi-localizing, γ-adaptin ear homology domain, ARF-binding) such as GGA1 and GGA3, which are important sorting adapters known to facilitate the formation of clathrin-coated vesicles in the TGN [70]. GGA proteins recognize the DISLL sequence in BACE1 and facilitate its recycling between the TGN and endosomes [66, 71, 72]. Phosphorylation of the serine residue (S498) in this sequence does not affect BACE1 endocytosis [66], but it helps BACE1 bind to GGA1 at the endosomes for trafficking to the TGN [73]. Both GGA1 and GGA3 are decreased in AD brains [71, 74]. When GGA3 is depleted, BACE1 sorting to lysosomes is impaired, hence preventing BACE1 degradation [71, 75]. GGA1 overexpression has been shown to decrease Aβ levels [74, 76], possibly due to the increased retrograde transport of BACE1 from the endosomes to the TGN [73]. Taken together, it is reasonable to speculate that Golgi defects in AD affect both APP and BACE1 trafficking and processing and thus impact APP amyloidogenic cleavage, as revealed in our study [23].

Following BACE1-mediated processing, APP is further cleaved by γ-secretase for Aβ production. The γ-secretase complex is composed of four integral membrane proteins: presenilin, nicastrin, APH-1 (anterior pharynx-defective 1), and PEN-2 (presenilin enhancer 2). These membrane-associated components are assembled in a stepwise fashion during membrane trafficking through the ER and Golgi apparatus to form the active γ-secretase complex, and then transported to other cellular membrane organelles [77]. So far, γ-secretase activity has been detected in virtually every membrane organelle in the endocytic and exocytic pathways, including the endosome, lysosome, TGN, cell surface, and even extracellular vesicles after exosome release [37, 38, 78–80]. This could possibly be explained by protein missorting by the fragmented Golgi. Hence, Golgi defects may not only impact trafficking, glycosylation and sorting of APP, but also its processing enzymes.

Golgi defects may impact trafficking and sorting of proteins essential for neuronal function

The Golgi is an essential membranous organelle in all cell types including neurons. Its primary function is membrane trafficking that targets a large number of proteins and lipids to their final destinations. Dendritic Golgi outposts are involved in the trafficking of many integral membrane proteins and secretory proteins. These include the synaptic machineries for neuronal communication, ion channels and transporters for membrane and action potentials, membrane receptors and cell adhesion molecules for cell survival and other activities, and extracellular matrix proteins, neurotransmitters, hormones and growth factors essential for neuronal function. Golgi defects observed in AD may impact the trafficking of all these proteins and disrupt their functions in normal neuronal activity (Fig. 3).

Proper glycosylation of neuronal proteins in the Golgi is required for the functioning of these proteins. For example, Glucose transporter (Glut) expression in the brain is essential for a number of glucoregulatory responses, including glucagon secretion [81], feeding [82], and thermoregulation [83]. Glut2 activation triggers parasympathetic nerve firing and glucagon secretion. Glut-2 is N-glycosylated in the ER and the Golgi before reaching the cell surface; a deficiency in a Golgi-resident glucosyltransferase, GnT-4a, results in a reduced cell expression and hence functioning of Glut-2 [84]. Another example is polysialic acid (PSA), polymers of neuraminic acid derivatives associated with a wide range of biological components, including glycoproteins and lipids. PSA plays crucial roles in nervous system development and function and facilitate cell migration, neurite outgrowth, and synaptic plasticity. Polysialylation occurs via two Golgi-associated polysialyltransferases [85]. Hence, improper glycosylation resulting from Golgi defects may affect the functioning of neurons and impact the overall health of the nervous system.

In addition to protein trafficking, glycosylation and sorting, the Golgi is also involved in some other important cellular functions, such as cleavage and maturation of Notch during cell proliferation and differentiation, cleavage of ATF6 upon unfolded protein response (UPR), and cleavage and release of the transcription factor Sterol Regulatory Element-Binding Protein (SREBP) upon lipid deprivation. Golgi defects may impact the proper functioning of these pathways. For example, Notch is an important protein in neuronal and non-neuronal cell proliferation and differentiation. In the Notch signaling pathway, Notch is first cleaved by a Furin-like proteinase (S1 cleavage) in the Golgi before being transported to the cell surface. This cleavage is essential for producing a functional receptor [86–88]. Notch activity is also affected by its glycosylation in the Golgi during

neurogenesis [89, 90]. Notch signaling regulates neuronal cell differentiation during neurogenesis. This is controlled by Numb, an inhibitor of the Notch pathway. Numb is normally associated with a Golgi resident protein, ACBD3, but is released to the cytosol when the Golgi is fragmented during cell division to allow Numb-Notch interaction and thus Notch inhibition [91]. As the Golgi is fragmented in AD, whether Numb is released from the Golgi and how it affects Notch signaling are so far unknown. Interestingly, Notch is also cleaved by the γ-secretase (S2 cleavage) [92], a fact that hinders the use of γ-secretase inhibitors in AD treatment because of the requirement of Notch signaling in cell survival [93]. Put succinctly, studies on the causes and effects of Golgi fragmentation in AD may provide valuable information about AD pathogenesis and insight into other diseases with Golgi defects.

Conclusion and prospects

The Golgi apparatus plays a critical role in post-translational modification and sorting of a diverse range of proteins vital for neuronal function. While it has long been appreciated that intracellular trafficking of APP involves the Golgi apparatus and trans-Golgi network (recently reviewed by Haass et al. [52]), at present virtually nothing is known regarding the impact of toxic Aβ peptides on Golgi function. Similarly, there is a lack of information regarding how disruptions in Golgi function might alter APP processing and β-amyloid production, and how Golgi defects might affect trafficking, modification, and sorting of many other proteins critical for neuronal functions. In this review, we present a novel hypothesis that impaired APP processing, trafficking and clearance lead to hyperactivation of Cdk5, which then phosphorylates Golgi structural proteins, in particular GRASP65, resulting in Golgi fragmentation. Golgi fragmentation, in turn, affects the trafficking and processing of APP and its processing enzymes, and thus increases Aβ production. More importantly, Golgi defects also impact trafficking, processing and sorting of many other essential neuronal proteins, leading to compromised neuronal func-

tions. This deleterious feedback loop may underlie a so far unrecognized toxicity of Aβ peptides (Fig. 3). Restoring Golgi structure can effectively reduce Aβ production [23], therefore rescuing Golgi structure and function may represent a novel approach to alter Aβ production and toxicity and delay disease development. It is anticipated that the insights gained from this type of research will identify novel therapeutic targets that can be leveraged in the treatment of AD and will provide deeper insight into other diseases with trafficking defects.

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Hypotheses

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