frontiers in Molecular Neuroscience

REVIEW
published: 15 September 2017
doi: 10.3389/fnmol.2017.00294

# Modifications and Trafficking of APP in the Pathogenesis of Alzheimer's Disease

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Alzheimer's disease (AD), the most common neurodegenerative disorder, is the leading cause of dementia. Neuritic plaque, one of the major characteristics of AD neuropathology, mainly consists of amyloid β (Aβ) protein. Aβ is derived from amyloid precursor protein (APP) by sequential cleavages of β- and γ-secretase. Although APP upregulation can promote AD pathogenesis by facilitating Aβ production, growing evidence indicates that aberrant post-translational modifications and trafficking of APP play a pivotal role in AD pathogenesis by dysregulating APP processing and Aβ generation. In this report, we reviewed the current knowledge of APP modifications and trafficking as well as their role in APP processing. More importantly, we discussed the effect of aberrant APP modifications and trafficking on Aβ generation and the underlying mechanisms, which may provide novel strategies for drug development in AD.

**Keywords: Alzheimer's disease, APP, Aβ, post-translational modifications, trafficking**

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***Received:*** *01 June 2017*
***Accepted:*** *31 August 2017*
***Published:*** *15 September 2017*

***Citation:***
*Wang X, Zhou X, Li G, Zhang Y, Wu Y and Song W (2017) Modifications and Trafficking of APP in the Pathogenesis of Alzheimer's Disease. Front. Mol. Neurosci. 10:294. doi: 10.3389/fnmol.2017.00294*

## INTRODUCTION

Alzheimer's disease (AD), the most common neurodegenerative disorder leading to dementia, accounts for ∼75% of dementia cases (ADI World Alzheimer Report, 2014; Korvatska et al., 2015). The rapid increase of AD prevalence is a challenge to the public health and causes a huge socioeconomic burden worldwide. However, no effective treatment has been developed. Progressive memory loss is often the earliest sign of AD, while the impairment of other cognitive functions and psychosis are also presented (Hort et al., 2010; McKhann et al., 2011; Segal-Gidan et al., 2011).

Early-onset AD (EOAD) and late-onset AD (LOAD), occurring before and after the age of 65 years, respectively, are the two types of AD. Less than 5% of AD cases are EOAD (Alzheimer's Association, 2016). EOAD is caused by genetic alterations, including pathogenic mutations in the amyloid-β precursor protein (*APP*) gene (Goldgaber et al., 1987; Kang et al., 1987; Robakis et al., 1987; St. George-Hyslop et al., 1987; Tanzi et al., 1987), presenilin 1 (*PSEN1*) gene (Mullan et al., 1992; Schellenberg et al., 1992; St. George-Hyslop et al., 1992; Li et al., 1995; Sherrington et al., 1995) and presenilin 2 (*PSEN2*) gene (Levy-Lahad et al., 1995a,b; Rogaev et al., 1995), a duplication of *APP* locus, as well as trisomy of chromosome 21 causing Down syndrome (DS; Campion et al., 1999; Bettens et al., 2013). The etiology of LOAD is not yet fully understood. A combination of multiple factors is believed to contribute to the pathogenesis of LOAD, including aging, genetics, nutrition, lifestyle and chronic metabolic disorders (ADI;

Qiu et al., 2009; Yang and Song, 2013; Kang et al., 2017; Zeng et al., 2017; Zhang and Song, 2017). Among them, aging has been demonstrated as the greatest risk factor of AD. Due to the rapid increase in global aging population, the AD prevalence will be continuously increased worldwide (ADI; Korvatska et al., 2015).

Both EOAD and LOAD share the same pathological hallmarks in the brain, including extraneuronal neuritic plaques, intraneuronal neurofibrillary tangles and synaptic/neuronal loss leading to brain atrophy. As one of the major characteristics of AD neuropathology, neuritic plaque is mainly composed of amyloid β (Aβ), which was first identified by Glenner and Wong (1984). Thus, it has been proposed that Aβ overloading and plaque formation initiate the cascade of AD pathogenesis and also contribute to other pathological features, such as neurofibrillary tangles and synaptic/neuronal loss (Hardy and Higgins, 1992). Recent studies suggested that soluble Aβ oligomers might be the main culprit of neuron toxicity. Thus, amyloid hypothesis has been revised to propose that Aβ oligomers play a more important role in AD pathogenesis than mature amyloid fibrils do, indicating that reducing Aβ generation, facilitating Aβ clearance and blocking Aβ oligomerization would be potential strategies to inhibit the pathogenesis of AD (Sun et al., 2006a; Walsh and Selkoe, 2007; Qing et al., 2008; Karran et al., 2011; Ly et al., 2013; Dong et al., 2015).

Aβ is derived from sequential cleavages of the amyloid precursor protein (APP) by β- and γ-secretase. Over 30 pathogenic mutations in APP have been identified to cause early-onset familial AD due to the dysregulation of Aβ generation (Deng et al., 2013; Zhang S. et al., 2017). Overexpression of APP results in the elevation of Aβ levels, which is also implicated in AD pathogenesis (Brouwers et al., 2006; Rovelet-Lecrux et al., 2006, 2007; Sleegers et al., 2006; Ryoo et al., 2007; Kasuga et al., 2009; Sun et al., 2011, 2014; Long et al., 2012; Wu and Song, 2013; Yang et al., 2013; Wu et al., 2014, 2015; Song et al., 2015). For example, rare cases with *APP* locus duplication develop EOAD (Rovelet-Lecrux et al., 2006, 2007; Sleegers et al., 2006; Kasuga et al., 2009). In addition, DS patients with an extra copy of *APP* gene show the increase of APP expression and Aβ generation in the brain, which is associated with the development of AD neuropathology (Ryoo et al., 2007; Sun et al., 2011, 2014; Wu and Song, 2013; Wu et al., 2014, 2015; Song et al., 2015). Moreover, AD-associated mutations within *APP* gene promoter region also enhance APP expression (Brouwers et al., 2006). The downregulation of MiR-106b or MiR-153, targeting *APP* mRNA, has been observed in patients of sporadic AD with the elevation of *APP* mRNA (Long et al., 2012). More importantly, emerging evidence shows that alterations of APP trafficking and post-translational modifications have significant effects on APP processing and Aβ production. Therefore, we aim to introduce the current knowledge of APP modifications and trafficking, review their important roles in APP processing and Aβ generation, and discuss the effect of aberrant post-translational modifications and trafficking on Aβ generation, which may provide novel strategies for drug development in AD.

## APP GENE AND PROTEIN

The human *APP* gene is located on chromosome 21q21.3, spanning approximately 290,586bp of genomic DNA (Goldgaber et al., 1987; Kang et al., 1987; Robakis et al., 1987; Tanzi et al., 1987; Yoshikai et al., 1990; Lamb et al., 1993). By alternative splicing, approximate ten *APP* variants are generated, encoding APP isoforms with 639–770 amino acids. The three major isoforms are APP695, APP751 and APP770, all of which can generate Aβ after sequential cleavages by β- and γ- secretase (Neve et al., 1988; Tanzi et al., 1988; Zimmermann et al., 1988; Kang and Müller-Hill, 1990; Sisodia et al., 1993).

APP is ubiquitously expressed in human tissues with high expression in the central nervous system (CNS). Both APP751 and APP770 isoforms are mainly expressed in non-neuronal cells, while APP695 isoform is predominantly expressed in neurons. As the major isoform in human brains, APP695 expression is markedly increased during neuronal differentiation (Kang and Müller-Hill, 1990; Sisodia et al., 1993). The three isoforms share the conserved protein structure with a larger extracellular domain, a short transmembrane domain and a cytoplasmic domain (Muresan and Ladescu Muresan, 2015). The large ectodomain includes a cysteine-rich globular domain (E1), an acidic domain (AC), a helix-rich domain (E2) and a part of the Aβ domain extending into the transmembrane domain. The short cytoplasmic domain (the intracellular C-terminal domain) contains a conserved YENPTY motif responsible for the protein interactions. E1 domain contains a heparin-binding site (HBD) and a metal-binding domain (MBD) with copper and zinc binding sites. The E2 domain is composed of six α-helices forming a coiled-coil substructure. Both APP770 and APP751 contain a Kunitz-type serine protease inhibitors (KPI) domain following the AC. In addition, APP770 contains an OX2 domain following the KPI domain. APP plays numerous functions, such as neuronal differentiation, neurogenesis, synaptic function, apoptosis and cell proliferation (Bolós et al., 2014; Milosch et al., 2014; Fanutza et al., 2015; Wu et al., 2015, 2016).

## APP PROCESSING AND Aβ GENERATION

### Overview of APP Processing and Aβ Generation

Although Aβ is a well-known proteolytic product of APP, APP indeed undergoes both non-amyloidogenic and amyloidogenic pathways mediated by sequential cleavages of α-/β-/θ-/η-secretase and γ-secretase (**Figure 1**). The majority of APP undergoes non-amyloidogenic pathway. First, APP is cleaved by α-secretase to generate a N-terminal secreted APP (sAPPα) and C-terminal fragment (CTF) of 83 amino acids (C83) which is further cleaved by γ-secretase to release a 3 kDa product (P3) and APP intracellular domain (AICD). In addition, beta-site APP cleaving enzyme 2 (BACE2) is a θ-secretase, which is implicated in APP processing without Aβ generation (Sun et al., 2006b; Liu et al., 2013). The minority of APP is cleaved by β-secretase at Asp1 (β site) and Glu11 (β' site, numbering for Aβ)

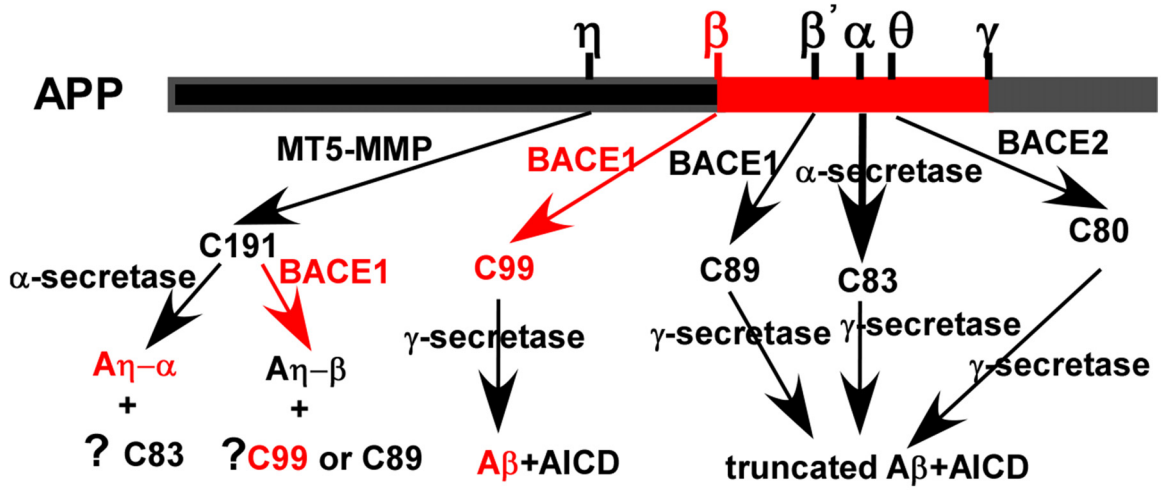


**FIGURE 1 |** Amyloid precursor protein (APP) processing and amyloid β (Aβ) generation. APP is mainly cleaved by α-secretase to generate secreted APP (sAPPα) and C-terminal fragment (CTF) of 83 amino acids (C83). C83 is further cleaved by γ-secretases to generates a truncated Aβ and APP intracellular domain (AICD), respectively. The minority of APP is cleaved by beta-site APP cleaving enzyme 1 (BACE1; β-secretase) at Asp1 and Glu11 (numbering for Aβ) sites to generate a CTF with 99 and 89 amino acids (C99 and C89), respectively. They were further cleaved by γ-secretase to produce Aβ and a truncated form of Aβ, respectively. APP is proteolyzed by BACE2 (θ-secretase) to generate a CTF with 80 amino acids (C80), which is further cleaved by γ-secretase to produce a truncated form of Aβ. Membrane type 5 matrix metalloproteinase (MT5-MMP; η-secretase) is revealed to cleave APP generating C191, which is further cleaved by α-secretase and β-secretase to produce Aη-α and Aη-β, respectively. However, the generation of C83, C99, C89 and their downstream cleavage products following η- cleavage remains elusive.

sites, respectively. Glu11 is the major β-cleavage site to yield a CTF with 89 amino acids (C89), which is further cleaved by γ-secretase to produce a truncated $A\beta_{11-40/42}$. Asp1 is the minor β-cleavage site to generate a CTF with 99 amino acids (C99; Deng et al., 2013). C99 is further cleaved by γ-secretase to produce Aβ (Liu et al., 2002; Deng et al., 2013). Recently, η-secretase, e.g., membrane type 5 matrix metalloproteinase (MT5-MMP), is revealed to be involved in APP processing (Willem et al., 2015).

## α-Secretase

Although α-secretase is not yet fully defined, three a disintegrin and metalloproteinase (ADAM) family members (ADAM9, ADAM10 and ADAM17) may feature α-secretase activity. α-cleavage predominantly occurs at plasma membrane (PM) to generate sAPPα and C83 excluding Aβ production, which is the major proteolytic process of APP at PM (Sisodia, 1992). The activity of α-secretase in Trans-Golgi-Network (TGN) v is regulated by multiple factors such as protein kinase C (PKC; Skovronsky et al., 2000).

## Beta-Site APP Cleaving Enzyme 1

BACE1, the dominant β-secretase *in vivo*, is a type-I transmembrane protein with 501 amino acids (Sun et al., 2012). The trafficking of BACE1 has been reviewed previously (Zhang and Song, 2013; Agostinho et al., 2015; Toh and Gleeson, 2016). Briefly, it is synthesized in the endoplasmic reticulum (ER)-bound polysomes as immature BACE1. Along the secretory pathway, BACE1 undergoes a series of post-translational- modifications in the ER and Golgi apparatus, becoming mature. Mature BACE1 is internalized through endosomes to lysosomes for degradation. It is also transported to the TGN via the retrograde route or recycled to the PM. BACE1 mainly localizes in the TGN and endosome where the acidic environment is optimal for BACE1 activity (Vassar et al., 1999). Thus, β-cleavage mainly occurs in the post-Golgi secretory compartments and endosomal/lysosome organelles (Koo and Squazzo, 1994; Haass et al., 1995; Munger et al., 1995). However, accumulated evidence has shown that intracellular β-secretase cleavage also occurs in ER/ER-Golgi Intermediate Compartment (ERGIC), indicating that acidic pH is not essential for BACE1 activity (Chyung et al., 1997). More importantly, the site preference of β-cleavage is mainly determined by the subcellular location and modification of BACE1 (Huse et al., 2002; Wang et al., 2014). Asp1 is the major BACE1 cleavage site in the ER resulting in C99 generation, which contributes to Aβ generation. However, Glu11 is the predominant β-cleavage site in the TGN, leading to C89 generation without the subsequent Aβ production. In addition, lipid raft is the preferred microdomain for β-secretase cleavage (Zhang and Song, 2013). Many BACE1 inhibitors have been developed for AD treatment by inhibiting the generation of Aβ (Godyń et al., 2016; Hung and Fu, 2017). However, none of them is approved so far.

## θ-Secretase

BACE2, the homolog of BACE1, consists of 518 amino acids (Sun et al., 2005). BACE2 is a θ-secretase, which predominantly cleaves APP at Phe19 within the Aβ domain to yield CTF of

80 amino acids (C80) excluding Aβ generation (**Figure 1**; Sun et al., 2006b; Liu et al., 2013). Consistently, no Aβ overproduction and cognitive deficits were observed in BACE2 transgenic mice (Azkona et al., 2010; Bacher et al., 2010). Despite of high homology, the expression of BACE2 and BACE1 is differentially regulated at transcriptional and post-transcriptional levels (Sun et al., 2005, 2006b). BACE1 is predominantly expressed in neurons, whereas BACE2 expression is extremely low in the brain (Bennett et al., 2000; Marcinkiewicz and Seidah, 2000).

### γ-Secretase

γ-secretase is a protein complex consisting of presenilins (PSEN1 and PSEN2), nicastrin, APH-1 and PEN-2, which cleaves APP following α-/β-/θ-secretase cleavage. PSEN1 and PSEN2 are the core catalytic subunits of γ-secretase, while nicastrin, APH-1 and PEN-2 are the regulatory subunits playing a key role in the maturation and stabilization of the complex (De Strooper et al., 1998, 1999; Song et al., 1999; Zhang et al., 2000, 2013; Takasugi et al., 2003). In addition, Chen et al. (2006) showed that TMP21, a vesicle trafficking protein, is a component of γ-secretase, and its dysregulation and SNPs significantly affect Aβ generation (Chen et al., 2006; Zhang X. et al., 2017). Studies suggest that all the components of the complex are synthesized and mainly localized in the ER (Walter et al., 1996). Most evidence supports that the assembly and maturation processes, mainly occurring in the compartments of the secretory pathway, are crucial for γ-secretase activity, although one report showed that presenilins are not required for Aβ generation in the early secretory pathway (Wilson et al., 2002; Zhao et al., 2004; Capell et al., 2005). The details of γ-secretase trafficking and assembly have been extensively reviewed (Zhang et al., 2013; Agostinho et al., 2015). The active γ-secretase is mainly localized in late endosome and lysosome system (Kanatsu et al., 2014). However, the γ-cleavage has also been observed in the ER, TGN and at PM (Munger et al., 1995; Walter et al., 1996; Maltese et al., 2001; Zhao et al., 2004; Capell et al., 2005; Kaether et al., 2006). Targeting γ-cleavage is a well-known strategy for AD treatment by inhibiting Aβ generation. Although many γ-cleavage inhibitors have been developed, none of them is approved for clinical application (Godyń et al., 2016; Hung and Fu, 2017).

### η-Secretase

Membrane type 1 matrix metalloproteinase (MT1-MMP) was first revealed to be involved in APP processing (Higashi and Miyazaki, 2003). Later on, Ahmad et al. (2006) found that MT3-MMP and MT5-MMP also contribute to APP processing. However, MT3-MMP has no effect on Aβ generation (Ahmad et al., 2006). Recently, Willem et al. (2015) demonstrated that MT5-MMP has η-secretase activity, which cleaves APP695 at amino acids 504–505 to generate a higher molecular mass carboxy-terminal fragment of APP, termed CTF-η (C191). C191 is enriched in an AD mouse model and human AD brains and it could be further processed by α- and β-secretase generating Aη-α and Aη-β, respectively (**Figure 1**; Willem et al., 2015). Aη-α significantly inhibits long-term potentiation *in vitro* and *in vivo* (Willem et al., 2015). However, the generation of C83, C99, C89 and downstream cleavage products following η-cleavage remains elusive although Baranger et al. (2016, 2017) reported that MT5-MMP is a pro-amyloidogenic secretase, promoting amyloid pathology and cognitive decline in AD model mice.

### Aβ Generation Along the Secretory Pathway

Aβ, the major component of senile plaques in AD brains, is generated through sequential cleavages of APP by β- and γ-secretase. The trafficking-dependent co-residence of APP and secreatases plays a key role in the Aβ generation. However, the subcellular location of Aβ production is not fully defined. Several studies have shown that Aβ is mainly generated in the endosome/lysosome where the acidic environment facilitates the activity of β- and γ-secretase (Golde et al., 1992; Koo et al., 1996; Yamazaki et al., 1996; Kanatsu et al., 2014). In contrast, a large body of evidence indicates that Aβ is also generated along the secretory pathway, including ER, Golgi and TGN (Busciglio et al., 1993; Stephens and Austen, 1996; Cook et al., 1997; Hartmann et al., 1997; Tomita et al., 1998; Greenfield et al., 1999; McFarlane et al., 1999). It has been found that Aβ42 instead of Aβ40 is generated in the ER of neurons (Cook et al., 1997; Hartmann et al., 1997; Greenfield et al., 1999). Consistently, ER retention signal or BFA treatment-induced ER retention of APP increases the level of Aβ42. Since both α- and β-secretases exert their cleavages in the TGN, there is a competition between α-secretase and β-secretase for APP cleavage (Skovronsky et al., 2000).

## APP MODIFICATIONS AND TRAFFICKING IN Aβ GENERATION

### APP Trafficking and Co-Residence with Secretases

As a type I transmembrane protein, APP trafficks through the classic secretory, endo-lysosome and recycling pathways (**Figure 2**). After synthesized in the membrane-bound polysomes, the N-terminal signal peptide is removed during its translocation into the ER. Then, it transports to the Golgi apparatus and TGN via ERGIC. Finally, approximately 10% nascent APP reaches the PM, while the majority of APP resides in the Golgi apparatus and TGN. APP at PM is mainly cleaved by α-secretase to release sAPP and C83. The uncleaved APP on the cell surface is rapidly internalized into the endosome, which is mediated by its C-terminal "YENPTY" motif. The internalized APP is sorted into three pathways. Most APP is sorted into late endosome-lysosome pathway for degradation while a small fraction of APP is recycled back to the cell surface or retrograded to the TGN (Haass et al., 1992). Moreover, part of APP at TGN could be directly sorted into the endosome. APP and its fragments have also been detected in the mitochondria, cytoplasm and nuclear. However, the detailed routes and processes remain elusive since the holo-APP and multiple APP fragments may traffic through different pathways (Muresan and Ladescu Muresan, 2015). Furthermore, the trafficking of APP plays a key role in APP processing as the co-residence of APP with secretases (e.g., α-, β-, γ-secretase) along the secretory

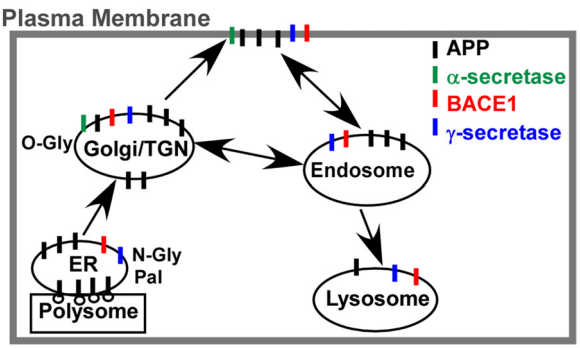


**FIGURE 2 |** APP trafficking and co-residence with secretases. After synthesized in the membrane-bound polysomes, the N-terminal signal peptide is removed during its translocation into the endoplasmic reticulum (ER). N-glycosylation (N-Gly) and palmitoylation (Pal) is crucial for APP transporting to the Golgi apparatus and Trans-Golgi-Network (TGN), while O-glycosylation (O-Gly) in Golgi apparatus is essential for APP transporting to the plasma membrane (PM). The uncleaved APP is rapidly internalized by the endosome and sorted into three pathways. Most APP is sorted into late endosome-lysosome pathway for degradation while a small fraction of APP is recycled back to the PM or retrograded to the TGN. Moreover, the co-residence of APP with secretases (e.g., α-, β-, γ-secretase) along the secretory pathway might contribute to the APP processing and Aβ generation.

pathway and organelle-specific secretase activity significantly affect APP processing and Aβ generation (**Figure 2**; Zhang and Song, 2013; Zhang et al., 2013; Agostinho et al., 2015). For example, α-cleavage predominantly occurs at PM (Sisodia, 1992), while β-cleavage mainly occurs in the endosome and lysosome (Koo and Squazzo, 1994; Haass et al., 1995; Munger et al., 1995).

## Post-Translational Modifications of APP

During the constitutive secretory pathway, APP undergoes extensive post-translational modifications, including N-glycosylation (N-Gly) and O-glycosylation (O-Gly), phosphorylation, sulfation, palmitoylation, ubiquitination and sumoylation. The residue numbering in the following corresponds to the APP695, unless otherwise indicated.

### Glycosylation

When the nascent APP translocates into the ER, N-Gly is catalyzed by the oligosaccharyl transferase (OST) complex with the addition of a precursor oligosaccharide to the luminal side of a polypeptide chain, forming the immature APP. Two asparagine sites, Asn467 and Asn496, are predicted to be glycosylated although only the former one has been confirmed (Pahlsson et al., 1992). However, Yazaki et al. (1996) showed that deletion of either Asn467 or Asn496 leads to a decrease of APP molecular weight in COS-1 cells, which indirectly indicates that both sites are N-glycosylated. O-Gly of APP occurs in Golgi apparatus to form the mature APP. Multiple O-Gly sites of APP have been identified by both *in vitro* and *in vivo* studies. Thr291, Thr292, Thr576 and Thr353 (numbering of APP770) are found to be O-Glycosylated in cultures (Perdivara et al., 2009). The O-Gly of Ser597, Ser606, Ser611, Thr616, Thr634, Thr635, Ser662 and Ser680 (numbering of APP770) has also been identified in human CSF (Halim et al., 2011). In addition to the classical O-GalNAcylation, O-GlcNAcylation is another form of O-Gly and characterized by the addition of a single β-N-acetylglucosamine (GlcNAc) to the residue of serine or threonine (Griffith et al., 1995). Alteration of O-GlcNAcylation in APP plays an important role in regulating APP processing and Aβ generation (Jacobsen and Iverfeldt, 2011; Chun et al., 2015b).

### Phosphorylation

Although APP is a phosphoprotein, the fully glycosylated (N-and O-glycosylated) APP is preferred to be phosphorylated (Gandy et al., 1988; Weidemann et al., 1989; Hung and Selkoe, 1994; Oishi et al., 1997). Ten phosphorylated sites of APP have been identified, including two sites in the ectodomain (Ser198 and Ser206) and eight sites in the cytoplasmic domain (Tyr653, Tyr682, Tyr687, Ser655, Ser675, Thr654, Thr668 and Thr686; Gandy et al., 1988; Walter et al., 1997; Lee et al., 2003). Under basal conditions, two phosphorylated serine residues (Ser198 and Ser206) could be detected in the ectodomain of APP and Ser198 is the major phosphorylated site compared with Ser206 (Hung and Selkoe, 1994; Walter et al., 1997).

More studies about APP phosphorylation focus on the residues within the cytoplasmic domain. Ser655 can be phosphorylated by PKC, while $Ca^{++}$/calmodulin-dependent protein kinase II is involved in the phosphorylation of both Ser655 and Thr654 during *in vitro* culture (Gandy et al., 1988; Suzuki et al., 1992). Moreover, Ser655 can be phosphorylated by APP kinase I *in vivo* (Isohara et al., 1999). Oishi et al. (1997) has reported that okadaic acid, a protein phosphatase 1 (PP1) and PP2A inhibitor, increases Ser655 phosphorylation, suggesting that phosphatases are also involved in the regulation of Ser655 phosphorylation. Phosphorylation of Ser655 is mainly detected in the mature APP, whereas Thr668 is the most common phosphorylated site in the immature APP (Oishi et al., 1997). The phosphorylation of Thr668 occurs in the ER and is cell-cycle dependent (Muresan and Muresan, 2012). Multiple kinases, such as glycogen synthase kinase 3β (GSK3β), cyclin dependent kinase 5 (CDK5), CDK1, stress-activated protein kinase1β (SAPK1β), dual-specificity tyrosinephosphorylation-regulated kinase 1A (DYRK1A) and c-Jun N-terminal protein kinase (JNK) are involved in the process of its phosphorylation (Suzuki et al., 1994; Aplin et al., 1996; Oishi et al., 1997; Iijima et al., 2000; Standen et al., 2001; Ryoo et al., 2008; Mazzitelli et al., 2011). In addition, a couple of phosphatases, such as PP1, PP2A, PP2B are also involved in the regulation of Thr668 phosphorylation (Oliveira et al., 2015).

### Palmitoylation

Palmitoylation is a common way of protein modifications with the addition of fatty acids to a cysteine residue, which is regulated by both palmitoyl acyltransferases and acyl protein thioesterases. Protein palmitoylation is involved in the regulation of protein trafficking and protein-protein interactions. Around 10% of APP undergoes palmitoylation, mainly occurring in ER (Bhattacharyya et al., 2013). Recently,

Bhattacharyya et al. (2013) has reported that APP palmitoylation is mediated by two palmitoyl acyltransferases, DHHC-7 and DHHC-21, and Cys186 and Cys187 are two palmitoylated sites in APP.

## Ubiquitination

Ubiquitination can modify the target protein by attaching ubiquitin, a small protein with 76 amino acids, to the lysine residues. It is catalyzed by ubiquitin-activating enzymes, ubiquitin-conjugating enzymes and ubiquitin ligases. Protein ubiquitination is implicated in the processes including protein degradation, trafficking and protein-protein interactions. Recent studies have identified a couple of ubiquitination sites within the cytoplasmic domain of APP, including Lys649–651, Lys651 and Lys688 (Kaneko et al., 2010; El Ayadi et al., 2012; Watanabe et al., 2012; Morel et al., 2013).

## Sumoylation

Protein sumoylation is characterized by the covalent modification of lysine residues on target proteins with small ubiquitin-like modifier (SUMO; SUMO-1, -2 and -3). It is an important modification to regulate protein functions and catalyzed by SUMO E1, E2 and E3 enzymes. It has been identified that both SUMO-1 and -2 are implicated in APP sumoylation with two sumoylated sites, Lys587 and Lys595 (Zhang and Sarge, 2008).

## Sulfation

Tyrosine sulfation is a common post-translational modification of cell surface protein occurring in the late Golgi compartments, which is implicated in protein trafficking as well as proteolysis process. APP is a tyrosine sulfated protein with two potential sulfated residues, Tyr217 and Tyr262 (Weidemann et al., 1989). However, the exact sulfated sites and the function of APP sulfation have not been fully investigated.

## Interplay of Post-Translational Modifications

Growing evidence indicates that the interplay of post-translational modifications, including phosphorylation and O-GlcNAcylation, phosphorylation and sumoylation, sumoylation and ubiquitination, and O-GlcNAcylation and sumoylation, is involved in complex physiological processes and the pathogenesis of multiple diseases (Zeidan and Hart, 2010; Hart et al., 2011; Ruan et al., 2013; Luo et al., 2014; Liebelt and

**TABLE 1 |** Amyloid precursor protein (APP) modifications and trafficking in amyloid β (Aβ) generation.

| Modification | Trafficking | Affected cleavage | Aβ | Cells/Organs | References |
|---|---|---|---|---|---|
| Glycosylation | | | | | |
| N-Glycosylation | | | | COS-7<br>HEK293 | Pahlsson et al. (1992) |
| O-Glycosylation | PM | α-secretase | ↓ | PC12<br>CHO<br>Hela | Weidemann et al. (1989)<br>Perdivara et al. (2009)<br>Jacobsen and Iverfeldt (2011)<br>Chun et al. (2015a,b) |
| Palmitoylation | | | | | |
| Cys186/187 | Lipid raft | β-secretase | ↑ | CHO<br>PC12<br>Primary neuron | Bhattacharyya et al. (2013) |
| Phosphorylation | | | | | |
| Ser655 | TGN | α-secretase | ↓ | PC12<br>Rat brains | Gandy et al. (1988)<br>Buxbaum et al. (1990)<br>Suzuki et al. (1992)<br>Isohara et al. (1999) |
| Thr668 | | β-/γ-secretase | ↑ | SKSH-SY5Y<br>H4<br>AD brains<br>Mouse brains<br>Primary neuron | Lee et al. (2003)<br>Vingtdeux et al. (2005)<br>Judge et al. (2011)<br>Mazzitelli et al. (2011) |
| Tyr687 | ER/Golgi | α-/γ-secretase | ↓ | AD brains<br>COS-7<br>HEK293 | Zambrano et al. (2001)<br>Tarr et al. (2002)<br>Lee et al. (2003)<br>Rebelo et al. (2007)<br>Takahashi et al. (2008) |
| Ubiquitination | | | | | |
| Lys651 | PM<br>↓ Lipid raft | α-secretase<br>↓β-secretase | ↓<br>↓ | Primary neuron<br>Hela<br>HEK293<br>N2a<br>Mouse brains | Watanabe et al. (2012)<br>Morel et al. (2013) |
| Lys688 | Golgi | | ↓ | PC12, H4 and HEK293 | Hiltunen et al. (2006)<br>El Ayadi et al. (2012) |
| Sumoylation | | | | | |
| Lys587/Lys595 | | | ↓ | Hela | Zhang and Sarge (2008) |
| Sulfation | | | | PC12 | Weidemann et al. (1989) |

Vertegaal, 2016). For example, alternative phosphorylation and O-GlcNAcylation of insulin receptors substrates is implicated in the risk of AD and diabetes (Jahangir et al., 2014). In addition, sumoylation of Tau protein promotes Tau phosphorylation and inhibits its ubiquitination, contributing to AD-associated Tau hyperphosphorylation and accumulation (Luo et al., 2014). As phosphorylation, O-GlcNAcylation, sumoylation and ubiquitination are all involved in APP modification, the interplay of APP modifications may affect APP processing and Aβ generation. For example, both phosphorylation and O-GlcNAcylation occur on serine and threonine residues, suggesting that these two types of post-translational modifications may have reciprocal effects at the same site contributing to the regulation of APP processing and Aβ generation (Weidemann et al., 1989; Chou et al., 1995; Cheng and Hart, 2001; Zeidan and Hart, 2010). However, the interplay of APP modifications has not been reported so far. Therefore, further investigation is essential to elucidate the interplay among APP modifications and its role in AD pathogenesis, providing a novel insight into AD treatment.

## Modifications and Trafficking

Along the secretory pathway, APP is subject to post-translational modifications. On the other hand, APP modifications affect its sorting and trafficking (**Table 1**). Thus, APP modifications and trafficking are mutually regulated, contributing to the regulation of Aβ generation.

### Glycosylation and Trafficking

N-Gly and O-Gly primarily occurs in the ER and Golgi/TGN, respectively. Both of them are essential for APP trafficking (Greenfield et al., 1999). The N-glycosylated APP (i.e., immature APP) is mainly located in the ER, while the mature APP generated through N-Gly, O-Gly and other modifications (e.g., sulfation, palmitoylation and phosphorylation) is mainly located in the TGN and at the PM (Tomita et al., 1998). Weidemann et al. (1989) reported that only the mature form of APP is detected on the cell surface, indicating that both N- and O-Gly are required for APP trafficking to the PM. A study by Chun et al. (2015b) has also shown that O-GlcNAcylation facilitates APP trafficking from the TGN to the PM, but inhibits the endocytosis of APP from the PM. Moreover, the sorting of APP from the Golgi apparatus to the cell surface is prevented due to the deletion of two N-glycosylated sites, Asn467 and Asn496 (Yazaki et al., 1996; McFarlane et al., 1999). Blockade of N-Gly can also inhibit the transport of APP to axonal synaptic membrane (McFarlane et al., 2000). Aforementioned evidence indicates that glycosylation plays a pivotal role in APP trafficking.

### Phosphorylation and Trafficking

APP phosphorylation occurs along the secretory pathway and modulates its sorting and trafficking (Knops et al., 1993; Walter et al., 1997). For example, a APP mutant mimicking the constitutive phosphorylated Tyr687 retains APP in the ER and Golgi. In contrast, a dephosphomimetic APP mutant, the whose phosphorylation site Tyr687 was substituted by alanine, markedly reduces the expression of APP on the cell surface (Rebelo et al., 2007; Takahashi et al., 2008). Phosphorylation of Ser655 also potentiates APP sorting and trafficking from the endosome to the TGN, but attenuates its trafficking to the lysosomes (Vieira et al., 2010). Accordingly, the dephosphomimetic Ser655Ala mutant is preferred to be targeted for lysosomal degradation (Vieira et al., 2010).

### Palmitoylation and Trafficking

APP palmitoylation is essential for APP trafficking and maturation. Double mutations at two palmitoylation sites, Cys186 and Cys187, result in the ER retention of APP and the blockade of APP maturation. Moreover, palmitoylated APP is highly enriched in lipid rafts (Bhattacharyya et al., 2013).

### Ubiquitination and Trafficking

Ubiquitination of APP also affects its sorting and trafficking. Abolishing ubiquitination by substituting Lys649–651 with arginines inhibits the sorting of APP into endosomal intraluminal vesicles (ILVs) in both Hela cells and hippocampal neurons (Morel et al., 2013). In addition, K63-linked polyubiquitination of APP inhibits APP maturation and impairs APP trafficking by sequestering it in the early secretory pathway, such as the Golgi apparatus. The substitution of Lys688 with arginine dramatically reduces APP ubiquitination and Golgi sequestration (El Ayadi et al., 2012). Moreover, FBL2-induced APP ubiquitination inhibits APP endocytosis resulting in an increase of APP on the cell surface and a decrease of APP in lipid rafts (Watanabe et al., 2012).

## Aberrant APP Modifications and Trafficking Dysregulate Aβ Generation in AD

Aberrant APP modifications and impairment of APP trafficking have been found in AD patients (Lee et al., 2003; Placido et al., 2014; Joshi and Wang, 2015), which plays an important role in the regulation of APP processing and Aβ generation (**Table 1**). Compared with the impairment of APP trafficking, the role of aberrant APP modifications in AD pathogenesis have been studied more extensively. Therefore, the dysregulation of trafficking is incorporated into the alteration of modifications.

### Phosphorylation and Aβ Generation

Dysregulation of multiple kinases and phosphatases has been observed in AD brains, including GSK3, PKC, DYRK1A, PP1, PP2A (Wang et al., 1994; Pei et al., 1999; Ferrer et al., 2005; Braithwaite et al., 2012). The abnormality of SET and RCAN1, two phosphatase regulators, has also been found in AD and DS brains, which possibly contributes to the dysregulation of phosphatase activity (Tanimukai et al., 2005; Wu and Song, 2013; Wu et al., 2015; Zhang et al., 2015). As the substrate of above

kinases and phosphatases, APP phosphorylation is impaired in AD, leading to the aberrant APP processing and Aβ generation.

The phosphorylation status of APP differentially affects APP processing and Aβ generation. Previous studies have suggested that reduction of Ser655 and ectodomain phosphorylation may stimulate Aβ generation in AD. For example, a deficiency of PKC in AD brains may reduce the phosphorylation of Ser655 and ectodomain (Wang et al., 1994), promoting Aβ generation (Buxbaum et al., 1990), while PKC-induced increased α-secretase cleavage in the TGN results in the reduction of β-cleavage and Aβ generation (Skovronsky et al., 2000). Moreover, PP1 and PP2A inhibitors have the same effect as PKC activation, such as increasing the secretion of soluble APP and reducing Aβ generation (Buxbaum et al., 1990, 1993; Hung et al., 1993; Hung and Selkoe, 1994).

Increased Thr668 phosphorylation has been detected in AD and DS brains, which is resulted from the imbalance between kinases and phosphatases. For example, increased DYRK1A in DS and AD promotes Thr668 phosphorylation (Ferrer et al., 2005; Ryoo et al., 2008; Wegiel et al., 2008). A number of studies have identified that Thr668 phosphorylation increases Aβ generation both *in vitro* and *in vivo* (Lee et al., 2003; Vingtdeux et al., 2005; Judge et al., 2011; Mazzitelli et al., 2011). Although phosphorylation of Thr668 residue reduces APP secretion, it facilitates β- and γ-secretase cleavages in neurons (Ando et al., 2001; Lee et al., 2003; Vingtdeux et al., 2005; Ryoo et al., 2008; Mazzitelli et al., 2011; Kim et al., 2016; Triaca et al., 2016), leading to enhanced generation of C99 sand Aβ (Suzuki et al., 1994; Colombo et al., 2009; Mazzitelli et al., 2011). However, two studies have reported that Thr668 phosphorylation reduces Aβ generation by inhibiting γ-secretase cleavage (Feyt et al., 2007; Matsushima et al., 2012). It has to be noted that Thr668 phosphorylation inhibits γ-secretase cleavage in CHO cells, which is different from that in neurons Although Thr668E could mimic Thr668 phosphorylation, it may have differential effects on APP conformation change compared with phosphorylated Thr668. Moreover, kinase activation and inhibition may also directly modulate the activity of α-, β- and γ-secretases in addition to APP phosphorylation.

Phosphorylated Tyr682 and Tyr687 have been detected in AD brains but not in healthy controls (Zambrano et al., 2001; Tarr et al., 2002; Lee et al., 2003; Rebelo et al., 2007). Compared with Tyr682, Tyr687 is the major tyrosine phosphorylation site (Takahashi et al., 2008). However, Tyr682 and Tyr687 phosphorylation cannot be detected in the cell lines overexpressing APP, suggesting that phosphorylation of these two residues may be exclusive in AD brains. Intriguingly, a couple of studies suggested that the phosphorylation of Tyr682 and Tyr687 negatively regulates APP processing and Aβ generation. For example, Trk A phosphorylates APP at Tyr682 and reduces the level of AICD (Tarr et al., 2002). In addition, APP mutant which mimics constitutive Tyr687 phosphorylation increases its half-life, but reduces Aβ generation in COS cells (Rebelo et al., 2007). However, a APP mutant with constitutive dephosphorylated Tyr687 (tyrosine replaced by alanine) also reduces both α- and γ-cleavages on APP (Takahashi et al., 2008). Since the cell type and conformation change of APP mutants may differently affect APP processing and Aβ generation, the role of Tyr682 and Tyr687 phosphorylation in Aβ generation remains elusive, which needs to be further investigated.

## Glycosylation and Aβ Generation

Reduced O-GlcNAcylation is observed in AD brains (Liu et al., 2009). Recent studies showed that APP O-GlcNAcylation plays a key role in APP processing. Jacobsen and Iverfeldt (2011) reported that increased O-GlcNAcylated APP by O-GlcNAcase (OGN) inhibitor or siRNA stimulated α-cleavage of APP and reduced Aβ generation. Consistently, Chun et al. (2015b) showed that the OGN inhibitor increased α-cleavage of APP and inhibited the β-cleavage of APP. These effects are due to the inhibition of APP endocytosis, which further increases the level of APP at PM (Sisodia, 1992). In addition, O-GlcNAcylation status of α-, β- and γ-secretases may also be implicated in OGN inhibitor-induced alteration of APP processing (Dias et al., 2009; Tarrant et al., 2012). Recently, Chun et al. (2015a) showed that the APP O-glycosylated site mutant (Thr576Ala) reduced APP expression on cell surface but increased its accumulation in the early endosome, leading to the increase of Aβ generation. It indicates that APP glycosylation does affect its processing and Aβ generation, which is associated with the alteration of APP trafficking.

## Palmitoylation and Aβ Generation

Emerging evidence indicates that APP palmitoylation plays an important role in Aβ generation. Abolishing APP palmitoylation by site-direct mutagenesis inhibits APP cleavage by α- and β-secretase, resulting in the dramatic reduction of APP-CTFs. On the hand, APP palmitoylation preferentially targets APP into the lipid rafts where BACE1 is enriched, facilitating β-cleavage by BACE1 and Aβ generation (Bhattacharyya et al., 2013).

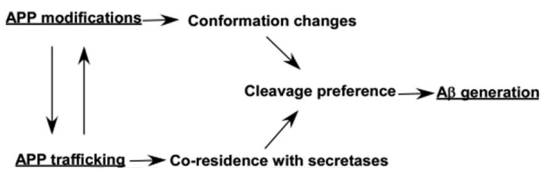


**FIGURE 3 |** Effects of modifications and trafficking of APP on its processing and Aβ generation. Post-translational modifications of APP occur along the constitutive secretory pathway, while the modifications also affect APP trafficking. Both APP modifications-induced conformation changes and trafficking-dependent co-residence with different secretases may alter the cleavage preference of each secretase, resulting in the alteration of APP processing and Aβ generation.

### Ubiquitination and Aβ Generation

It has been demonstrated that APP ubiquitination inhibits APP processing and Aβ generation. HRD1-induced APP ubiquitination facilitates APP degradation, leading to the reduction of Aβ generation (Kaneko et al., 2010). In addition, blocking APP ubiquitination by the substitution of Lys649–651with arginines impairs APP sorting and enhances Aβ generation (Morel et al., 2013), while the ubiquitination of Lys651 is essential for FBL2-induced reduction of Aβ generation (Watanabe et al., 2012). Moreover, ubiquilin 1-mediated K63-linked polyubiquitination of APP delays its proteolytic processing, while reduced UBQLN1 increases Aβ generation (Hiltunen et al., 2006; El Ayadi et al., 2012).

### Sumoylation and Aβ Generation

Two sumoylation sites of APP, Lys587 and Lys595, are close to the β-cleavage site, suggesting that sumoylation of APP may affect β-cleavage of APP and Aβ generation. Simultaneous overexpression of Ubc9 and SUMO-1 promotes APP sumoylation, which is associated with the reduction of Aβ generation. The result indicates that APP sumoylation may negatively regulate Aβ generation (Zhang and Sarge, 2008). In addition, SUMO3 reduces APP turnover rate, which may also contribute to the alteration of Aβ generation (Dorval et al., 2007).

## Therapeutic Strategies for AD by Targeting Aβ

Currently, only four symptomatic drugs, rivastigmine, donepezil, galantamine and memantine, are available for AD treatment by regulating cholinergic and glutamatergic systems, which only leads to a temporary slowdown in the loss of cognitive function. However, these drugs neither delay the progression of dementia nor represent a cure (Godyń et al., 2016; Hung and Fu, 2017). As there is no effective treatment for AD, it is urgent to develop novel drugs for AD treatment. Targeting Aβ generation e is a major strategy for drug development in addition to accelerating Aβ clearance, anti-Tau pathology and anti-inflammation strategies (Godyń et al., 2016; Hung and Fu, 2017). Up to now, more than a hundred of BACE1 inhibitors and γ-secretase modulators have been developed for AD treatment by inhibiting the generation of Aβ. However, none of them is approved although a couple of inhibitors are still in the clinical trial (Godyń et al., 2016; Hung and Fu, 2017). The failure of many inhibitors in clinical trial suggests that several key issues need to be considered. First, as there are tons of known and unknown substrates of BACE1 and γ-secretase, the effects of the inhibitors on other substrates and associated processes should be considered. For example, the severe side effect of the first generation of γ-secretase inhibitor is caused by inhibiting Notch cleavage, a major substrate of γ-secretase (Song et al., 1999; Qing et al., 2008). Second, the inhibition effect on site preference of BACE1 and γ-secretase should be considered because only β-site and γ-site cleavages by BACE1 and γ-secretase contribute to Aβ generation but not β'-site and ε-site cleavages by BACE1 and γ-secretase, respectively (Deng et al., 2013; Zhang et al., 2013). In addition, the effect of the inhibitors on the co-residence of APP with BACE1 and γ-secretase should be considered, which is more important than the general inhibition of BACE1 and γ-secretase activity. Moreover, modulating APP modification might be a novel strategy to complement current strategies of inhibiting BACE1 and γ-secretase activity, which has two major advantages. First, it would have less or no effect on the processing of other BACE1 and γ-secretase substrates, resulting in less or no side effect. Second, it could reduce the co-residence of APP with BACE1 and γ-secretase by altering APP trafficking leading to the reduction of Aβ generation. Importantly, it may alter the cleavage site preference contributing to the reduction of Aβ generation as two recent studies reported that the APP mutants significantly affect cleavage site preference of BACE1 (Kimura et al., 2016; Zhang S. et al., 2017).

## CONCLUSION

Post-translational modifications of APP occur along the constitutive secretory pathway, while it also affect APP trafficking (**Figure 2** and **Table 1**), indicating that APP modifications and trafficking are mutually regulated (**Figure 3**). The modifications and trafficking of APP are precisely controlled to execute its physiological functions and maintain its normal processing. A growing body of evidence has shown that modifications and trafficking of APP have significant effects on APP processing and Aβ production. Aberrant APP modifications-induced trafficking and conformation changes may alter the cleavage preference of each secretase, resulting in the dysregulation of APP processing and Aβ generation (**Figure 3**). Moreover, alteration of APP trafficking has significant effects on its co-residence with different secretases, contributing to the alterations of APP processing and Aβ generation (**Figure 3**). Thus, the regulation of APP modifications and trafficking needs to be further investigated in order to develop novel therapeutic approaches for AD by modulating APP modification and trafficking.

## AUTHOR CONTRIBUTIONS

XW, XZ, GL, YZ and YW wrote the manuscript. YW and WS formatted and revised the manuscript.

## ACKNOWLEDGMENTS

This work was supported by grants from National Natural Science Fund of China (81571334), Natural Science Foundation of Shandong Province (ZR2016HM30, ZR2011HM023), Science and Technology Project of Higher education of Shandong Province (J10LF01), the Development of Medical Science and Technology Project of Shandong Province (2011HZ011), Postgraduate Education Innovation Program of Shandong Province (SDYY15012) and Research Project of Teaching Reform in Undergraduate Colleges and Universities in Shandong Province (2015M049). WS is the holder of the Tier 1 Canada Research Chair in Alzheimer's Disease, and YZ is the recipient of Michael Smith Foundation for Health Research/The Pacific Alzheimer Research Foundation Post-Doctoral Fellowship Award.

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**Conflict of Interest Statement**: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.