

## ● REVIEW

# Physiological effects of amyloid precursor protein and its derivatives on neural stem cell biology and signaling pathways involved

Raquel Coronel, Charlotte Palmer, Adela Bernabeu-Zornoza, María Monteagudo, Andreea Rosca, Alberto Zambrano, Isabel Liste\*  
Unidad de Regeneración Neural, Unidad Funcional de Investigación de Enfermedades Crónicas, Instituto de Salud Carlos III (ISCIII),  
Majadahonda, Madrid, Spain

**Funding:** This work was supported by grants from the Ministerio de Ciencia e Innovación-Instituto de Salud Carlos III (PI-10/00291 and MPY1412/09), Ministerio de Economía y Competitividad (SAF2015-71140-R) and Comunidad de Madrid (Neurostem-Comunidad de Madrid consortium; S2010/BMD-2336). RC was supported by grants from Plan de Empleo Juvenil-Ministerio de Economía y Competitividad.

## Abstract

The pathological implication of amyloid precursor protein (APP) in Alzheimer's disease has been widely documented due to its involvement in the generation of amyloid- $\beta$  peptide. However, the physiological functions of APP are still poorly understood. APP is considered a multimodal protein due to its role in a wide variety of processes, both in the embryo and in the adult brain. Specifically, APP seems to play a key role in the proliferation, differentiation and maturation of neural stem cells. In addition, APP can be processed through two canonical processing pathways, generating different functionally active fragments: soluble APP- $\alpha$ , soluble APP- $\beta$ , amyloid- $\beta$  peptide and the APP intracellular C-terminal domain. These fragments also appear to modulate various functions in neural stem cells, including the processes of proliferation, neurogenesis, gliogenesis or cell death. However, the molecular mechanisms involved in these effects are still unclear. In this review, we summarize the physiological functions of APP and its main proteolytic derivatives in neural stem cells, as well as the possible signaling pathways that could be implicated in these effects. The knowledge of these functions and signaling pathways involved in the onset or during the development of Alzheimer's disease is essential to advance the understanding of the pathogenesis of Alzheimer's disease, and in the search for potential therapeutic targets.

**Key Words:** amyloid precursor protein; APP; soluble APP alpha; soluble APP beta; amyloid beta peptide; APP intracellular domain; neural stem cells; neural progenitor cells; neurogenesis; signaling pathways

## Introduction

Amyloid precursor protein (APP) has been one of the most intensively studied proteins in the last 30 years because of its involvement in the generation of amyloid- $\beta$  (A $\beta$ ) peptide. A $\beta$  peptide is one of the main proteolytic derivatives of APP, whose accumulation and extracellular aggregation causes senile plaques (Tharp and Sarkar, 2013). The formation of these plaques in the brain is the main histopathological feature of Alzheimer's disease (AD) (Gouras et al., 2015), although they also appear in other neurological conditions such as Down syndrome (Lee et al., 2017) or traumatic brain injury (Johnson et al., 2010).

Apart from being the precursor of A $\beta$  peptide, APP itself also seems to contribute to the pathological progression of AD. Mutations in the APP gene are associated with the appearance of familial AD, which represents 5% of AD cases and is characterized by its early onset (before 65 years of age) (Karch and Goate, 2015). In addition, several studies have shown that APP causes the deregulation of several signaling pathways, producing cellular and molecular alterations characteristic of AD (Bukhari et al., 2017). However, the mechanisms involved in both the onset and the development of AD are still not well known, mainly because the physiological functions of APP are poorly understood.

APP has been described as a multimodal protein, and

seems to regulate a wide range of biological activities, including intracellular transport, neuronal development and cell signaling processes (Chen et al., 2017; Müller et al., 2017). These functions may be related to the production of different fragments that result from the proteolytic processing of APP. These fragments include not only A $\beta$  peptide, but also the so-called non-amyloid derivatives of APP, including soluble APP- $\alpha$  (sAPP $\alpha$ ), soluble APP- $\beta$  (sAPP $\beta$ ) and the APP intracellular C-terminal domain (AICD).

As described above, the involvement of APP in the pathogenesis of AD is increasingly recognized, but the physiological functions of this protein are still poorly understood. In the embryo, the expression of APP is elevated during early stages of nervous system development (Kirazov et al., 2001; van der Kant and Goldstein, 2015), which suggests an important role of this protein in neural growth and maturation. Specifically, APP seems to play a key role in the proliferation, differentiation and maturation of neural stem cells (NSCs) (Small et al., 2014; Coronel et al., 2018). In the adult brain, APP is also abundantly expressed and various functions have been described, including its role as a regulatory protein of axonal outgrowth after injury (Leysen et al., 2005) and in the proliferation of neural progenitor cells (NPCs) (Wang et al., 2016).

Despite the existing studies, a more detailed understand-

\*Correspondence to:

Isabel Liste, PhD, [iliste@isciii.es](mailto:iliste@isciii.es).

orcid:

0000-0002-3294-9558

(Isabel Liste)

doi: 10.4103/1673-5374.257511

Received: December 21, 2018

Accepted: March 7, 2019

ing of APP function and activity in the physiology of the central nervous system (CNS) is necessary, and NSCs/NPCs could be a good study model for that purpose. NSCs/NPCs are multipotent cells capable of self-renewal and differentiation into all cell type of the CNS, including neurons and macroglia. These cells can be sourced from fetal, neonatal and adult brains, as well as from the differentiation of pluripotent stem cells (Martínez-Morales et al., 2013).

In this review, we show an updated summary of the physiological functions of APP and its main proteolytic derivatives in NSCs/NPCs, as well as the possible signaling pathways that could be involved in these effects. The knowledge of these functions is of vital importance to improve the understanding of the pathogenesis of AD.

### Expression of Amyloid Precursor Protein

APP belongs to the small family of APP proteins that includes, apart from APP, the so-called amyloid precursor-like protein 1 and 2 (APLP1 and APLP2, respectively). Both APP and APLPs are single-pass transmembrane glycoproteins, with a large extracellular domain at the N-terminal and a small intracellular domain at the C-terminal (Nguyen, 2015). Despite having elevated structural homology, highly conserved amino acid sequences and biological functions seemingly similar or related to APLPs, APP is the only member of the family that contains the coding sequence for A $\beta$  peptide, located in the transmembrane domain of APP (van der Kant and Goldstein, 2015). For this reason, APP has been more extensively studied in the context of AD.

In humans, APP is encoded by a single gene located on chromosome 21 (locus 21q21.3), contains 18 exons and comprises a distance of approximately 240 kb (Dawkins and Small, 2014). Due to its location on chromosome 21, several authors associate cognitive deterioration and the early onset of AD in people with Down syndrome to this extra copy of the APP gene (Sosa et al., 2014). In addition, as discussed above, mutations in the APP gene (with the exception of A673T mutation) (Jonsson et al., 2012) are associated with the appearance of familial AD, since they all favor the production of A $\beta$  peptide (Van Dam and De Deyn, 2006). Thus, given that modifications in the levels and sequence of APP gene are pathological, its physiological functions seem to be essential for the correct cerebral functioning.

Regarding the transcription process, the alternative splicing of APP mRNA can give rise to approximately ten different isoforms, defined by the number of amino acids (639–770) it contains (Wang et al., 2017a). The main isoforms produced are APP695, APP751 and APP770, with APP695 being the isoform predominantly expressed in the CNS (Chen et al., 2013; Nalivaeva and Turner, 2013). APP mRNA and the protein itself are ubiquitously expressed in a wide variety of tissues, including the nervous system, muscle, liver, immune system, lung, kidney and thyroid gland (Puig and Combs, 2013). However, different isoforms of APP are expressed in different proportions and in different cell types. APP695 is the isoform predominantly expressed in neurons, while APP751 and APP770 are predominantly expressed in

cells of peripheral tissues. It should be noted that in terms of glial lineage, *in vitro* studies have documented the expression of the three major isoforms of APP in astrocyte and microglial cultures (Müller et al., 2017). Therefore, studying APP expression in astrocytes is also important, since these cells are vital for maintaining the regenerative capacity in the reservoirs of new neurons (Papadimitriou et al., 2018).

### Processing of Amyloid Precursor Protein

APP is usually synthesized in the endoplasmic reticulum and is transported to the cell membrane through the secretory pathway. This pathway is responsible for the folding, post-translational modification and protein translocation to the membrane of a diverse range of proteins, generally glycoproteins (Barlowe and Miller, 2013). In the case of APP, post-translational modifications such as phosphorylations, N-glycosylations, O-glycosylations, sulfations in tyrosine residues and palmitoylations give rise to the mature form of the protein (Buoso et al., 2010; Bhattacharyya et al., 2013; Wang et al., 2017a).

After the formation of mature APP, it can be processed by enzymes called secretases, giving rise to two canonical processing pathways. These pathways generate different biologically active proteolytic fragments whose functions are specific and, in some cases, opposing.

The non-amyloidogenic pathway occurs in the cell membrane and is characterized by the non-production of A $\beta$  peptide. In this pathway,  $\alpha$ -secretase cleaves APP within the A $\beta$  domain, preventing its formation and aggregation. This process produces the sAPP $\alpha$  fragment and releases it to the extracellular medium, leaving the C-terminal fragment  $\alpha$  (also known as C83) in the cell membrane. Next, the  $\gamma$ -secretase enzymatic complex cleaves the C83 fragment, releasing the soluble peptide p3 to the extracellular medium and the AICD fragment to the cytoplasm (Haass et al., 2012; Grimm et al., 2013). It is important to note that, although there are two canonical processing pathways, the non-amyloidogenic pathway predominates under physiological conditions (Agostinho et al., 2015) (**Figure 1**).

The amyloidogenic pathway, on the other hand, is characterized by the production of A $\beta$  peptide. In this pathway,  $\beta$ -secretase cleaves APP outside the A $\beta$  domain and, therefore, its formation is not prevented.  $\beta$ -secretase produces the sAPP $\beta$  fragment and releases it to the extracellular medium, while the remaining C-terminal fragment  $\beta$  (also known as C99) stays in the cell membrane. Next, the  $\gamma$ -secretase enzymatic complex cleaves the C99 fragment, releasing A $\beta$  peptide to the extracellular medium and the AICD fragment to the cytoplasm (Haass et al., 2012; Grimm et al., 2013) (**Figure 1**). Several authors have proposed that the amyloidogenic pathway takes place in endosomes and this hypothesis is increasingly accepted (Morel et al., 2013). These compartments are characterized by having a lower pH (more acidic), optimal for  $\beta$ -secretase activity. Therefore, the cellular distribution of APP seems to influence its proteolytic processing. The non-amyloidogenic pathway is favored by the accumulation of APP in the cell membrane, while the amyloidogenic

pathway is favored when APP accumulates in endosomes as a result of clathrin-mediated endocytosis (van der Kant and Goldstein, 2015).

Studies have shown that post-translational modifications such as ubiquitination affect the trafficking and processing of APP, inhibiting its clathrin-mediated endocytosis and increasing its presence in the cell membrane (Watanabe et al., 2012). Due to this, some authors highlight the importance of ubiquitination as a key factor in differential APP processing (Williamson et al., 2017). However, post-translational modifications of APP is not solely responsible for its differential processing, as varying activity levels of different secretases are also responsible.

As can be observed, APP proteolytic processing is a highly controlled and modulated process. Therefore, deregulation of the processing of APP and imbalance in the normal physiological levels of APP and its derivatives could be one of the first pre-amyloidogenic changes involved in the onset of AD (Tijms et al., 2018).

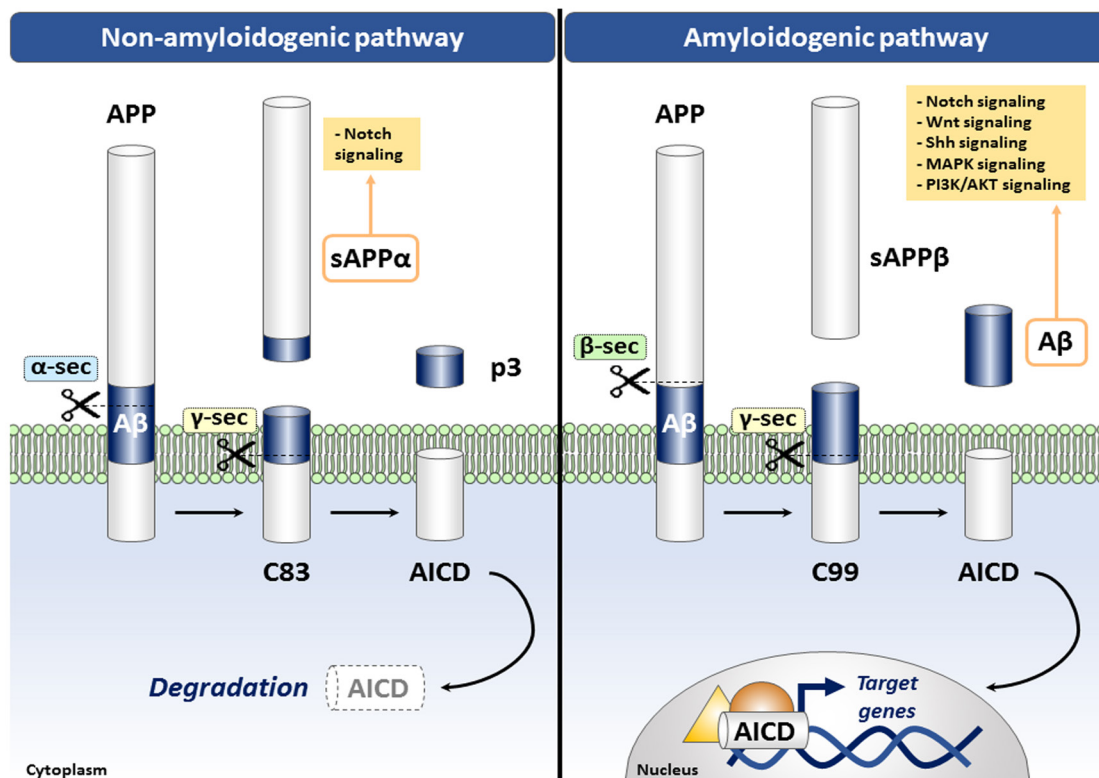
In addition to the canonical processing pathways described above, APP can also be processed in several non-canonical pathways. The  $\eta$ -secretase pathway (Willem et al., 2015), the  $\delta$ -secretase pathway (Zhang et al., 2015) and the meprin pathway (Jefferson et al., 2011) have been discovered

in which additional N-terminal derivatives of APP are generated. Thus, a firm knowledge of APP and the regulation of its processing is needed in order to understand the physiological functions of APP.

## Functions of Amyloid Precursor Protein and Its Derivatives on the Biology of Neural Stem Cells/Neural Progenitor Cells

As discussed above, there is still no clear consensus about the physiological functions of APP. The studies carried out during recent years have established APP as a multimodal protein that can modulate a variety of biological processes, ranging from transcriptional regulation to synaptic functions (Müller et al., 2017).

There is evidence indicating the involvement of APP in neural development (Nicolas and Hassan, 2014; Soldano and Hassan, 2014), its participation as a trophic factor during the proliferation and differentiation of stem cells (Small et al., 2014), its ability to adhere to the extracellular matrix (Sosa et al., 2013) and its role in neuronal migration, neurite growth and synaptogenesis (Chen and Dou, 2012; Tyan et al., 2012). APP has also been described to interact with N-methyl-D-aspartate receptors (Cousins et al., 2015)



**Figure 1** Proteolytic processing of APP by non-amyloidogenic pathway and amyloidogenic pathway.

In the non-amyloidogenic pathway, APP is sequentially cleaved by  $\alpha$ -sec and  $\gamma$ -sec generating sAPP $\alpha$ , p3 and AICD fragments. In the amyloidogenic pathway, APP is sequentially cleaved by  $\beta$ -sec and  $\gamma$ -sec generating sAPP $\beta$ , A $\beta$  and AICD fragments. In both cases, the APP processing releases functionally active fragments to the extracellular medium (sAPP $\alpha$ , p3, sAPP $\beta$  and A $\beta$  peptide) which seem to modulate or influence several signaling pathways (marked in yellow boxes). Also the release of the intracellular fragment AICD seems to act differently according to its processing pathway. In the non-amyloidogenic pathway, AICD is rapidly degraded, while in the amyloidogenic pathway, AICD acts as a transcriptional regulator of several target genes. APP: Amyloid precursor protein;  $\alpha$ -sec:  $\alpha$ -secretase;  $\beta$ -sec:  $\beta$ -secretase;  $\gamma$ -sec:  $\gamma$ -secretase; sAPP $\alpha$ : soluble APP- $\alpha$ ; sAPP $\beta$ : soluble APP- $\beta$ ; AICD: intracellular C-terminal domain; A $\beta$ : amyloid- $\beta$ ; Shh: sonic hedgehog; MAPK: mitogen-activated protein kinase; PI3K/AKT: phosphoinositide 3-kinase/protein kinase B.



and have effects on neuroplasticity (Marik et al., 2016). Moreover, APP has been shown to have implications in the process of neurogenesis both *in vitro* (Bolós et al., 2014) and *in vivo* (Wang et al., 2014).

Due to its structural homology with transmembrane type I receptors and the identification of certain APP ligands, APP has also been proposed as a receptor involved in cell signaling (Deyts et al., 2016), as well as its participation in transcriptional regulation, specifically by the AICD fragment (Shu et al., 2015). However, the molecular mechanisms by which APP and/or its proteolytic derivatives regulate all these processes have not yet been described and it is therefore necessary to use new techniques to deepen the study of this complex protein.

NSCs/NPCs are undifferentiated neural cells capable of proliferating and self-renewing, maintaining a multipotent state and give rise to neurons, astrocytes and oligodendrocytes (Itokazu and Yu, 2014). In the context of AD, NSCs/NPCs lose plasticity and regenerative capacity, while these cells in healthy brains constitute an endogenous reservoir for neurons that could be potentially used for regenerative therapies in neurodegenerative diseases (Papadimitriou et al., 2018). As mentioned above, NSCs/NPCs can be sourced from fetal, neonatal and adult brains, as well as from the differentiation of pluripotent stem cells (Martínez-Morales et al., 2013), and they have been widely used as models to study human brain development and several pathologies *in vitro* (Seto and Eiraku, 2019).

It has been described that APP plays an essential role in normal brain development and possibly also in the plasticity of adult brains (Shariati and De Strooper, 2013). Recent studies in our group show that APP is abundantly expressed in human NSCs and seems to exert a dual role in the differentiation of these cells, favoring gliogenesis and inhibiting the generation of neurons (Coronel et al., 2018). In addition, APP expression is also elevated during neuronal differentiation of induced pluripotent stem cells toward NPCs (Ochalek et al., 2017) and cortical neurons (Bergström et al., 2016), and has been proposed as a new and specific biomarker of the pluripotent state in human stem cells (Venkataramani et al., 2012). Therefore, NSCs/NPCs are a good model to study the physiological function of APP and its proteolytic derivatives, and to help deepen the knowledge of the molecular mechanisms involved. In the following sections, we summarize some functions of the main proteolytic fragments of APP in NSCs/NPCs.

### Functions of sAPP $\alpha$ and sAPP $\beta$

sAPP $\alpha$  is one of the fragments released to the extracellular medium after the proteolytic processing of APP by the non-amyloidogenic pathway. This processing pathway occurs predominantly under physiological conditions and many authors associate the sAPP $\alpha$  fragment with neuroprotective and neurotrophic functions (Hick et al., 2015; Plummer et al., 2016; Hefter and Draguhn, 2017). sAPP $\beta$  is another fragment released into the extracellular medium after the proteolytic processing of APP, but in this case, by the amyloidogenic

pathway, which later releases A $\beta$  peptide. Regarding this processing pathway, most studies have focused on discovering the functions of A $\beta$  peptide, but the functions of sAPP $\beta$  are still poorly understood (Hesse et al., 2018).

The specific roles of sAPP $\alpha$  and sAPP $\beta$  remain unclear. Many studies have demonstrated a role of sAPP $\alpha$  in the proliferation of NPCs both *in vitro* and *in vivo* (Caillé et al., 2004; Demars et al., 2011; Baratchi et al., 2012). In contrast, sAPP $\beta$  does not appear to elicit the same proliferative effect in NPCs, either *in vitro* or *in vivo*, when used at an equimolar concentration with respect to sAPP $\alpha$  (Demars et al., 2013). Recent studies show how soluble forms of APP increase the formation of individual neurospheres *in vitro*, but decrease their size by suppressing the growth of NSCs. Similarly, the same authors determined that soluble forms of APP negatively regulate the growth of NSCs by controlling the number of cells in the subventricular zone of adult mice (Sato et al., 2017).

Apart from possible roles as proliferative regulators of NSCs/NPCs, the functions of sAPP $\alpha$  and sAPP $\beta$  in neuronal differentiation of human embryonic stem cells has also been described (Freude et al., 2011). They have been shown to be involved in cell fate specification processes, promoting gliogenesis and suppressing neurogenesis of human NSCs (Kwak et al., 2006). In addition, high sAPP $\alpha$  secretions have been detected in both neurons and astrocytes derived from induced pluripotent stem cells (Liao et al., 2016), further indicating its importance during the process of cell differentiation.

Recently, it has been described that the sAPP $\alpha$  fragment also regulates various synaptic functions, modulating synaptic plasticity, spine density and cognition in the adult mouse brain. However, these effects are not reproducible with the sAPP $\beta$  fragment (Richter et al., 2018).

Since the physiological functions of both soluble fragments are still not well understood, it is not known whether some of the symptoms of AD could be due to functional changes caused, for example, by a reduction in sAPP $\alpha$  levels or an alteration of the sAPP $\alpha$ /sAPP $\beta$  ratio (Mockett et al., 2017). Therefore, it is important to know the physiological functions of both soluble fragments that are secreted to the extracellular medium upon APP processing.

### Functions of A $\beta$ peptide

A $\beta$  peptide is released into the extracellular medium after  $\gamma$ -secretase cleavage in the amyloidogenic pathway and can be found in different aggregation states. Monomeric A $\beta$  peptides are known to aggregate to form oligomers, which can be further aggregated to form fibrils, which in turn are part of the senile plaques in AD. These different aggregation states are generated by the increasing concentration of monomeric A $\beta$  peptides and all of them have been shown to produce diverse effects in the brain (Chen et al., 2017). Recent evidences support that A $\beta$  peptides in their oligomeric and fibrillar forms are the main cause of neurodegeneration in AD, as these aggregation states are the most toxic (Selkoe and Hardy, 2016; Wang et al., 2017b; Mroczko et al., 2018).

Therefore, since this review focuses on the physiological functions of APP and its derivatives, here we mainly discuss the effects of A $\beta$  peptide in its monomeric form.

The aggregation state of A $\beta$  peptide is not the only factor that influences its functionality, but also its isoform. Processing of the C99 fragment by  $\gamma$ -secretase at multiple sites generate cleavage fragments of 43, 45, 46, 48, 49 and 51 amino acids, which are further cleaved to the main A $\beta$  isoforms, A $\beta_{40}$  and A $\beta_{42}$  (A $\beta$  peptides of 40 and 42 amino acids, respectively) (Chen et al., 2017). As with the aggregation states, it has been described that different isoforms of A $\beta$  peptide are functionally diverse, where longer A $\beta$  peptides (A $\beta_{42}$  and A $\beta_{43}$ ) are more toxic and prone to self-aggregation, whereas A $\beta_{40}$ , on the other hand, could be considered anti-amyloidogenic, and even neuroprotective (Kim et al., 2007).

Apart from the pathological function already described, monomeric A $\beta_{40}$  and A $\beta_{42}$  peptides are present in brains and cerebrospinal fluid of healthy individuals, suggesting that both also have a physiologically active function (Wirth et al., 2013). Our group recently showed that monomeric A $\beta_{42}$  peptide (at a concentration of 1  $\mu$ M) promotes the proliferation of human NSCs, specifically, increasing the pool of proliferating glial precursors, without affecting neurogenesis (Bernabeu-Zornoza et al., 2018). Similar effects have been described in both mouse NSCs and rat NPCs, where treatment with monomeric A $\beta_{42}$  peptide (at a concentration of 1.5  $\mu$ M) favors the proliferation and gliogenesis of these cells, in contrast to monomeric A $\beta_{40}$  peptide that preferentially promotes neuronal differentiation (Chen and Dong, 2009; Fonseca et al., 2013).

In addition, this proliferative effect has also been described in mouse NSCs at high concentrations (10  $\mu$ M) of A $\beta_{42}$  peptide, capable of significantly increasing the number of cells (Itokazu and Yu, 2014). However, recent studies have shown the opposite, where at a concentration of 10  $\mu$ M, A $\beta_{42}$  peptide compromised proliferation and blocked neuronal differentiation of mouse NSCs (Ribeiro et al., 2018). A possible explanation could be that, at high concentrations (10  $\mu$ M), monomeric A $\beta$  peptides aggregate to form oligomers, which would be similar to the effects observed in studies done in human NSCs with A $\beta_{42}$  peptide in its oligomeric state, which decreases proliferation, favors glial differentiation and suppresses the migratory capacity of these cells (Lee et al., 2013).

Thus, it is important to keep in mind that, although controversies exist in the field, the different results could be due to the A $\beta$  peptide isoforms, the concentration of peptides, the aggregation state and the type of NSCs/NPCs used in each experiment.

### Functions of AICD

Unlike other proteolytic fragments, AICD is released into the cytoplasm after it has been cleaved by  $\gamma$ -secretase in both canonical processing pathways. However, it seems that the AICD fragment is only transcriptionally active when it comes from the amyloidogenic pathway (Beckett et al., 2012; Nalivaeva and Turner, 2013). The involvement of AICD

in the pathological development of AD has been widely documented but its physiological role in nuclear signaling, transcriptional regulation, apoptosis and DNA repair is still controversial (Bukhari et al., 2017).

Like A $\beta$  peptide, several isoforms of AICD have been described, including AICD59, AICD57, AICD51, AICD50 and AICD31, according to the number of amino acids it contains (Bukhari et al., 2016, 2017). It should be noted that the AICD57/59 isoforms correspond to the A $\beta_{40/42}$  fragments resulting from  $\gamma$ -secretase cleavage, but the isoforms mainly detected are the AICD<sub>50/51</sub> fragments, which result from  $\gamma$ -secretase cleavage at the  $\epsilon$ -site (Dimitrov et al., 2013; Pinix et al., 2013). Cleavage at the  $\epsilon$ -site is similar to the cleavage caused by  $\gamma$ -secretase at Notch receptors, which results in the formation of the Notch intracellular C-terminal domain (NICD) (Gu et al., 2001). For this reason, many believe that AICD has a transcriptional regulation function similar to the NICD fragment (Nagase and Nakayama, 2014).

It has been described that AICD, when it has not been degraded in the cytoplasm, is capable of forming a transcriptionally active complex with Fe65 and Tip60 that regulates the expression of several genes, including APP itself, GSK3B (glycogen synthase kinase 3 $\beta$ ; serine/threonine kinase associated with hyperphosphorylation of Tau protein) (Kim et al., 2003), PTCH1 (Ptch1, protein patched homolog 1; receptor that suppresses the sonic hedgehog (Shh) signaling in absence of Shh ligands) (Trazzi et al., 2011), STMN1 (stathmin1; phosphoprotein that regulates microtubule dynamics by destabilizing microtubules) (Müller et al., 2013), NEP (nephrilysin; metalloprotease that degrades A $\beta$  peptide) (Grimm et al., 2015), TP53 (p53; tumor suppressor protein that induces apoptotic cell death by caspase activation) (Herold et al., 2015) and SOX2 (SRY-box2; transcription factor that regulates embryonic development and cell fate determination) (Sarлак et al., 2016).

Unlike sAPP $\alpha$  and sAPP $\beta$ , AICD seems to impair the proliferation of mouse NPCs *in vitro*. Some authors show that AICD activates the transcriptional regulation of the PTCH1 promoter, which would inhibit the Shh signaling pathway and cell division (Trazzi et al., 2011). In addition, proliferative defect caused by AICD could also be due to the negative transcriptional regulation that it exerts on SOX2 (Sarлак et al., 2016) or EGFR (epidermal growth factor receptor), which initiate the signaling processes of cell proliferation, differentiation and survival (Zhang et al., 2007). Studies conducted in human NSCs have shown that AICD suppresses the neuronal differentiation of these cells *via* positive transcriptional regulation of miR-663, a microRNA responsible for suppressing the expression of multiple genes involved in neurogenesis (Shu et al., 2015). As we have previously commented, gain-of-function studies performed by our group show a possible dual role of APP in the differentiation of human NSCs, favoring gliogenesis and inhibiting neurogenesis. These effects could be mediated by the AICD fragment, as our results showed high levels of this fragment at the nuclear level in cells overexpressing APP (Coronel et al., 2018). In addition, elevated levels of AICD were accompanied by

high expression levels of several of its target genes, including *GSK3B*. Therefore, we believe that the AICD fragment could be involved in the process of differentiation in human NSCs, regulating the transcription of *GSK3B* and decreasing the generation of neurons (Coronel et al., 2018).

Interestingly, a study performed by Giacomini et al. (2015) showed that the effects of AICD in the processes of proliferation and neurogenesis could be reversed by blocking  $\gamma$ -secretase activity, thus inhibiting AICD formation, in mouse NSCs. Despite affecting cell proliferation and neurogenesis in NSCs/NPCs, AICD has also been associated with apoptosis, both by positive transcriptional regulation of pro-apoptotic genes such as *TP53* (Herold et al., 2015) and by the generation of the AICD31 fragment, which contributes to apoptotic cell death (Lu et al., 2003). Thus, it is necessary to deepen our understanding of the physiological functions of AICD since it could be a key factor in many processes related to the biology of NSCs.

### Signaling Pathways Implicated in the Effects of Amyloid Precursor Protein and Its Derivatives in Neural Stem Cells/Neural Progenitor Cells

Although some of the functions exerted by APP and its proteolytic fragments have been described, the molecular mechanisms and the signaling pathways involved in these effects remain mostly unknown. Therefore, it is necessary to better understand how APP and its derivatives affect these pathways and how the alteration of these effects could contribute to early development of AD pathogenesis.

Some of the signaling pathways in which APP and its derivatives seem to modulate the functions described above are Notch signaling, Wnt signaling and Shh signaling, with *GSK3 $\beta$*  being a key regulator of these processes.

#### Notch signaling

Notch signaling is one of the most important and conserved signaling pathways in mammalian cells. This signaling pathway is well known for its role as a master regulator of NSCs and neural development, although it also plays an important role in mature differentiated cells of the CNS and in the adult brain. Specifically, some of the functions modulated by Notch signaling include the maintenance of NSCs in neurogenic niches through the regulation of proliferation, migration and neuronal differentiation, as well as synaptic plasticity (Ables et al., 2011).

In addition, it has been seen that Notch signaling can be influenced or even regulated by APP due to its ability to interact with Notch receptors located in the cell membrane (Oh et al., 2005). In particular, it has been determined that sAPP $\alpha$  activates Notch signaling and causes glial differentiation of human NPCs through the formation of the proteolytic fragment NICD (Kwak et al., 2011).

Curiously, although sAPP $\alpha$  could be postulated as one of the ligands of Notch receptors, the treatment of mouse NSCs with A $\beta_{42}$  peptide also seems to modulate Notch sig-

naling, stimulating self-renewal and proliferation in these cells (Itokazu and Yu, 2014) (**Figure 2A**).

As for the intracellular fragment AICD (which is generated in a similar way as the NICD fragment), it has been shown to co-localize with NICD in the same transcription complexes, which could suggest that both intracellular fragments regulate the transcription of similar genes (Konietzko et al., 2010) (**Figure 2B**).

#### Wnt signaling

Wnt signaling is a highly conserved pathway involved in the main processes of embryonic development and adult cell homeostasis, as well as in the self-renewal, maintenance, maturation and differentiation of NSCs.

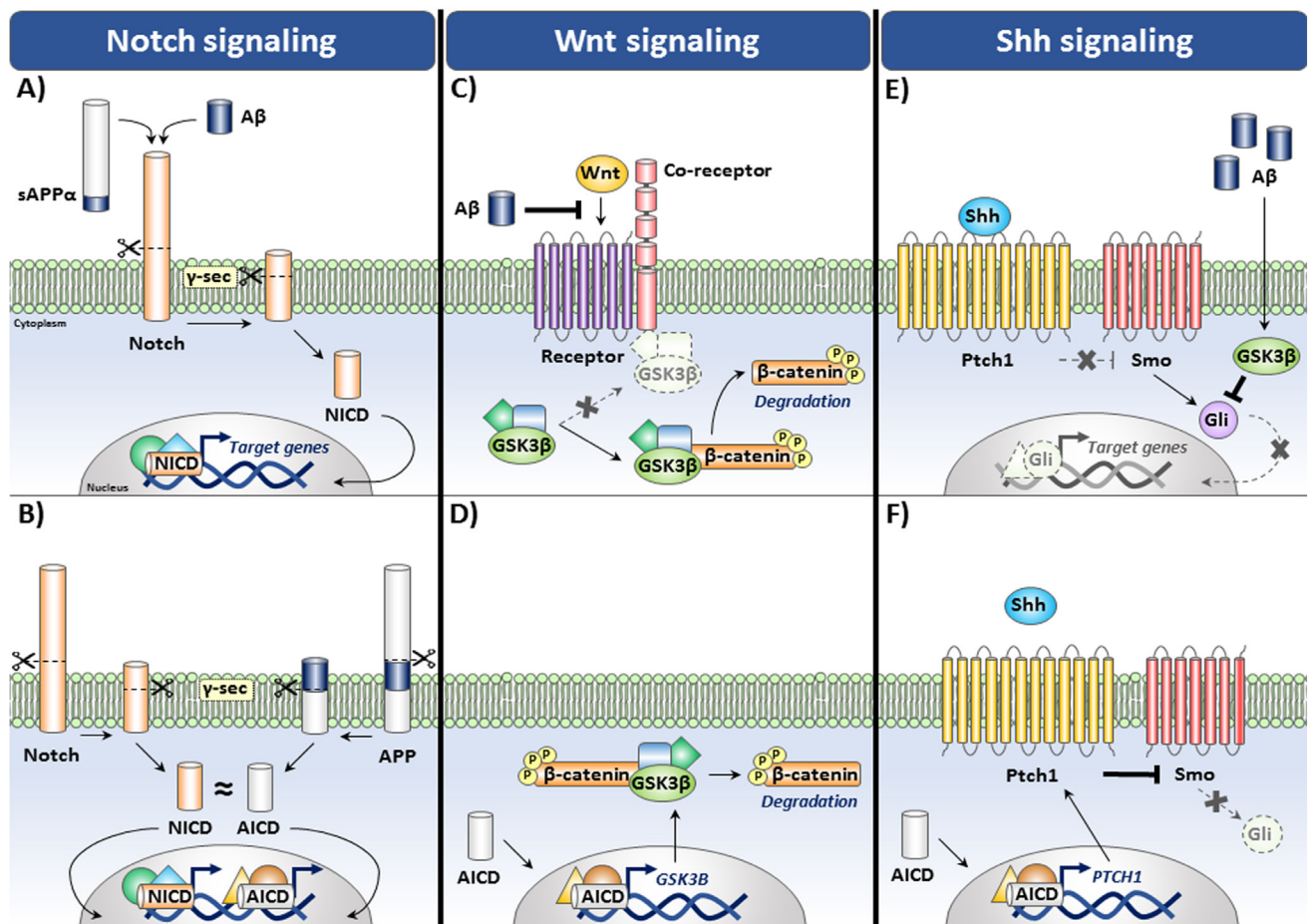
There are several Wnt signaling pathways, including the  $\beta$ -catenin dependent (also known as canonical) and  $\beta$ -catenin independent (also known as non-canonical) pathways. Both signaling pathways occur in NSCs and they are mainly differentiated by the Wnt ligands involved and the receptors that recognize these ligands on the cell surface (Bengoa-Vergniory and Kypta, 2015). Studies show that APP acts as a co-activator in both Wnt pathways, and several specific interactions of APP with Wnt co-receptor proteins (Elliott et al., 2018) and APP interactions with  $\beta$ -catenin (Zhang et al., 2018) have been described. However, it should be noted that the influence of APP in Wnt signaling is reciprocal, since this pathway is also fundamental in the processing of APP. Dysfunctions in Wnt signaling favors the amyloidogenic pathway of APP, resulting in an increase in the production and aggregation of A $\beta$  peptides (Tapia-Rojas et al., 2016). In addition, it has been seen that A $\beta_{42}$  peptide itself, in its monomeric and oligomeric forms, increases proliferation and reduces neurogenesis in mouse NPCs *via* Wnt signaling, whose pathway components are affected after exposure to A $\beta$  peptides (Shruster et al., 2011) (**Figure 2C**). As for the intracellular fragment AICD, it also seems to affect Wnt signaling, acting as a negative regulator of the canonical signaling pathway (Wnt/ $\beta$ -catenin). Specifically, the authors describe that AICD could activate *GSK3 $\beta$* , a kinase responsible for the phosphorylation and subsequent degradation of  $\beta$ -catenin in Wnt signaling, inhibiting the pathway and causing the effects observed in proliferation and neural differentiation (Zhou et al., 2012) (**Figure 2D**).

Both Notch and Wnt signaling are necessary for the maintenance of precursors and the interaction between these two pathways has been detected at several levels. It has been seen that, on one hand, Wnt stimulates the expression of Notch ligands and promotes Notch signaling in NSCs and, on the other hand, Notch activation modulates the Wnt signaling threshold (Ables et al., 2011). In addition, both pathways seem to interact through *GSK3 $\beta$*  to regulate the dynamics of NSCs.

#### Shh signaling

Shh is the main activating ligand in the brain. Shh signaling is essential for the development of the CNS and occurs simultaneously with other signaling pathways. Although Shh is





**Figure 2** Possible implications of APP proteolytic derivatives in Notch signaling, Wnt signaling and Shh signaling.

It should be noted that this scheme summarizes some hypotheses about the action exerted by different proteolytic fragments of APP in different signaling pathways, but there is still no clear consensus. (A) In Notch signaling, sAPP $\alpha$  and A $\beta$  peptide could activate the Notch receptor and initiate the signaling cascade that culminates with NICD-mediated transcriptional regulation in the nucleus. (B) The intracellular fragment AICD could act as a transcriptional regulator similar to the NICD fragment, based on their similar routes of generation. In Wnt signaling, A $\beta$  peptide could prevent the interaction of Wnt with its receptor, blocking activation of the signaling cascade. (C) Consequently, GSK3 $\beta$  is not recruited by the receptor and can hyperphosphorylate  $\beta$ -catenin, preventing its translocation to the nucleus and causing its degradation. (D) The intracellular fragment AICD could act as transcriptional regulator of *GSK3B*, favoring the hyperphosphorylation and degradation of  $\beta$ -catenin mediated by GSK3 $\beta$ . (E) In Shh signaling, a high concentration of A $\beta$  peptide could activate GSK3 $\beta$  (directly or indirectly), which causes the inhibition of Gli transcription factors. (F) The intracellular fragment AICD could act as transcriptional regulator of *PTCH1*, favoring an increase of Ptch1 receptor thus suppressing the signaling cascade in the absence of Shh ligand. APP: Amyloid precursor protein;  $\gamma$ -sec:  $\gamma$ -secretase; sAPP $\alpha$ : soluble APP- $\alpha$ ; AICD: intracellular C-terminal domain; A $\beta$ : amyloid- $\beta$ ; Shh: sonic hedgehog; NICD: Notch intracellular C-terminal domain; GSK3 $\beta$ : glycogen synthase kinase 3 $\beta$ ; Smo: smoothened; Ptch1: protein patched homolog 1.

mainly known for its morphogenic and proliferative action in NSCs/NPCs, Shh also contributes in other functions such as neuronal differentiation, axonal formation and synaptic processes (Belgacem et al., 2016). This signaling pathway starts when secreted Shh interacts with the Ptch1 receptor located in the cell membrane, relieving the inhibition it exerts on a second receptor called Smo and activating, ultimately, Gli transcription factors (Álvarez-Buylla and Ibrie, 2014).

Several studies have determined that A $\beta_{42}$  peptide regulates Shh signaling (He et al., 2014; Vorobyeva and Saunders, 2018). One study performed in mouse NSCs showed that A $\beta_{42}$  peptide, at low concentrations, favors Shh signaling by increasing the expression of several Shh signaling components. However, the same authors also determined that a high concentration of A $\beta_{42}$  peptide causes a dysfunction in Shh signaling in these cells, accompanied by a decrease in

proliferation. This could be due to the fact that, as demonstrated by the authors, A $\beta$  peptide promotes the activation of GSK3 $\beta$ , which in turn would inhibit Gli transcription factors, which would ultimately prevent the transcription of both proliferative genes and genes that code for Shh signaling components (He et al., 2014) (Figure 2E).

As for the intracellular fragment AICD, it has been described that it positively regulates the transcription of *PTCH1* which, as mentioned above, codes for the Ptch1 receptor that keeps Shh signaling repressed (Trazzi et al., 2011). Furthermore, it has been seen that inhibition of APP  $\gamma$ -secretase in NPCs is accompanied by a reduction in the levels of AICD, and therefore of *PTCH1*, restoring Shh signaling in these cells as well as the process of neurogenesis (Giacomini et al., 2015) (Figure 2F).

It should be noted that Shh exerts multiple functions on

the proliferation, differentiation and configuration of the nervous system and it is due both to a coordinated regulation of Shh signaling and to the interaction of this pathway with other signaling pathways, including Notch signaling and Wnt signaling (Belgacem et al., 2016).

### GSK3 $\beta$ as a key regulator

GSK3 $\beta$  is a serine/threonine kinase involved in a wide variety of cellular processes, including glucose metabolism, apoptosis, microtubule stability, and in the proliferation and differentiation processes of NSCs (Ahn et al., 2014). The activity of GSK3 $\beta$  is negatively regulated by several signaling pathways (phosphoinositide 3-kinase signaling or mitogen-activated protein kinase signaling) (Shi and He, 2016) but when activated, it can influence several signaling pathways (such as Wnt signaling or Shh signaling) as discussed above.

*In vitro* and *in vivo* studies show how GSK3 $\beta$  negatively regulates the neuronal differentiation of mouse NPCs (Ahn et al., 2014) and several authors associate this fact with an increase in APP expression. Studies performed in mouse NPCs with overexpression of APP show an increase in the levels of the intracellular fragment AICD, which, as previously mentioned, regulates the transcription of *GSK3B*. Therefore, the authors state that the alteration of GSK3 $\beta$  activity, in an APP-AICD-dependent manner, seems to be responsible for neurogenic impediment in these cells (Trazzi et al., 2014). Similar results were obtained by our group, where we showed that the overexpression of APP in human NSCs was accompanied by high levels of the AICD fragment and GSK3 $\beta$  expression (Coronel et al, 2018). We believe the increase in GSK3 $\beta$  expression mediated by AICD could favor glial differentiation over neurogenesis in these cells. To verify the involvement of GSK3 $\beta$  in the differentiation process of human NSCs, we used the GSK3 $\beta$  inhibitor CHIR99021. After treating cells overexpressing APP with the GSK3 $\beta$  inhibitor, we saw the opposite effects on cell differentiation, where these cells now favored neurogenesis over gliogenesis (Coronel et al, 2018). Therefore, our results could elucidate a potential molecular mechanism of APP-AICD-GSK3 $\beta$  in the context of NSCs.

Apart from the results obtained regarding to the intracellular fragment AICD, our group has also recently shown that by activation of GSK3 $\beta$ , A $\beta$ <sub>42</sub> peptide in its monomeric form favors the proliferation of human NSCs, increasing the pool of proliferating glial precursor. After the treatment of human NSCs with monomeric A $\beta$ <sub>42</sub> peptide combined with GSK3 $\beta$  inhibitor, we observed a significant decrease in gliogenesis in these cells, reversing the effects of A $\beta$ <sub>42</sub> peptide alone (Bernabeu-Zornoza et al., 2018). Thus, in summary, our work defends that the different proteolytic derivatives of APP, specifically AICD and A $\beta$ <sub>42</sub> peptide, regulate several physiological processes in human NSCs through GSK3 $\beta$ .

The effects described above seem to indicate that GSK3 $\beta$  is a key regulator in the biology of NSCs, since it appears to act as an “integrator molecule” of different molecular signals, while simultaneously influencing several signaling pathways. Consequently, a deregulation the physiological

activity of GSK3 $\beta$  could explain the common neurogenic deficit observed due to aging, as well as the neurogenic deficit observed in early stages of AD.

### Conclusion

APP has been one of the most widely studied proteins in the context of AD and other neurological conditions (such as Down syndrome or traumatic brain injury) because it produces A $\beta$  peptide, the main component of senile plaques. In fact, there are several pathogenic mutations in the human APP gene associated with the early onset of AD. However, despite the considerable progress made concerning the pathological functions of APP and its proteolytic derivatives in recent years, there is still no clear consensus on its physiological function. As we have discussed in this review, APP and its derivatives have diverse functions in NSCs/NPCs. APP is known to be processed by two different pathways, each giving rise to different proteolytic derivatives with varying functions. However, the exact mechanisms involved in favoring one processing pathway over another, and the physiological functions of the derivatives produced from these pathways, remains somewhat unknown.

Although it has been demonstrated that APP and its derivatives have varying effects on proliferation, differentiation and cell death, the molecular mechanisms and signaling pathways responsible for these effects remain unclear. Our group has recently shown that the APP derivative AICD may modulate the cell fate specification of NSCs, inhibiting neurogenesis, and this could be mediated by GSK3 $\beta$ . Similarly, we have shown that monomeric A $\beta$  peptide may regulate the proliferation and glial specification of NSCs through GSK3 $\beta$ .

GSK3 $\beta$  appears to be an important molecular integrator of several signals in the context of APP, in addition to integrating and interconnecting different signaling pathways that are important in stem cell biology. For that reason, better knowledge of this kinase in the context of APP could be key to understanding the physiological functions of APP and its derivatives. Similarly, due to the possible role of GSK3 $\beta$  as a master regulator in this context, a deregulation of its expression or activity could be associated with early stages of AD development. To understand the molecular mechanisms involved in the physiological functions of APP and its proteolytic derivatives, as well as the role played by GSK3 $\beta$  as modulator of these effects, stem cell models can be used as platforms of phenotypic testing to identify and confirm signaling pathways involved.

Moreover, the knowledge of the physiological functions of APP and its derivatives, along with signaling pathways involved, is essential to advance the understanding of the pathogenesis of AD since APP could provide potential targets in the search and development of new treatment options for AD.

**Acknowledgments:** The authors are grateful to Javier Hernández (Instituto de Salud Carlos III, Spain) for his technical assistance.

**Author contributions:** Review design and definition of intellectual content: RC; concepts and literature search: RC, IL; manuscript preparation and editing: RC, CP, ABZ, IL; manuscript review: RC, CP, ABZ, MM,



Coronel R, Palmer C, Bernabeu-Zornoza A, Monteagudo M, Rosca A, Zambrano A, Liste I (2019) Physiological effects of amyloid precursor protein and its derivatives on neural stem cell biology and signaling pathways involved. *Neural Regen Res* 14(10):1661-1671. doi:10.4103/1673-5374.257511

AR, AZ, IL.

**Conflicts of interest:** All authors disclose any conflicts of interest.

**Financial support:** This work was supported by grants from the Ministerio de Ciencia e Innovación-Instituto de Salud Carlos III (PI-10/00291 and MPY1412/09), Ministerio de Economía y Competitividad (SAF2015-71140-R) and Comunidad de Madrid (Neurostem-Comunidad de Madrid consortium; S2010/BMD-2336). RC was supported by grants from Plan de Empleo Juvenil-Ministerio de Economía y Competitividad.

**Copyright license agreement:** The Copyright License Agreement has been signed by all authors before publication.

**Plagiarism check:** Checked twice by iThenticate.

**Peer review:** Externally peer reviewed.

**Open access statement:** This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non-Commercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

**Open peer reviewers:** Larry Baum, University of Hong Kong, China; T. Bucky Jones, Midwestern University, USA.

**Additional file:** Open peer review reports 1 and 2.

## References

- Ables JL, Breunig JJ, Eisch AJ, Rakic P (2011) Not(ch) just development: Notch signalling in the adult brain. *Nat Rev Neurosci* 12:269-283.
- Agostinho P, Pliássova A, Oliveira CR, Cunha RA (2015) Localization and trafficking of amyloid- $\beta$  protein precursor and secretases: impact on Alzheimer's disease. *J Alzheimers Dis* 45:329-347.
- Ahn J, Jang J, Choi J, Lee J, Oh SH, Lee J, Yoon K, Kim S (2014) GSK3 $\beta$ , but not GSK3 $\alpha$ , inhibits the neuronal differentiation of neural progenitor cells as a downstream target of mammalian target of rapamycin complex1. *Stem Cells Dev* 23:1121-1133.
- Álvarez-Buylla A, Ihrie RA (2014) Sonic hedgehog signaling in the postnatal brain. *Semin Cell Dev Biol* 33:105-111.
- Baratchi S, Evans J, Tate WP, Abraham WC, Connor B (2012) Secreted amyloid precursor proteins promote proliferation and glial differentiation of adult hippocampal neural progenitor cells. *Hippocampus* 22:1517-1527.
- Barlowe CK, Miller EA (2013) Secretory protein biogenesis and traffic in the early secretory pathway. *Genetics* 193:383-410.
- Beckett C, Nalivaeva NN, Belyaev ND, Turner AJ (2012) Nuclear signalling by membrane protein intracellular domains: The AICD enigma. *Cell Signal* 24:402-409.
- Belgacem YH, Hamilton AM, Shim S, Spencer KA, Borodinsky LN (2016) The many hats of Sonic hedgehog signaling in nervous system development and disease. *J Dev Biol* 4:E35.
- Bengoa-Vergniory N, Kypita RM (2015) Canonical and noncanonical Wnt signaling in neural stem/progenitor cells. *Cell Mol Life Sci* 72:4157-4172.
- Bergström P, Agholme L, Nazir FH, Satir TM, Toombs J, Wellington H, Strandberg J, Bontell TO, Kvartsberg H, Holmström M, Boreström C, Simonsson S, Kunath T, Lindahl A, Blennow K, Hanse E, Portelius E, Wray S, Zetterberg H (2016) Amyloid precursor protein expression and processing are differentially regulated during cortical neuron differentiation. *Sci Rep* 6:29200.
- Bernabeu-Zornoza A, Coronel R, Palmer C, Calero M, Martínez-Serrano A, Cano E, Zambrano A, Liste I (2018) A $\beta$ 42 peptide promotes proliferation and gliogenesis in human neural stem cells. *Mol Neurobiol* doi: 10.1007/s12035-018-1355-7.
- Bhattacharyya R, Barren C, Kovacs DM (2013) Palmitoylation of amyloid precursor protein regulates amyloidogenic processing in lipid rafts. *J Neurosci* 33:11169-11183.
- Bolós M, Hu Y, Young KM, Foa L, Small DH (2014) Neurogenin 2 mediates amyloid- $\beta$  precursor protein-stimulated neurogenesis. *J Biol Chem* 289:31253-31261.
- Bukhari H, Glotzbach A, Kolbe K, Leonhardt G, Loosse C, Müller T (2017) Small things matter: Implications of APP intracellular domain AICD nuclear signaling in the progression and pathogenesis of Alzheimer's disease. *Prog Neurobiol* 156:189-213.
- Bukhari H, Kolbe K, Leonhardt G, Loosse C, Schröder E, Knauer S, Marcus K, Müller T (2016) Membrane tethering of APP c-terminal fragments is a prerequisite for T668 phosphorylation preventing nuclear sphere generation. *Cell Signal* 28:1725-1734.
- Buoso E, Lanni C, Schettini G, Govoni S, Racchi M (2010) Beta-amyloid precursor protein metabolism: focus on the functions and degradation of its intracellular domain. *Pharmacol Res* 62:308-317.
- Caillé I, Allinquant B, Dupont E, Bouillot C, Langer A, Müller U, Prochiantz A (2004) Soluble form of amyloid precursor protein regulates proliferation of progenitors in the adult subventricular zone. *Development* 131:2173-2181.
- Chen GF, Xu TH, Yan Y, Zhou YR, Jiang Y, Melcher K, Xu HE (2017) Amyloid beta: structure, biology and structure-based therapeutic development. *Acta Pharmacol Sin* 38:1205-1235.
- Chen J, Wang M, Turko IV (2013) Quantification of amyloid precursor protein isoforms using quantification concatamer internal standard. *Anal Chem* 85:303-307.
- Chen KP, Dou F (2012) Selective interaction of amyloid precursor protein with different isoforms of neural cell adhesion molecule. *J Mol Neurosci* 46:203-209.
- Chen Y, Dong C (2009) Abeta40 promotes neuronal cell fate in neural progenitor cells. *Cell Death Differ* 16:386-394.
- Coronel R, Lachgar M, Bernabeu-Zornoza A, Palmer C, Domínguez-Alvaro M, Revilla A, Ocaña I, Fernández A, Martínez-Serrano A, Cano E, Liste I (2018) Neuronal and glial differentiation of human neural stem cells is regulated by amyloid precursor protein (APP) levels. *Mol Neurobiol* doi: 10.1007/s12035-018-1167-9.
- Cousins SL, Dai W, Stephenson FA (2015) APLP1 and APLP2, members of the APP family of proteins, behave similarly to APP in that they associate with NMDA receptors and enhance NMDA receptor surface expression. *J Neurochem* 133:879-885.
- Dawkins E, Small DH (2014) Insights into the physiological function of the  $\beta$ -amyloid precursor protein: beyond Alzheimer's disease. *J Neurochem* 129:756-769.
- Demars MP, Bartholomew A, Strakova Z, Lazarov O (2011) Soluble amyloid precursor protein: A novel proliferation factor of adult progenitor cells of ectodermal and mesodermal origin. *Stem Cell Res Ther* 2:36.
- Demars MP, Hollands C, Zhao Kda T, Lazarov O (2013) Soluble amyloid precursor protein- $\alpha$  rescues age-linked decline in neural progenitor cell proliferation. *Neurobiol Aging* 34:2431-2440.
- Deyts C, Thinakaran G, Parent AT (2016) APP receptor? To be or not to be. *Trends Pharmacol Sci* 37:390-411.
- Dimitrov M, Alattia JR, Lemmin T, Lehal R, Fligier A, Houacine J, Hussain I, Radtke F, Dal Peraro M, Behr D, Fraering PC (2013) Alzheimer's disease mutations in APP but not  $\gamma$ -secretase modulators affect epsilon-cleavage-dependent AICD production. *Nat Commun* 4:2246.
- Elliott C, Rojo AI, Ribe E, Broadstock M, Xia W, Morin P, Semenov M, Baillie G, Cuadrado A, Al-Shawi R, Ballard CG, Simons P, Killick R (2018) A role for APP in Wnt signaling links synapse loss with  $\beta$ -amyloid production. *Transl Psychiatry* 8:179.
- Fonseca MB, Solá S, Xavier JM, Dionísio PA, Rodrigues CM (2013) Amyloid  $\beta$  peptides promote autophagy-dependent differentiation of mouse neural stem cells: A $\beta$ -mediated neural differentiation. *Mol Neurobiol* 48:829-840.
- Freude KK, Penjwini M, Davis JL, LaFerla FM, Blurton-Jones M (2011) Soluble amyloid precursor protein induces rapid neural differentiation of human embryonic stem cells. *J Biol Chem* 286:24264-24274.
- Giacomini A, Stagni F, Trazzi S, Guidi S, Emili M, Brigham E, Ciani E, Bartesaghi R (2015) Inhibition of APP gamma-secretase restores Sonic Hedgehog signaling and neurogenesis in the Ts65Dn mouse model of Down syndrome. *Neurobiol Dis* 82:385-396.
- Gouras GK, Olsson TT, Hansson O (2015)  $\beta$ -Amyloid peptides and amyloid plaques in Alzheimer's disease. *Neurotherapeutics* 12:3-11.
- Grimm MO, Mett J, Stahlmann CP, Grösgen S, Hauptenthal VJ, Blümel T, Hundsdörfer B, Zimmer VC, Mylonas NT, Tanila H, Müller U, Grimm HS, Hartmann T (2015) APP intracellular domain derived from amyloidogenic  $\beta$ - and  $\gamma$ -secretase cleavage regulates neprilysin expression. *Front Aging Neurosci* 7:77.
- Grimm MO, Mett J, Stahlmann CP, Hauptenthal VJ, Zimmer VC, Hartmann T (2013) Neprilysin and A $\beta$  clearance: impact of the APP intracellular domain in NEP regulation and implications in Alzheimer's disease. *Front Aging Neurosci* 5:98.

Coronel R, Palmer C, Bernabeu-Zornoza A, Monteagudo M, Rosca A, Zambrano A, Liste I (2019) Physiological effects of amyloid precursor protein and its derivatives on neural stem cell biology and signaling pathways involved. *Neural Regen Res* 14(10):1661-1671. doi:10.4103/1673-5374.257511

- Gu Y, Misonou H, Sato T, Dohmae N, Takio K, Ihara Y (2001) Distinct intramembrane cleavage of the beta-amyloid precursor protein family resembling gamma-secretase-like cleavage of Notch. *J Biol Chem* 276:35235-35238.
- Haass C, Kaether C, Thinakaran G, Sisodia S (2012) Trafficking and proteolytic processing of APP. *Cold Spring Harb Perspect Med* 2:a006270.
- He P, Staufenbiel M, Li R, Shen Y (2014) Deficiency of patched 1-induced Gli1 signal transduction results in astrogenesis in Swedish mutated APP transgenic mice. *Hum Mol Genet* 23:6512-6527.
- Hefter D, Draguhn A (2017) APP as a protective factor in acute neuronal insults. *Front Mol Neurosci* 10:22.
- Herold S, Kumar P, Wichert SP, Kretzschmar B, Bähr M, Rossner MJ, Hein K (2015) Neurodegeneration in autoimmune optic neuritis is associated with altered APP cleavage in neurons and up-regulation of p53. *PLoS One* 10:e0138852.
- Hesse R, von Einem B, Wagner F, Bott P, Schwanzar D, Jackson RJ, Föhr KJ, Lausser L, Kroker KS, Proepper C, Walther P, Kestler HA, Spires-Jones TL, Boeckers T, Rosenbrock H, von Arnim CAF (2018) sAPP $\beta$  and sAPP $\alpha$  increase structural complexity and E/I input ratio in primary hippocampal neurons and alter Ca<sup>2+</sup> homeostasis and CREB1-signaling. *Exp Neurol* 304:1-13.
- Hick M, Herrmann U, Weyer SW, Mallm JP, Tschäpe JA, Borgers M, Mercken M, Roth FC, Draguhn A, Slomianka L, Wolfner DP, Korte M, Müller UC (2015) Acute function of secreted amyloid precursor protein fragment APP $\alpha$  in synapsis plasticity. *Acta Neuropathol* 129:21-37.
- Itokazu Y, Yu RK (2014) Amyloid  $\beta$ -peptide 1-42 modulates the proliferation of mouse neural stem cells: Upregulation of fucosyltransferase IX and notch signaling. *Mol Neurobiol* 50:186-196.
- Jefferson T, Čaušević M, auf dem Keller U, Schilling O, Isbert S, Geyer R, Maier W, Tschickardt S, Jumpertz T, Weggen S, Bond JS, Overall CM, Pietrzik CU, Becker-Pauly C (2011) Metalloprotease meprin beta generates nontoxic N-terminal amyloid precursor protein fragments in vivo. *J Biol Chem* 286:27741-27750.
- Johnson VE, Stewart W, Smith DH (2010) Traumatic brain injury and amyloid- $\beta$  pathology: a link to Alzheimer's disease? *Nat Rev Neurosci* 11:361-370.
- Jonsson T, Atwal JK, Steinberg S, Snaedal J, Jonsson PV, Bjornsson S, Stefansson H, Sulem P, Gudbjartsson D, Maloney J, Hoyte K, Gustafson A, Liu Y, Lu Y, Bhangale T, Graham RR, Huttenlocher J, Bjornsdottir G, Andreassen OA, Jönsson EG, et al. (2012) A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature* 488:96-99.
- Karch CM, Goate AM (2015) Alzheimer's disease risk genes and mechanisms of disease pathogenesis. *Biol Psychiatry* 77:43-51.
- Kim HS, Kim EM, Lee JP, Park CH, Kim S, Seo JH, Chang KA, Yu E, Jeong SJ, Chong YH, Suh YH (2003) C-terminal fragments of amyloid precursor protein exert neurotoxicity by inducing glycogen synthase kinase-3 $\beta$  expression. *FASEB J* 17:1951-1953.
- Kim J, Onstead L, Randle S, Prince R, Smithson L, Zwizinski C, Dickson DW, Golde T, McGowan E (2007) Abeta40 inhibits amyloid deposition in vivo. *J Neurosci* 27:627-633.
- Kirazov E, Kirazov L, Bigl V, Schliebs R (2001) Oncogenetic changes in protein level of amyloid precursor protein (APP) in growth cones and synaptosomes from rat brain and prenatal expression pattern of APP mRNA isoforms in developing rat embryo. *Int J Dev Neurosci* 19:287-296.
- Konietzko U, Goodger ZV, Meyer M, Kohli BM, Bosset J, Lahiri DK, Nitsch RM (2010) Co-localization of the amyloid precursor protein and Notch intracellular domains in nuclear transcription factories. *Neurobiol Aging* 31:58-73.
- Kwak YD, Brannen CL, Qu T, Kim HM, Dong X, Soba P, Majumdar A, Kaplan A, Beyreuther K, Sugaya K (2006) Amyloid precursor protein regulates differentiation of human neural stem cells. *Stem Cells Dev* 15:381-389.
- Kwak YD, Marutle A, Dantuma E, Merchant S, Bushnev S, Sugaya K (2011) Involvement of notch signaling pathway in amyloid precursor protein induced glial differentiation. *Eur J Pharmacol* 650:18-27.
- Lee IS, Jung K, Kim IS, Park KI (2013) Amyloid- $\beta$  oligomers regulate the properties of human neural stem cells through GSK-3 $\beta$  signaling. *Exp Mol Med* 45:e60.
- Lee NC, Chien YH, Hwu WL (2017) A review of biomarkers for Alzheimer's disease in Down syndrome. *Neurol Ther* 6:S69-81.
- Leyssen M, Ayaz D, Hébert SS, Reeve S, De Strooper B, Hassan BA (2005) Amyloid precursor protein promotes post-developmental neurite arborization in the Drosophila brain. *EMBO J* 24:2944-2955.
- Liao MC, Muratore CR, Gierahn TM, Sullivan SE, Srikanth P, De Jager PL, Love JC, Young-Pearse TL (2016) Single-cell detection of secreted A $\beta$  and sAPP $\alpha$  from human iPSCs-derived neurons and astrocytes. *J Neurosci* 36:1730-1746.
- Lu DC, Soriano S, Bredesen DE, Koo EH (2003) Caspase cleavage of the amyloid precursor protein modulates amyloid beta-protein toxicity. *J Neurochem* 87:733-741.
- Marik SA, Olsen O, Tessier-Lavigne M, Gilbert CD (2016) Physiological role for amyloid precursor protein in adult experience-dependent plasticity. *Proc Natl Acad Sci U S A* 113:7912-7917.
- Martínez-Morales PL, Revilla A, Ocaña I, González C, Sainz P, McGuire D, Liste I (2013) Progress in stem cell therapy for major human neurological disorders. *Stem Cell Rev* 9:685-699.
- Mockett BG, Richter M, Abraham WC, Müller UC (2017) Therapeutic potential of secreted amyloid precursor protein APP $\alpha$ . *Front Mol Neurosci* 10:30.
- Morel E, Chamoun Z, Lasiecka ZM, Chan RB, Williamson RL, Vatanovetz C, Dall'Armi C, Simoes S, Point Du Jour KS, McCabe BD, Small SA, Di Paolo G (2013) Phosphatidylinositol-3-phosphate regulates sorting and processing of amyloid precursor protein through the endosomal system. *Nat Commun* 4:2250.
- Mroczo B, Groblewska M, Litman-Zawadzka A, Kornhuber J, Lewczuk P (2018) Amyloid  $\beta$  oligomers (A $\beta$ Os) in Alzheimer's disease. *J Neural Transm (Vienna)* 125:177-191.
- Müller T, Schrötter A, Loosse C, Pfeiffer K, Theiss C, Kauth M, Meyer HE, Marcus K (2013) A ternary complex consisting of AICD, FE65, and TIP60 down-regulates Stathmin1. *Biochim Biophys Acta* 1834:387-394.
- Müller UC, Deller T, Korte M (2017) Not just amyloid: physiological functions of the amyloid precursor protein family. *Nat Rev Neurosci* 18:281-298.
- Nagase H, Nakayama K (2014) The intracellular domain of amyloid precursor protein is a potential therapeutic target in Alzheimer's disease. *Curr Drug Discov Technol* 11:243-258.
- Nalivaeva NN, Turner AJ (2013) The amyloid precursor protein: A biochemical enigma in brain development, function and disease. *FEBS Lett* 587:2046-2054.
- Nguyen KV (2015) The human  $\beta$ -amyloid precursor protein: biomolecular and epigenetic aspects. *Biomol Concepts* 6:11-32.
- Nicolas M, Hassan BA (2014) Amyloid precursor protein and neural development. *Development* 141:2543-2548.
- Ochalek A, Mihalik B, Avci HX, Chandrasekaran A, Téglási A, Block I, Giudice ML, Tancos Z, Molnár K, László L, Nielsen JE, Holst B, Freude K, Hyttel P, Kobolák J, Dinnyés A (2017) Neurons derived from sporadic Alzheimer's disease iPSCs reveal elevated TAU hyperphosphorylation, increased amyloid levels, and GSK3B activation. *Alzheimers Res Ther* 9:90.
- Oh SY, Ellenstein A, Chen CD, Hinman JD, Berg EA, Costello CE, Yamin R, Neve RL, Abraham CR (2005) Amyloid precursor protein interacts with notch receptors. *J Neurosci Res* 82:32-42.
- Papadimitriou C, Celikkaya H, Cosacak MI, Mashkaryan V, Bray L, Bhattarai P, Brandt K, Hollak H, Chen X, He S, Antos CL, Lin W, Thomas AK, Dahl A, Kurth T, Friedrichs J, Zhang Y, Freudenberg U, Werner C, Kizil C (2018) 3D culture method for Alzheimer's disease modeling reveals interleukin-4 rescues A $\beta$ 42-induced loss of human neural stem cell plasticity. *Dev Cell* 46:85-101.
- Pinnix I, Ghiso JA, Pappolla MA, Sambamurti K (2013) Major carboxyl terminal fragments generated by  $\gamma$ -secretase processing of the Alzheimer amyloid precursor are 50 and 51 amino acids long. *Am J Geriatr Psychiatry* 21:474-483.

- Coronel R, Palmer C, Bernabeu-Zornoza A, Monteagudo M, Rosca A, Zambrano A, Liste I (2019) Physiological effects of amyloid precursor protein and its derivatives on neural stem cell biology and signaling pathways involved. *Neural Regen Res* 14(10):1661-1671. doi:10.4103/1673-5374.257511
- Plummer S, Van den Heuvel C, Thornton E, Corrigan F, Cappai R (2016) The neuroprotective properties of the amyloid precursor protein following traumatic brain injury. *Aging Dis* 7:163-179.
- Puig KL, Combs CK (2013) Expression and function of APP and its metabolites outside the central nervous system. *Exp Gerontol* 48:608-611.
- Ribeiro MF, Genebra T, Rego AC, Rodrigues CMP, Solá S (2018) Amyloid  $\beta$  peptide compromises neural stem cell fate by irreversibly disturbing mitochondrial oxidative state and blocking mitochondrial biogenesis and dynamics. *Mol Neurobiol* doi: 10.1007/s12035-018-1342-z.
- Richter MC, Ludewig S, Winschel A, Abel T, Bold C, Salzburger LR, Klein S, Han K, Weyer SW, Fritz AK, Laube B, Wolfer DP, Buchholz CJ, Korte M, Müller UC (2018) Distinct in vivo roles of secreted APP ectodomain variants APPs $\alpha$  and APPs $\beta$  in regulation of spine density, synaptic plasticity, and cognition. *EMBO J* 37:e98335.
- Sarlak G, Htoo HH, Hernandez JF, Iizasa H, Checler F, Konietzko U, Song W, Vincent B (2016) Sox2 functionally interacts with  $\beta$ APP, the  $\beta$ APP intracellular domain and ADAM10 at a transcriptional level in human cells. *Neuroscience* 312:153-164.
- Sato Y, Uchida Y, Hu J, Young-Pearse TL, Niikura T, Mukoyama YS (2017) Soluble APP functions as a vascular niche signal that controls adult neural stem cell number. *Development* 144:2730-2736.
- Selkoe DJ, Hardy J (2016) The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med* 8:595-608.
- Seto Y, Eiraku M (2019) Human brain development and its in vitro recapitulation. *Neurosci Res* 138:33-42.
- Shariati SA, De Strooper B (2013) Redundancy and divergence in the amyloid precursor protein family. *FEBS Lett* 587:2036-2045.
- Shi Y, He MX (2016) PflRR interacts with HrIGF-I and activates the MAP-kinase and PI3-kinase signaling pathways to regulate glyco-gen metabolism in *Pinctada fucata*. *Sci Rep* 6:22063.
- Shruster A, Eldar-Finkelman H, Melamed E, Offen D (2011) Wnt signaling pathway overcomes the disruption of neuronal differentiation of neural progenitor cells induced by oligomeric amyloid  $\beta$ -peptide. *J Neurochem* 116:522-529.
- Shu R, Wong W, Ma QH, Yang ZZ, Zhu H, Liu FJ, Wang P, Ma J, Yan S, Polo JM, Bernard CC, Stanton LW, Dawe GS, Xiao ZC (2015) APP intracellular domain acts as a transcriptional regulator of miR-663 suppressing neuronal differentiation. *Cell Death Dis* 6:e1651.
- Small DH, Hu Y, Bolós M, Dawkins E, Foa L, Young KM (2014)  $\beta$ -Amyloid precursor protein: function in stem cell development and Alzheimer's disease brain. *Neurodegener Dis* 13:96-98.
- Soldano A, Hassan BA (2014) Beyond pathology: APP, brain development and Alzheimer's disease. *Curr Opin Neurobiol* 27:61-67.
- Sosa LJ, Bergman J, Estrada-Bernal A, Glorioso TJ, Kittelson JM, Pfenninger KH (2013) Amyloid precursor protein is an autonomous growth cone adhesion molecule engaged in contact guidance. *PLoS One* 8:e64521.
- Sosa LJ, Postma NL, Estrada-Bernal A, Hanna M, Guo R, Busciglio J, Pfenninger KH (2014) Dosage of amyloid precursor protein affects axonal contact guidance in Down syndrome. *FASEB J* 28:195-205.
- Tapia-Rojas C, Burgos PV, Inestrosa NC (2016) Inhibition of Wnt signaling induces amyloidogenic processing of amyloid precursor protein and the production and aggregation of Amyloid- $\beta$  (A $\beta$ )42 peptides. *J Neurochem* 139:1175-1191.
- Tharp WG, Sarkar IN (2013) Origins of amyloid- $\beta$ . *BMC Genomics* 14:290.
- Tijms BM, Vermunt L, Zwan MD, van Harten AC, van der Flier WM, Teunissen CE, Scheltens P, Visser PJ (2018) Pre-amyloid stage of Alzheimer's disease in cognitively normal individuals. *Ann Clin Transl Neurol* 5:1037-1047.
- Trazzi S, Fuchs C, De Franceschi M, Mitrugno VM, Bartesaghi R, Ciani E (2014) APP-dependent alteration of GSK3 $\beta$  activity impairs neurogenesis in the Ts65Dn mouse model of Down syndrome. *Neurobiol Dis* 67:24-36.
- Trazzi S, Mitrugno VM, Valli E, Fuchs C, Rizzi S, Guidi S, Perini G, Bartesaghi R, Ciani E (2011) APP-dependent up-regulation of Pth1 underlies proliferation impairment of neural precursor in Down syndrome. *Hum Mol Genet* 20:1560-1573.
- Tyan SH, Shih AY, Walsh JJ, Maruyama H, Sarsoza F, Ku L, Eggert S, Hof PR, Koo EH, Dickstein DL (2012) Amyloid precursor protein (APP) regulates synaptic structure and function. *Mol Cell Neurosci* 51:43-52.
- Van Dam D, De Deyn PP (2006) Drug discovery in dementia: The role of rodent models. *Nat Rev Drug Discov* 5:956-970.
- van der Kant R, Goldstein LS (2015) Cellular functions of the amyloid precursor protein from development to dementia. *Dev Cell* 32:502-515.
- Venkataramani V, Thiele K, Behnes CL, Wulf GG, Thelen P, Opitz L, Salinas-Riester G, Wirths O, Bayer TA, Schweyer S (2012) Amyloid precursor protein is a biomarker for transformed human pluripotent stem cells. *Am J Pathol* 180:1636-1652.
- Vorobyeva AG, Saunders AJ (2018) Amyloid- $\beta$  interrupts canonical Sonic hedgehog signaling by distorting primary cilia structure. *Cilia* 7:5.
- Wang B, Wang Z, Sun L, Yang L, Li H, Cole AL, Rodriguez-Rivera J, Lu HC, Zheng H (2014) The amyloid precursor protein controls adult hippocampal neurogenesis through GABAergic interneurons. *J Neurosci* 34:13314-13325.
- Wang MJ, Yi S, Han JY, Park SY, Jang JW, Chun IK, Kim SE, Lee BS, Kim GJ, Yu JS, Lim K, Kang SM, Park YH, Youn YC, An SSA, Kim S (2017b) Oligomeric forms of amyloid- $\beta$  protein in plasma as a potential blood-based biomarker for Alzheimer's disease. *Alzheimers Res Ther* 9:98.
- Wang S, Bolós M, Clark R, Cullen CL, Southam KA, Foa L, Dickson TC, Young KM (2016) Amyloid  $\beta$  precursor protein regulates neuron survival and maturation in the adult mouse brain. *Mol Cell Neurosci* 77:21-33.
- Wang X, Zhou X, Li G, Zhang Y, Wu Y, Song W (2017a) Modifications and trafficking of APP in the pathogenesis of Alzheimer's disease. *Front Mol Neurosci* 10:294.
- Watanabe T, Hikichi Y, Willuweit A, Shintani Y, Horiguchi T (2012) FBL2 regulates amyloid precursor protein (APP) metabolism by promoting ubiquitination-dependent APP degradation and inhibition of APP endocytosis. *J Neurosci* 32:3352-3365.
- Willem M, Tahirovic S, Busche MA, Ovsepian SV, Chafai M, Kootar S, Hornburg D, Evans LD, Moore S, Daria A, Hampel H, Müller V, Giudici C, Nuscher B, Wenninger-Weinzierl A, Kremmer E, Heneka MT, Thal DR, Giedraitis V, Lannfelt L, et al. (2015)  $\eta$ -Secretase processing of APP inhibits neuronal activity in the hippocampus. *Nature* 526:443-447.
- Williamson RL, Laulagnier K, Miranda AM, Fernandez MA, Wolfe MS, Sadoul R, Di Paolo G (2017) Disruption of amyloid precursor protein ubiquitination selectively increases amyloid  $\beta$  (A $\beta$ ) 40 levels via presenilin 2-mediated cleavage. *J Biol Chem* 292:19873-19889.
- Wirth M, Villeneuve S, Haese CM, Madison CM, Oh H, Landau SM, Rabinovici GD, Jagust WJ (2013) Associations between Alzheimer disease biomarkers, neurodegeneration, and cognition in cognitively normal older people. *JAMA Neurol* 70:1512-1519.
- Zhang N, Parr CJC, Birch AM, Goldfiner MH, Sastre M (2018) The amyloid precursor protein binds to  $\beta$ -catenin and modulates its cellular distribution. *Neurosci Lett* 685:190-195.
- Zhang YW, Wang R, Liu Q, Zhang H, Liao FF, Xu H (2007) Presenilin/gamma-secretase-dependent processing of beta-amyloid precursor protein regulates EGF receptor expression. *Proc Natl Acad Sci U S A* 104:10613-10618.
- Zhang X, Song M, Liu X, Su Kang S, Duong DM, Seyfried NT, Cao X, Cheng L, Sun YE, Ping Yu S, Jia J, Levey AI, Ye K (2015) Delta-secretase cleaves amyloid precursor protein and regulates the pathogenesis in Alzheimer's disease. *Nat Commun* 6:8762.
- Zhou F, Gong K, Song B, Ma T, van Laar T, Gong Y, Zhang L (2012) The APP intracellular domain (AICD) inhibits Wnt signalling and promotes neurite outgrowth. *Biochim Biophys Acta* 1823:1233-1241.

P-Reviewers: Baum L, Jones TB; C-Editors: Zhao M, Yu J; T-Editor: Liu XL