REVIEW

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

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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-β 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-α, soluble APP-β, amyloid-β 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*

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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-β (Aβ) peptide. Aβ 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 Aβ 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 characteristics 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 Aβ peptide, but also the so-called non-amyloid derivatives of APP, including soluble APP-α (sAPPα), soluble APP-β (sAPPβ) 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 (Leyssen et al., 2005) and in the proliferation of neural progenitor cells (NPCs) (Wang et al., 2016).

Despite the existing studies, a more detailed understand-

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 Aβ 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 Aβ 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 Aβ peptide. In this pathway, α-secretase cleaves APP within the Aβ domain, preventing its formation and aggregation. This process produces the sAPPα fragment and releases it to the extracellular medium, leaving the C-terminal fragment α (also known as C83) in the cell membrane. Next, the γ-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 Aβ peptide. In this pathway, β-secretase cleaves APP outside the Aβ domain and, therefore, its formation is not prevented. β-secretase produces the sAPPβ fragment and releases it to the extracellular medium, while the remaining C-terminal fragment β (also known as C99) stays in the cell membrane. Next, the γ-secretase enzymatic complex cleaves the C99 fragment, releasing Aβ 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 β-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 η-secretase pathway (Willem et al., 2015), the δ-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)

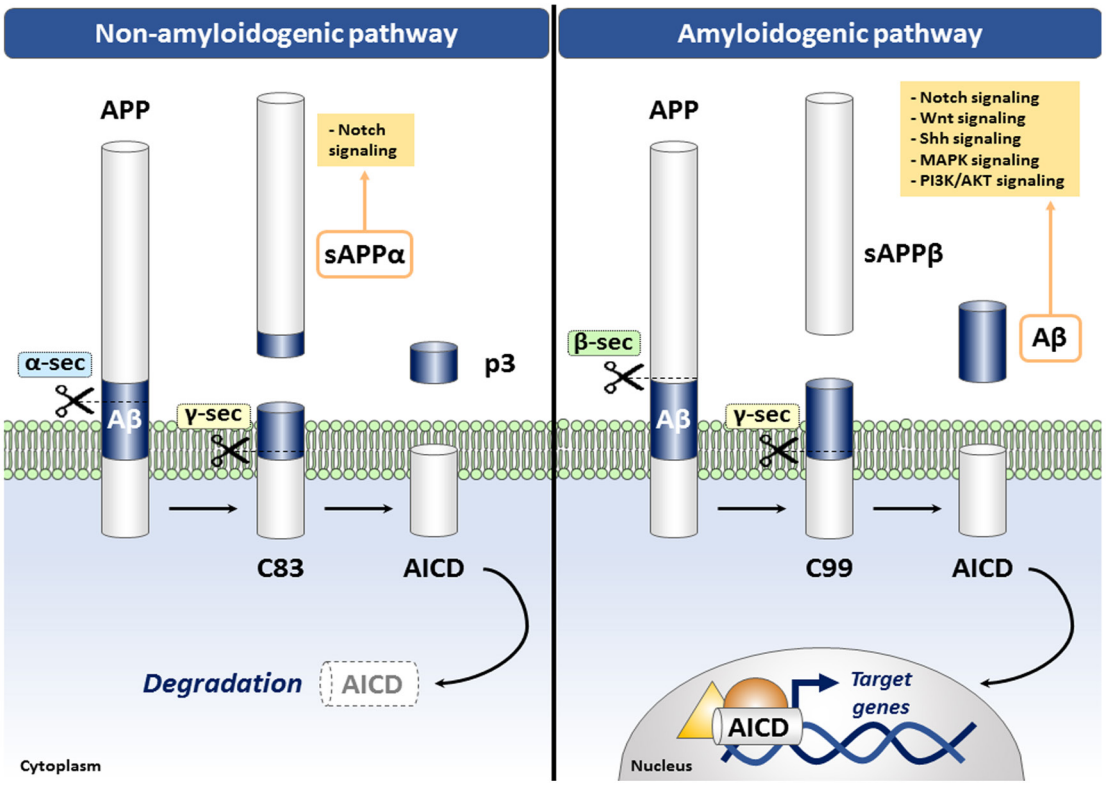

Figure 1 Proteolytic processing of APP by non-amyloidogenic pathway and amyloidogenic pathway.
In the non-amyloidogenic pathway, APP is sequentially cleaved by α-sec and γ-sec generating sAPPα, p3 and AICD fragments. In the amyloidogenic pathway, APP is sequentially cleaved by β-sec and γ-sec generating sAPPβ, Aβ and AICD fragments. In both cases, the APP processing releases functionally active fragments to the extracellular medium (sAPPα, p3, sAPPβ and Aβ 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; α-sec: α-secretase; β-sec: β-secretase; γ-sec: γ-secretase; sAPPα: soluble APP-α; sAPPβ: soluble APP-β; AICD: intracellular C-terminal domain; Aβ: amyloid-β; 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 sAPPα and sAPPβ

sAPPα 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 sAPPα fragment with neuroprotective and neurotrophic functions (Hick et al., 2015; Plummer et al., 2016; Hefter and Draguhn, 2017). sAPPβ is another fragments released into the extracellular medium after the proteolytic processing of APP, but in this case, by the amyloidogenic pathway, which later releases Aβ peptide. Regarding this processing pathway, most studies have focused on discovering the functions of Aβ peptide, but the functions of sAPPβ are still poorly understood (Hesse et al., 2018).

The specific roles of sAPPα and sAPPβ remain unclear. Many studies have demonstrated a role of sAPPα 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, sAPPβ 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 sAPPα (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 sAPPα and sAPPβ 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 sAPPα 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 sAPPα 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 sAPPβ 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 sAPPα levels or an alteration of the sAPPα/sAPPβ 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 Aβ peptide

Aβ peptide is released into the extracellular medium after γ-secretase cleavage in the amyloidogenic pathway and can be found in different aggregation states. Monomeric Aβ 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 Aβ peptides and all of them have been shown to produce diverse effects in the brain (Chen et al., 2017). Recent evidences support that Aβ 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 Aβ peptide in its monomeric form.

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

Apart from the pathological function already described, monomeric $Aβ_{40}$ and $Aβ_{42}$ 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 $Aβ_{42}$ peptide (at a concentration of 1 μM) 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 $Aβ_{42}$ peptide (at a concentration of 1.5 μM) favors the proliferation and gliogenesis of these cells, in contrast to monomeric $Aβ_{40}$ 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 μM) of $Aβ_{42}$ 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 μM, $Aβ_{42}$ peptide compromised proliferation and blocked neuronal differentiation of mouse NSCs (Ribeiro et al., 2018). A possible explanation could be that, at high concentrations (10 μM), monomeric Aβ peptides aggregate to form oligomers, which would be similar to the effects observed in studies done in human NSCs with $Aβ_{42}$ 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 Aβ 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 γ-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 Aβ 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 $Aβ_{40/42}$ fragments resulting from γ-secretase cleavage, but the isoforms mainly detected are the $AICD_{50/51}$ fragments, which result from γ-secretase cleavage at the ε-site (Dimitrov et al., 2013; Pinnix et al., 2013). Cleavage at the ε-site is similar to the cleavage caused by γ-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 3β; 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* (neprilysin; metalloprotease that degrades Aβ 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 (Sarlak et al., 2016).

Unlike sAPPα and sAPPβ, 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* (Sarlak 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 γ-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 GSK3β 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 sAPPα activates Notch signaling and causes glial differentiation of human NPCs through the formation of the proteolytic fragment NICD (Kwak et al., 2011).

Curiously, although sAPPα 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 signaling, 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 β-catenin dependent (also known as canonical) and β-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 β-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 Aβ 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 Aβ 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/β-catenin). Specifically, the authors describe that AICD could activate GSK3β, a kinase responsible for the phosphorylation and subsequent degradation of β-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 GSK3β 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

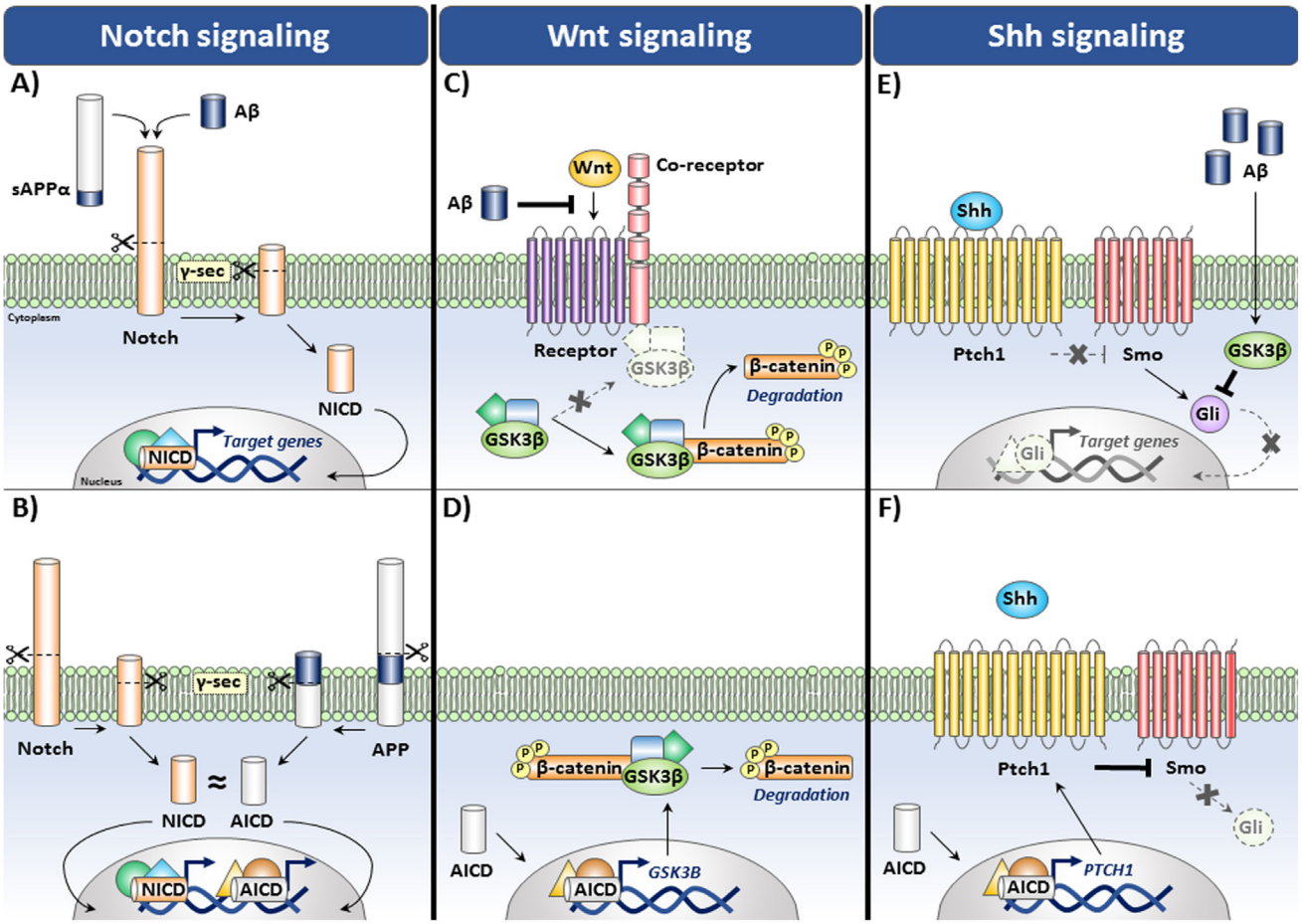

Figure 2 Possible implications of APP proteolytic derivatives in Notch signaling, Wnt signaling and Shh signaling.
It should be noted that this scheme summarizes some hypotheses about the action exerted by different proteolytic fragments of APP in different signaling pathways, but there is still no clear consensus. (A) In Notch signaling, sAPPα and Aβ 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, Aβ peptide could prevent the interaction of Wnt with its receptor, blocking activation of the signaling cascade. (C) Consequently, GSK3β is not recruited by the receptor and can hyperphosphorylate β-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 β-catenin mediated by GSK3β. (E) In Shh signaling, a high concentration of Aβ peptide could activate GSK3β (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; γ-sec: γ-secretase; sAPPα: soluble APP-α; AICD: intracellular C-terminal domain; Aβ: amyloid-β; Shh: sonic hedgehog; NICD: Notch intracellular C-terminal domain; GSK3β: glycogen synthase kinase 3β; 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 Ihrie, 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, Aβ peptide promotes the activation of GSK3β, 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 γ-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).

GSK3β as a key regulator

GSK3β 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 GSK3β 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 GSK3β 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 GSK3β 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 GSK3β expression (Coronel et al, 2018). We believe the increase in GSK3β expression mediated by AICD could favor glial differentiation over neurogenesis in these cells. To verify the involvement of GSK3β in the differentiation process of human NSCs, we used the GSK3β inhibitor CHIR99021. After treating cells overexpressing APP with the GSK3β 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-GSK3β 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 GSK3β, $A\beta_{42}$ 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 $A\beta_{42}$ peptide combined with GSK3β inhibitor, we observed a significant decrease in gliogenesis in these cells, reversing the effects of $A\beta_{42}$ peptide alone (Bernabeu-Zornoza et al., 2018). Thus, in summary, our work defends that the different proteolytic derivatives of APP, specifically AICD and $A\beta_{42}$ peptide, regulate several physiological processes in human NSCs through GSK3β.

The effects described above seem to indicate that GSK3β 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 GSK3β 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 Aβ 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 GSK3β. Similarly, we have shown that monomeric Aβ peptide may regulate the proliferation and glial specification of NSCs through GSK3β.

GSK3β 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 GSK3β 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 GSK3β 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.

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