REVIEW

Physiological and pathological effects of amyloid-β species in neural stem cell biology

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Abstract

Although amyloid-β peptide is considered neurotoxic, it may mediate several physiological processes during embryonic development and in the adult brain. The pathological function of amyloid-β peptide has been extensively studied due to its implication in Alzheimer's disease, but its physiological function remains poorly understood. Amyloid-β peptide can be detected in non-aggregated (monomeric) and aggregated (oligomeric and fibrillary) forms. Each form has different cytotoxic and/or physiological properties, so amyloid-β peptide and its role in Alzheimer's disease need to be studied further. Neural stem cells and neural precursor cells are good tools for the study on neurodegenerative diseases and can provide future therapeutic applications in diseases such as Alzheimer's disease. In this review, we provide an outline of the effects of amyloid-β peptide, in monomeric and aggregated forms, on the biology of neural stem cells/neural precursor cells, and discuss the controversies. We also describe the possible molecular targets that could be implicated in these effects, especially GSK3β. A better understanding of amyloid-β peptide (both physiological and pathological), and the signaling pathways involved are essential to advance the field of Alzheimer's disease.

Key Words: *amyloid-β peptide; Aβ; neural stem cells; neural progenitor cells; Alzheimer's disease; amyloid precursor protein; toxicity; neurogenesis; gliogenesis; GSK3β*

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Introduction

Alzheimer's disease (AD), first described by Alosis Alzheimer in 1906, is a neurodegenerative disorder that represents the most common cause of dementia in the elderly population (Burns et al., 2002). It is a global disease affecting more than 30 million people around the world, and about 10 million people in Europe (Nalivaeva et al., 2013). AD presents a series of pathological characteristics. Psychiatric and behavioral alterations are symptoms that become apparent at early stages, and as the disease progresses, difficulties appear in daily activities, such as eating (Burns et al., 2002). Finally, cognitive disabilities begin to appear, along with loss of self-consciousness and perception (Amemori et al., 2015).

Although the causes of AD remain unknown, age is the main risk factor, and the incidence doubles every 5 years after age of 65 years (Burns et al., 2002). Other risk factors have also been described, including type 2 diabetes, hypertension, hypercholesterolemia, alcohol consumption and smoking (Barnes and Yaffe, 2011; Daviglus et al., 2011; Durazzo et al., 2014).

AD is characterized by neuronal death and loss of brain tissue, leading to a progressive deterioration of cognitive functions and motor alterations (Zhang et al., 2011). It first affects the entorhinal cortex and the hippocampus, leading to memory loss. Later, it extends to areas of the cerebral cortex and the thalamus, producing cognitive decline and motor function alterations (Braak and Braak, 1991).

In the first case of AD described, the histopathological presence of plaques and tangles of unknown origin were detected in the brain of the patient (Burns et al., 2002; Amemori et al., 2015). Today, it is known that the histopathology of AD is defined by the presence of amyloid plaques formed by the extracellular deposit of amyloid-β (Aβ) peptide, and the presence of intracellular tangles of the hyperphosphorylated tau protein. In its physiological form, tau is an essential component of the neuronal cytoskeleton, but in its phosphorylated state it becomes toxic to neurons (Serrano-Pozo et al., 2011).

Although most of AD cases are sporadic, about 5% seem to have a genetic component. So far, three mutations have been identified that can lead to an increased production of Aβ peptide. These mutations are found in the gene that encodes amyloid precursor protein (APP) on chromosome 21, in the gene that encodes presenilin 1 on chromosome 14, and in the gene that encodes presenilin 2 on chromosome 1 (Hama and Saido, 2005; Bekris et al., 2010).

At present, there is no effective cure for AD. In recent years, some authors have identified several novel therapeutic approaches, focusing on reducing or inhibiting the formation of Aβ peptide aggregates using small molecules or immunotherapy. However, these methods are currently being developed and are still in pre-clinical stages (Chen et al., 2017). Furthermore, it is believed that the molecular and cellular characteristics involved in AD manifest themselves decades before clinical symptoms appear, but the pathogenic mechanism remains unclear. Therefore, it is of great interest to understand the underlying mechanisms of the disease development.

Currently, both APP and Aβ peptides are an issue of concern in AD researches, because they are critical for the disease development. However, results from several different studies are controversial. It has been shown that, although Aβ is classically considered neurotoxic, it may have physiological functions important for normal development, and in some cases may even be neuroprotective (Ohkawara et al., 2011). Consequently, it is necessary to design and perform consistent studies, using the same isoforms, same aggregation states and similar models for more conclusive results.

One of the most suitable models to study the physiological and pathological functions of APP and Aβ peptide are neural stem cells (NSCs) or neural precursor cells (NPCs). These models are highly suitable, because they show the potential to self-renew and to differentiate into the main cellular phenotypes (neurons, astrocytes, oligodendrocytes) of the central nervous system. These multipotent stem cells can be sourced from fetal, neonatal, and adult brains, or from the directed differentiation of pluripotent stem cells (Lindvall and Kokaia, 2010; Martínez-Morales et al., 2013). NSCs provide an unlimited supply of neural precursors, neurons and glial cells without the need for primary tissue preparation. Furthermore, it allows the study of both pathological and physiological processes (such as proliferation and cell fate specification), which in primary cultures is more difficult, since these cultures cannot expand and have limited differentiation capacity. NSCs can be used to standardize *in vitro* studies, including drug discovery, simulating brain development and adult neurogenesis (Martínez-Morales and Liste, 2012; Marsh and Blurton-Jones, 2017).

In particular, human NSCs have provided a useful tool to help advance clinical applications of stem cell-based therapies for several neurodegenerative disorders and have also facilitated a better understanding of human brain development and the molecular pathology associated with neurodegeneration (Villa et al., 2004; Martínez-Morales et al., 2013; Bernabeu-Zornoza et al., 2018; Coronel et al., 2018, 2019). Therefore, human NSCs may encourage new therapeutic applications for future treatments of neurodegenerative diseases, such as AD.

In this review, we used the database of PubMed with the keywords: Beta amyloid [and] neural stem cells, amyloid precursor protein [and] neural stem cells. We show an updated summary of Aβ peptide and its different aggregation states, their effects in NSCs/NPCs and finally the possible molecular targets that could be involved in the effects observed. The knowledge gained by studying the varying functions of Aβ peptide will be important for a better understanding of the pathogenesis of AD and for the development of future therapeutic treatments.

Amyloid Precursor Protein

APP is the precursor of Aβ peptide, which is the major component of amyloid plaques in the brain of patients with AD (Dawkins and Small, 2014), and the involvement of APP in the pathology of AD has been widely documented. However, the physiological function of APP still remains unclear.

APP is a member of the small family of APP proteins that also includes the amyloid precursor-like protein 1 and 2 (APLP1 and APLP2, respectively). This family of proteins is preserved in a great variety of species, including invertebrates such as *Caenorhabditis elegans* (APL-1) and *Drosophila melanogaster* (APPL) (Johnstone et al., 1991).

APP is a type I transmembrane glycoprotein, with a long N-terminal domain and a cytoplasmic C-terminal domain, which is found in different types of cells, including neurons and astrocytes (Frost and Li, 2017). The gene that encodes APP is located on chromosome 21 and has 19 exons. In neurons, APP can act as a trophic factor and is required in different events such as synaptogenesis, synapse remodeling and neurite outgrowth (Zheng and Koo, 2006; Tyan et al., 2012). Interestingly, APP seems to play a key role in the proliferation, differentiation and maturation of NSCs (Trazzi et al., 2013; Coronel et al., 2019). Recent results published by our group show that APP is endogenously expressed in human NSCs and elevated levels of APP affects the differentiation of these cells, favoring gliogenesis and inhibiting neurogenesis (Coronel et al., 2019).

After post-translational modification, APP is processed in one of two main processing pathways, the non-amyloidogenic pathway or the amyloidogenic pathway (**Figure 1**). Non-amyloidogenic processing is characterized by the non-production of Aβ peptide. APP is cleaved by the enzyme α-secretase within the Aβ domain, preventing the formation of Aβ peptide and releasing the soluble ectodomain of APP (sAPP-α). The remaining C-terminal fragment (CTF-α) is subsequently cleaved by γ-secretase, liberating the non-toxic peptide P3 and the APP intracellular domain (AICD) (Grimm et al., 2013; Dawkins and Small, 2014). It should be noted that the non-amyloidogenic pathway is the dominant processing pathway and competes with the amyloidogenic pathway for the APP substrate (Haass et al., 2012) (**Figure 1A**). Amyloidogenic processing is characterized by the production of Aβ peptide. APP is cleaved by the enzyme β-secretase within the extracellular domain, at position 671, shedding off the soluble ectodomain of APP (sAPP-β). The remaining C-terminal fragment (CTF-β) is subsequently processed by γ-secretase, releasing the Aβ peptide and the AICD fragment (Thinakaran and Koo, 2008; Grimm et al., 2013) (**Figure 1B**).

Proteolytic processing of APP is highly regulated and modulated, and the processes described above are the most well-known. However, in addition to these two canonical processing pathways, other non-canonical pathways have also been described. Each of these different pathways leads to the liberation of distinct derivatives of APP (Coronel et al., 2018), which could have varying implications in either physiological or pathological conditions. A deregulation, therefore, at any stage of the processing of APP or an imbalance in the products generated, could be the first characteristic molecular alterations of AD. Correctly identifying these early characteristics would provide new biomarkers to aid in diagnosing the disease sooner. A good understanding of the processing of APP and the effects of its derivatives are fundamental to a better understanding of AD and its development.

Amyloid-β Peptide

Aβ peptide, is a peptide with a length of 39–43 amino acids and a molecular weight of approximately 4 kDa (Manzano-León and Mas-Oliva, 2006). *In vivo* studies have shown that Aβ peptide is released in its monomeric form (Luheshi et al., 2010), and monomeric Aβ peptides are able to assemble into progressively more aggregated forms, ranging from dimers and oligomers to fibrils (Hardy and Selkoe, 2002) (**Figure 2A**). Aβ oligomers are soluble and may exist throughout the brain, while Aβ fibrils are larger and insoluble (Chen et al., 2017). As a result of aging and in diseases such as AD, these fibrils precipitate as plaques in the cerebral parenchyma causing neurotoxicity (Manzano-León and Mas-Oliva, 2006). The amyloid hypothesis of AD, extensively studied in the last decade, states that Aβ peptide becomes toxic when it adopts a fibrillary conformation and the deposition of Aβ fibrils in amyloid plaques causes neuronal degeneration (Kim et al., 2003). However, some studies suggest that oligomeric Aβ is the form that induces neurotoxicity and may be responsible for neurodegeneration (Kim et al., 2003; Kayed and Lasagna-Reeves, 2013).

In humans, the most common isoforms of Aβ peptide present in amyloid plaques are $Aβ_{40}$ and $Aβ_{42}$, and the latter is highly neurotoxic (Yanker et al., 1990). Despite the known neurotoxic effects of Aβ peptide, several studies have shown that both isoforms are present during embryonic development and are important for normal brain development (Chasseigneaux and Allinquant, 2012).

It is important to keep in mind that Aβ peptides exist in different isoforms, and they can be found in different aggregation states and in different concentrations depending on the developmental stage of the brain. All these factors contribute to the complexity of the peptide, and can have either cytotoxic or physiological properties.

Due to this complexity, the function of Aβ peptide remains very controversial. Some authors have observed that freshly prepared $Aβ_{40}$ preferentially enhances neurogenesis, while $Aβ_{42}$ appears to favor gliogenesis in NSCs/NPCs (Chen and Dong, 2009; Fonseca et al., 2013; Bernabeu-Zornoza et al., 2018). On the contrary, another group has found that neurogenesis is induced by $Aβ_{42}$ and not $Aβ_{40}$, and this activity seems to be a property of Aβ oligomers and not of fibrils (Lopez-Toledano and Shelanski, 2004).

Three Main States of Amyloid-β Peptide

Amyloid-β monomers

The generation of Aβ peptide is a process that occurs during embryogenesis and seems to be required for normal brain development. This suggests that Aβ peptide is not always associated with neurotoxicity, particularly at low concentrations, which do not allow the formation of oligomers (Chasseigneaux and Allinquant, 2012).

Previous studies have shown that monomeric forms of Aβ peptide can positively affect differentiation and proliferation of rat NPCs (Chen and Dong, 2009), mouse NSCs (Heo et al., 2007; Fonseca et al., 2013; Itokazu and Yu, 2014) and human NSCs (Bernabeu-Zornoza et al., 2018). Interestingly, it has been demonstrated that monomeric $Aβ_{40}$ stimulates neurogenesis in NSCs/NPCs, whereas $Aβ_{42}$ favors gliogenesis in the same cells (Chen and Dong, 2009; Bernabeu-Zornoza et al., 2018). Furthermore, our group has found that the exposure of human NSCs to $Aβ_{42}$ stimulates their differentiation towards glial cell fates, with no effect on neuronal differentiation. $Aβ_{42}$ was also shown to specifically enhance the proliferation of glial precursor cells (Bernabeu-Zornoza et al., 2018) (**Figure 2B**).

As mentioned above, Aβ peptide in its monomeric form and at low concentrations may be neuroprotective, and some studies have shown that monomeric Aβ peptide enhances the survival of hippocampal neurons *in vitro* (Whitson et al., 1990; Kim et al., 2007). Furthermore, other studies have shown that Aβ monomers enhance the survival of developing neurons deprived of trophic-factors and protect mature neurons from excitotoxic cell death (Giuffrida et al., 2009).

However, in pathological conditions, a change occurs in the levels of the two major isoforms ($Aβ_{40}$/$Aβ_{42}$). Although the concentration of both Aβ monomers is increased, $Aβ_{42}$ levels are notably higher, causing an imbalance in $Aβ_{40}$/$Aβ_{42}$. Furthermore, these increased levels can begin to aggregate, forming oligomers, protofibrils and amyloid fibrils (Yanker et al., 1990; Chen et al., 2017), which are best known for their cytotoxic effects related to the development of AD.

Amyloid-β oligomers

Aβ oligomers result from the aggregation of monomers, before forming fibrils. Aβ oligomers are soluble, can spread throughout the brain and their distribution is heterogeneous. It is believed that the accumulation of soluble Aβ occurs when it reaches approximately 100–200 kDa under relatively physiological conditions *in vitro* (Ferreira et al., 2015; Chen et al., 2017).

The function of Aβ oligomers is controversial and results differ significantly between studies (Kwak et al., 2006; Trazzi et al., 2011; Nicolas and Hassan, 2014). It has been shown that human NSCs exposed to oligomeric Aβ peptides reveal reduced proliferation and commitment to a glial phenotype, without affecting neuronal fates (Lee et al., 2013). This is consistent with a marked effect of Aβ oligomers on NSCs. However, another group found that in NSCs from rat hippocampus, neurogenesis is induced by oligomeric $Aβ_{42}$ (Lopez-Toledano and Shelanski, 2004; Lee et al., 2013). Besides, Heo et al. (2007) showed that at a concentration of 1μM, oligomeric Aβ peptide significantly increases the number of proliferating mouse NPCs (**Figure 2B**).

Additionally, several groups have observed that Aβ, in its oligomeric form and at low concentrations, does not appear to have an effect on apoptosis (López-Toledano and Shelanski, 2004; Fonseca et al., 2013), suggesting that low concentrations of Aβ peptide does not seem to be involved in the process of cell death, but rather, might have a neuroprotective effect by promoting the differentiation of NSCs/NPCs. However, upon an increase in its concentration, oligomeric Aβ peptide begins to show its characteristic cytotoxic effects.

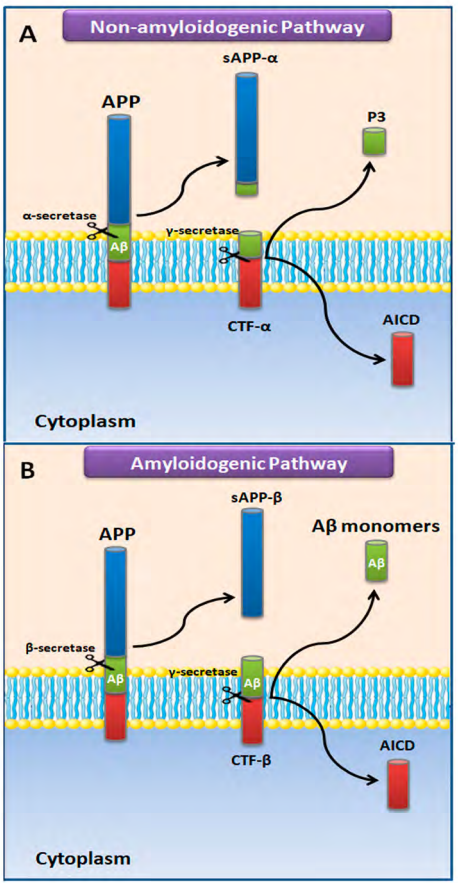


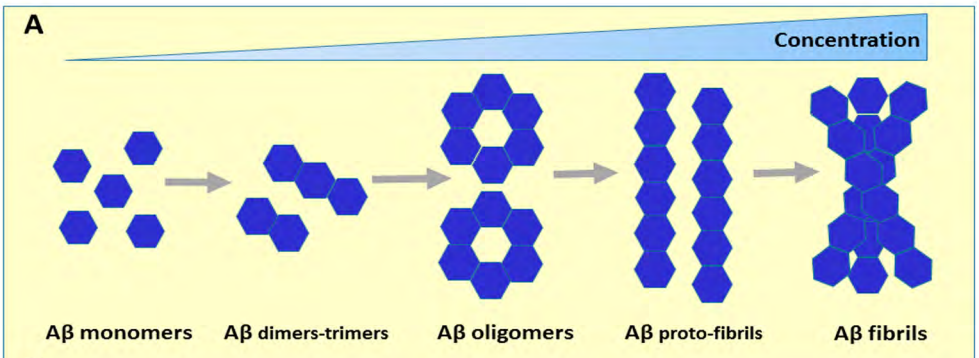
Figure 1 Proteolytic processing pathways of APP.
(A) Non-amyloidogenic pathway, where α-secretase and γ-secretase act, generating the proteolytic derivatives sAPP-α, p3 and AICD. (B) Amyloidogenic pathway, where β-secretase and γ-secretase act, generating the proteolytic derivatives sAPP-β, Aβ and AICD. APP: Amyloid precursor protein; AICD: amyloid precursor protein intracellular domain; Aβ: amyloid-β; sAPP: souble amyloid amyloid precursor protein; CTF: C-terminal fragment.

The toxic effects of oligomeric Aβ depends on whether the peptide is found in a soluble or insoluble form (Lee et al., 2013; Amemori et al., 2015). Some studies have shown that soluble forms of Aβ oligomers exhibit strong neurotoxic effects and an increase in soluble oligomeric Aβ levels could be a potential cause of AD (Kim et al., 2003; Kayed and Lasagna-Reeves, 2013) suggesting that soluble oligomeric Aβ peptides are the most toxic species (particularly to neurons) and is believed to be involved in problems of memory, dementia and synaptic depletion (Dahlgren et al., 2002; Walsh et al., 2002; Cleary et al., 2005; Heo et al., 2007; Lee et al., 2013).

Studies of the toxic effects of Aβ oligomers have been strongly promoted in AD research because it provides a potential explanation for one of the pathological causes of the disease (Benilova et al., 2012). Furthermore, Aβ oligomers could have a type of paracrine effect, capable of mediating toxicity by altering neurotransmission leading to cell death in another region of the brain, which has been shown both *in vitro* and *in vivo* (Kayed and Lasagna-Reeves, 2013). Although the involvement of Aβ peptide in its oligomeric form has been described in the context of AD, the cause of the disease is still unknown (Lee et al., 2013).

Amyloid-β fibrils

Aβ fibrils are larger than oligomers, insoluble, and assemble into amyloid plaques forming the histological lesions characteristic of AD (Ferreira et al., 2015). *In vitro* studies suggest that fibrillary Aβ peptide induces neurotoxicity mediated by its interaction with neuronal membrane proteins, including APP (Lorenzo et al., 2000), while Aβ fibrillary deposits lead to synaptic abnormalities by altering neuronal dendrites. Furthermore, it is widely documented that fibrillary Aβ aggregates, which assemble into amyloid plaques in the AD



B

Aβ monomers	Aβ oligomers	Aβ fibrils
Toxicity → Depends on concentration	Toxicity → Depends on solubility and concentration	↑ Neurotoxicity (Aβ42 more fibrillogenic than Aβ40)
↑ Proliferation	↑ Proliferation (mouse NSCs) ↓ Proliferation (human NSCs)	? Proliferation
Aβ40 → ↑ Neurogenesis Aβ42 → ↑ Gliogenesis	↑ Neurogenesis (rat NSCs) ↑ Gliogenesis (human NSCs)	↑ Gliogenesis

Figure 2 Summary of Aβ peptide aggregation forms and their effects on NSCs biology.
(A) Schematic indication of the possible forms of Aβ peptide. (B) Summary of the effects of Aβ peptide (monomeric, oligomeric and fibrillary) on cell death, proliferation and differentiation of NSCs. Positive effects are indicated by upward arrows. Negative effects are indicated by downward arrows. Unclear effects are indicated by question marks. Aβ: Amyloid-β; NSCs: neural stem cells.

brain, activate microglia, increasing the expression of inflammatory cytokines, which has severe neurodegenerative effects. Some studies also show that fibrillary Aβ peptide inhibit neurogenesis by promoting the differentiation of NSCs into glial cells (Malmsten et al., 2014). Preliminary studies by our group support these findings, indicating that fibrillary Aβ peptide seems to affect the differentiation and proliferation of human NSCs (**Figure 2B**).

Furthermore, Aβ fibrils are believed to be one of the early initiating events in AD pathogenesis. The "amyloid cascade" hypothesis has defined the aggregation of Aβ fibrils, later deposited into amyloid deposits (a histopathological hallmark of AD), as a toxic gain-of-function (Dahlgren et al., 2002). Interestingly, some studies reveal that $A\beta_{42}$ is more fibrillogenic than $A\beta_{40}$ (Dahlgren et al., 2002).

The accumulation of Aβ peptide as fibrils and the eventual formation of amyloid plaques are also believed to be involved in the hyperphosphorylation of tau protein and the formation of intracellular tangles (Chen et al., 2017). It is believed that tau protein becomes hyperphosphorylated in response to changes in kinase/phosphatase activity mediated by Aβ aggregation, leading to the formation of neurofibrillary tangles, further contributing to the pathogenesis of AD, and another reason why fibrillary Aβ peptide is considered so neurotoxic.

However, whether amyloid pathology is caused by insoluble fibrillar Aβ or soluble oligomeric Aβ is uncertain (Koffie et al., 2009), and which form is the most toxic remains controversial. Therefore, more studies are necessary in order to clarify the functions of these two aggregation states of the Aβ peptide.

GSK3β as a Target of Amyloid-β Peptides

The molecular pathways involved in Aβ peptide physiology are currently a hotspot in AD researches. Due to the role of Aβ peptide in the appearance of this disease, knowing the implication of these peptides in different signaling pathways is of great significance for developing new therapies to treat AD.

GSK3β is involved in a variety of physiological processes, such as regulating cell morphology, neuronal outgrowth, motility and synaptic plasticity (Engmann and Giese, 2009). The GSK3B gene is constitutively expressed in most tissues, and the protein is commonly regulated by inhibitory phosphorylation on serine 9. However, dysregulation of some signal transduction pathways results in failure to adequately inhibit GSK3β, thus yielding an abnormally active form, which is believed to contribute to various diseases (Llorens-Martins et al., 2014).

Currently, GSK3β is the most studied target of Aβ peptide, and it is believed that GSK3β is involved in the pathological symptoms of AD, Aβ plaque formation, tau hyperphosphorylation and neurodegeneration. It has also been suggested that the activation of GSK3β promotes the phosphorylation of APP, giving rise to plaques and tangles characteristic of AD. Many groups have observed that increased levels of APP and phosphorylated tau facilitate the appearance of Aβ peptide, which supposes a feedback loop, where a greater amount of Aβ peptide increases the activation of GSK3β, which further leads to neurodegeneration and synaptic failure (Lee et al., 2013; Kirouac et al., 2017). In addition, GSK3β could play a dual role in Aβ production by enhancing the activity of the enzymes β-secretase and γ-secretase and downregulating the activity of α-secretase (Cai et al., 2012).

The importance of GSK3β has also been demonstrated in cultured NPCs where inhibitors of GSK3β protect these cells from apoptosis (Jaeger et al., 2013) and facilitate neural progenitor differentiation towards a neuronal phenotype (Kim et al., 2004). *In vivo* overexpression of the GSK3B gene causes alterations in adult neurogenesis, leading to a depletion of the neurogenic niches and a decrease in the number of mature neurons (Fuster-Matanzo et al., 2013). Recent results obtained by our group indicate that treatment with freshly prepared $A\beta_{42}$ peptide increases the expression of the GSK3B gene in differentiating human NSCs. We observed that $A\beta_{42}$, mediated by GSK3β, might have an important role in cell fate specification of human NSCs, since GSK3β inhibition (with CHIR99021), reversed the effect of $A\beta_{42}$ alone (which favored gliogenesis) (Bernabeu-Zornoza et al., 2018).

Furthermore, *in vivo* studies using the GSK3β inhibitor Tideglusib, an orally administered drug, has shown positive results, reducing neuronal loss and tau phosphorylation in a mouse model (Domínguez et al., 2012). However, the majority of currently available inhibitors of GSK3β, including maleimide derivatives, indirubin and paullone, cause severe side effects, preventing them from entering clinical trials (Dolan and Johnson, 2010; Rajasekhar and Govindaraju, 2018). Therefore, although inhibiting GSK3β has shown promising results *in vitro* using NSCs/NPCs and some *in vivo* pre-clinical trials, its translational application to treat AD in clinical trials has proven difficult. The main reason for this is due to the fact that GSKβ affects the phosphorylation of a variety of substrates and is implicated in the regulation of diverse cellular functions, including proliferation, differentiation, metabolism and apoptosis. Inhibiting GSK3β in general terms, consequently, appears to be associated with highly cytotoxic side effects, hypoglycemia and tumorigenesis (Martinez et al., 2011).

GSK3β has also become an important target in the study of Aβ peptides because it is known to integrate different pathways, and interacts with other molecular targets (Thornton et al., 2008; Engmann and Giese, 2009).

An example of how GSK3β interacts with different pathways is with the MAPK pathway. This pathway is upstream of GSK3β activity, regulating its activation *via* phosphorylation (Goold and Gordon-Weeks, 2005; Thornton et al., 2008). However, MAPK signaling may have important implications as an early driver of AD pathology development independent of GSK3β activity. The MAPK pathway has a broad spectrum of stimulations, such as growth cytokines, stress, TGF-β, and ceramides (Xu et al., 2018). MAPK family members also play a vital role in cell proliferation, differentiation,

survival, and development by activating gene expression, mitosis, and metabolism (Wang, 2018; Xu et al., 2018). *In vitro* studies have reported that NSCs expressing APP show enhanced Ras expression and activation of ERK1/2 (Kirouac et al., 2017). These findings also hypothesize that APP or a metabolite of APP (such as Aβ peptide) would promote both MAPK signaling and increase proliferation in NSCs/NPCs. Increased expression of Ras that persist through later stages of AD, appears to be associated with Aβ generation implying a pathologic link between Aβ and altered Ras-MAPK signaling. Elevated Aβ levels in the brain correlate with increased expression of Ras and phosphorylation of APP and tau, thus indicating a proliferative role (Kirouac et al., 2017). Other studies demonstrate that, on an intracellular level, oligomeric Aβ peptide activates the Ras/ERK pathway in a positive-feedback loop, which in turn activates cyclin D1 through a mitogenic stimulus promoting cell cycle entry, leading to disruption and eventual cellular degeneration (Lee et al., 2013).

An example of a molecular target of GSK3β is CDK5, and it has been suggested that these two molecules have "cross-talk" and contribute to the development to AD (Engmann and Giese, 2009). CDK5 is named for its structural similarity to members of the serine/threonine cyclin-dependent kinase family. It reaches a peak kinase activity in neurons due to restricted expression of its activators p35 and p39, which are implicated in the formation of functional synapses (Zhang et al., 2008). CDK5 is a main player in processes of neural development, synaptic signaling, learning and memory (Fischer et al., 2005; Angelo et al., 2006). Since some of the clinical symptoms of AD include memory loss and impaired learning, it has been suggested that CDK5 is implicated in the molecular changes that appear as these symptoms develop. This theory is supported by increased CDK5 activity in AD brains (Patrick et al., 1999). Furthermore, some authors have observed the involvement of CDK5 in the differentiation of NSCs/NPCs, making these cells a good model to study the relationship between Aβ and CDK5, and to see how it may affect the cell fate specification of these cells (Quan et al., 2014). Several studies have found a link between Aβ and CDK5. Some authors have shown that Aβ peptide triggers CDK5 activation to induce p53 phosphorylation and stabilization, which leads to neuronal damage. The inhibition of the CDK5-p53 pathway may, therefore, represent a novel therapeutic strategy against Aβ-induced neurodegeneration (Engmann and Giese, 2009; Lapresa et al., 2018). Furthermore, some studies suggest that CDK5 might be the link between Aβ peptide and the phosphorylation of tau protein (Hernandez et al., 2009).

CDK5 inhibitors, like inhibitors of GSK3β, have been reported with potential therapeutic applications. For example, butyrolactone-I is a CDK5 inhibitor that could directly impact and lower Aβ peptide aggregation (Mushtaq et al., 2016). However, severe side effects have prevented their use in clinical trials. Therefore, novel molecular approaches that guides the specificity of these types of inhibitors as exclusive treatments for AD, without affecting other physiological functions, need to be discovered (Rajasekhar and Govindaraju, 2018).

Conclusion

It is known that AD is the most prevalent neurodegenerative diseases, especially in the elderly population. Several studies have tried to determine the main cause by which this neurodegenerative disorder is triggered, however, its etiology is still unknown. Aβ peptide plaques are one of the main targets of AD research, and therefore, extensive work has been done in order to determine the cause of their appearance and accumulation, and which form(s) are mainly responsible for neurotoxicity and neuronal death.

We know that Aβ peptide is generated after cleavage of APP by γ-secretase in the amyloidogenic pathway. Although most researchers associate Aβ peptide with neurotoxicity, it is now becoming more apparent that this peptide also has physiological functions necessary for normal development, including non-pathogenic processes, such as NSC proliferation and cell fate specification.

The study of Aβ peptide is incredibly complex. On one hand, it can be found in states ranging from monomers and dimers to oligomers and fibrils, and on the other hand, it can be found in different isoforms. Isoforms of Aβ peptide vary in length and appear in different ratios, which may contribute to their aggregation states. Furthermore, depending on the isoform, the aggregation state, the developmental stage of the brain and the concentration of Aβ peptide, it can either function normally or become cytotoxic, indicating that the homeostatic balance of this peptide is highly important for normal brain development and function.

Due to this complexity, the physiological and pathological functions of Aβ peptide remain highly controversial, mainly due to inconsistent and contradictory results in the field. The discrepancies observed are most likely due to the different model systems used (NSCs/NPCs from different species such as mouse, rat and human), the use of different peptide concentrations, when, how and how long the peptide was administered and the use of different aggregation states. For that reason, greater focus should be place on standardizing experiments, in order to gain a wholesome understanding of how Aβ peptide functions on a physiological and pathological level. It is also important to understand how this peptide functions on a molecular level to identify the mechanisms and signaling pathways implicated, in order to determine new biomarkers for earlier diagnosis, since it is believed that cellular and molecular alterations begin to appear long before the first cognitive and clinical symptoms appear. NSCs/NPCs are helpful tools to discover new biomarkers in the development of treatments for AD.

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