

Advances in Alzheimer's disease Research: Neural Stem Cells Culture and Gene Therapies

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Abstract: Alzheimer's disease (AD) is the most common form of dementia, involving a progressive loss of synapses in different regions of the central nervous system (CNS), with the onset in the hippocampus, with cognitive dysfunction and extensive neuronal loss. Animal models are often used in research to discover novel pharmacological treatments, but have limitations as long study times and less controlled variables related to environmental factors. The use of hippocampal subgranular and subventricular zone stem cells obtained from transgenic animals for AD cultivated in vitro may allow the better understating of the molecular mechanisms involved in the disease and to minimize the research timeframe. Epidemiological and genetic studies have documented the involvement of genetic risk factors to acquire AD, such as the Apolipoprotein E gene. Thus, the possible use of neural stem cells in promoting neurogenesis and repairing the affected brain areas to improve neuronal loss is key for AD treatment. Genetic therapy approaches concerning the regulation of genes associated with AD and brain neural stem cell regulation appears as a potential research advance to interrupt the course of disease or even its remission. However, animal models are still in need to support these findings.

Keywords: Alzheimer's disease; genetic; treatment; stem cell

I. INTRODUCTION

Neurodegenerative diseases are characterized by neuronal loss, reduction of synaptic connections in various areas of the central nervous system and brain atrophy [1,2]. Signs and symptoms such as impairment of cognitive functions related to behavioral changes and impaired memory are involved in these diseases [3]. Among the most common neurodegenerative diseases are Parkinson's disease, Multiple Sclerosis, and various types of dementia such as Alzheimer's disease [4]. Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, accompanied by an extensive neuronal loss [5]. Pathognomonic brain structural characteristics of AD involve the accumulation of amyloid plaques containing beta-amyloid peptide (A β) and neurofibrillary tangles formed by hyperphosphorylated tau protein [6,7].

The associated memory losses that occur in AD are caused by damage of various affected areas of the brain, particularly the hippocampus. The spatial memory is associated with the hippocampus, one of the parameters evaluated in AD research by using the Morris Water Maze test for rodents [8].

Research using in vitro studies with neural stem cells to better comprehend the road maps and signaling maps for the formation of new neurons following brain damage, has been developed [9, 10]. Studies with stem cells of the subventricular zone (SVZ) and the subgranular layer of the dentate hippocampal gyrus to replace apoptotic neuronal cells, support a possible way to repair partly the damaged brain areas following degenerative diseases [11]. In both regions, the complex phenomena of stem cell regulation, proliferation and differentiation the involved signaling pathways, such as the Wnt pathway, have yet to be further explored. Wnt glycoproteins represent a large family of signaling molecules that are involved in cell fate and neurogenesis [12].

The DA-related research has advanced, but the current knowledge about the genetic factors that are associated with this disease is still limited. Among the genes identified in the major pathway of Alzheimer's, Amyloid Precursor Protein (APP), Presinilin 1 (PS1), Presinilin 2 (PS2) and Apolipoprotein E (ApoE) have been the focus of several studies. ApoE has been considered as an important genetic risk factor for the later development of the disease. This protein plays an essential role for the normal metabolism of triglyceride-rich lipoproteins [2, 13].

ApoE is a glycosylated protein composed of 299 amino acids, it is mainly synthesized in the liver but also in the spleen, lungs, ovaries, testes, adrenal cells, smooth muscle and kidney, but its second highest

concentration is identified in the brain, particularly in astrocytes, reaching 1/3 of the amount found in the liver. ApoE participates in the transport of triglycerides liver to other tissues by binding to very low density lipoproteins (VLDL) and is a ligand with high affinity uptake to cellular lipoproteins [2, 5]. The APOE polymorphism has been associated as a risk factor for the development of late-onset AD, specifically the E4 allele, which has been implicated in brain inflammatory processes [14]. In humans, ApoE has three common isoforms: ApoE2, ApoE3 and ApoE4, the latter being involved in the clearance of A β . ApoE4 levels in the cerebrospinal fluid (CSF) may be a potential biomarker of AD during aging [15].

The need for studies and research in the search for new therapies that control, reverse or even cure this disease in humans causes culture of neural stem cells and gene therapy models are important in studies related to neurogenesis. In addition, in vitro model is an effective way to check the involvement of neurogenesis in AD [12].

This study aims to present the model of subventricular stem cell culture and hippocampal used in the study of neurogenesis and its involvement in AD, and the use of gene therapy as an effective way to develop new search technologies in therapeutic approach in this pathology.

II. ALZHEIMER'S DISEASE PATHOPHYSIOLOGY

According to the amyloid hypothesis, the progressive accumulation of A β is considered the early pathogenesis, leading to the formation of neurofibrillary tangles resulting in hyperphosphorylation of Tau protein, responsible for the structural conformation of microtubules, causing synaptic reduction and subsequent neuronal cell death [16]. Thus, when microtubules are destabilized, the pyramidal neurons of the hippocampus is changed, being observed in histological studies the loss of neuronal cells [17].

Due to high metabolic oxygen demand, the brain is particularly vulnerable to free radicals. Thus, oxidative stress promotes possibly important role in the progression of AD is associated with elevated levels of A β and loss of hippocampal neurons [18]. The neuroinflammation associated with AD is correlated with significant losses in cognitive functions through the release of inflammatory cytokines initiating the cascade of cellular and molecular events leading to increases in amyloid plaques and neurofibrillary tangles, promoting the loss of neurons [19,20].

III. GENETIC FACTORS

ApoE is considered the most genetic risk factor for AD [16, 21]. In AD, ApoE4 contributes to the changes related to cleavage by amyloidogenic pathway, producing the A β peptide, structural feature of the disease. The ApoE gene is responsible for encoding the ApoE protein that when combined with lipids in the body has the function to mediate the binding of lipoprotein to cell surface receptors [22].

In culture of neuronal cells expressing amyloid precursor protein (APP), the use of exogenous ApoE4, ApoE3 behaves as unchanged in the development of the disease. Expression of ApoE is associated with protection processes of aggressors stimulus cells or to repair damage, possibly through redistribution of lipids [23]. However, the difference in the ApoE3 and ApoE4 is possibly related to the action of proteolytic cleavage high ApoE4, resulting in fragments that enter the cytoplasm and often are neurotoxic.

It is known that ApoE production by neurons can be induced by environmental factors such as stress or associated with aging, oxidative stress and trauma. Thus, ApoE4 studies in cell culture showed that fragments move into the cytosol, mitochondria interact with and cause neurotoxicity. Thus, the mitochondria are important in synaptogenesis and ApoE4 promotes changes associated disorders at the molecular level resulting in neuronal cell death [24]. However, the polymorphism of ApoE, although associated with the pathogenesis of AD are not well defined. The relationship between high levels of A β and genetic factors are well studied.

However, recent studies have shown that ApoE promotes a possibility of drug therapy from the synthesis of mimetic peptide, such as the APOE COG 133, which corresponds to residues 133-149 of the ApoE holoprotein, demonstrating anti-inflammatory in brain injury models and intestinal mucositis [25]. Another study showed that the APOE COG 133 treatment also benefit inflammatory processes in animal models of spinal cord injury [26].

IV. EXPERIMENTAL PROCEDURE

Hippocampus and SVZ Cell Cultures

The SVZ and hippocampus cells are obtained from mice at 1-3 days of age. The brains are removed after decapitation and placed on plates for holding coronal sections 450 μ M performed in an equipment specific to brain sections (Tissue Chopper®) of specific regions, and then washed with a solution containing 0.025% trypsin and 0.265 mM EDTA. The fragments are isolated and dissociated in a petri dish and then washed with serum-free medium (SFM, Gibco, Rockville, MD, USA). Then they are supplemented with epidermal growth factor (EGF) (Gibco) [27].

Neurogenesis Evidenced

In a study of Neuropeptide Y (NPY), a peptide with 36 amino acids found in abundance in the brain and involved in the regulation of appetite, mood and sexual behavior, we used the 5-bromo-2'-deoxyuridine (BrdU; 10 μ M; Sigma-Aldrich) for detecting cell proliferation of SVZ region. Through immunohistochemistry, we determined an increase of the percentage of labeled cells, concluding that this neuropeptide is capable of activating neurogenesis, enabling new prospects for developing therapies for neurodegenerative diseases [10].

Another similar study, also conducted with Neuropeptide Y, by histological and biochemical tests was possible to prove neurogenesis in the SVZ, suggesting its importance in pathological conditions. In several studies, it has been identified the participation of neural stem cells in diseases such as Huntington's disease and epilepsy and engagement with the increase of neurogenesis NPY [28].

In a study of the use of hippocampal stem cells subgranular region of the dentate gyrus, it was observed that by activating the Wnt pathway with overexpression of the gene occurred Wnt3 neurogenesis after brain injury [12]. These data enable new research has been done looking for a way to use the neurogenesis induction processes in the hippocampus region, promoting improvement in cognitive functions associated with this region, as the AD [29].

V. CONCLUSION

The AD is a multifactorial disorder of unknown origin. A great amount of accumulating research has been done to seek adequate treatment and prevention. One potential approach that may advance AD research is neural stem cell studies for neuronal repair, focusing on mechanisms of neurogenesis and gene therapy, especially regarding the APOE gene.

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