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Preclinical models available to study therapeutics for Alzheimer's disease: For interference of drug discovery

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ABSTRACT: Neurodegenerative diseases such as Alzheimer disease have complex pathophysiology and aetiologies, which is why drugs to treat them are ineffectual. For a better knowledge of the aetiology and quicker development of treatment plans, in vitro models of neurodegenerative disorders like Alzheimer's disease have become more necessary. So, in this study we conducted a comprehensive review on available in vitro models for studying Alzheimer disease. Therefore, a thorough search was done in a number of databases, including Google Scholar, Scopus, Web of Science, and Science Direct, with the goal of filling in the research gaps. We evaluate the relative benefits of various models in this paper, ranging from classic cell cultures to the most recent high-throughput three-dimensional systems. We go over their benefits and drawbacks as well as their potential for delving into the intricate systems underlying Alzheimer's disease. This review of key components of AD pathophysiology and how these models have contributed to a better understanding of AD molecular pathways covers the fundamental challenges connected with using this technology and the efforts being made to overcome them. To better understand the molecular pathways involved in drug discovery and AD research, they can be helpful tools.

KEYWORDS: Alzheimer disease, in vitro, cell line, stem cell, neuroprotective

I. INTRODUCTION

The understanding of Alzheimer's disease (AD) has advanced significantly over the approximately 110 years since it was first described by Dr. Alzheimer in 1906. AD is a neurological condition that develops slowly and insidiously. Clinical signs include deterioration of daily living skills, mental and behavioural abnormalities, and impairment of cognitive processes. The pathogenesis of AD is complicated and still not completely understood. The deposition of amyloid beta (Aβ) in brain tissue is a commonly recognised idea among scientists, and it may be the fundamental mechanism behind both the onset and progression of AD [1].Moreover multiple risk factors, such as genetic predispositions and environmental triggers, together with ageing, contribute to susceptibility. However, the ineffectiveness of medications for the treatment of neurodegenerative illnesses is a reflection of their complicated pathophysiology and aetiology [2]. Given that gene interactions cause human AD to proceed, there is a strong likelihood that one or more equivalent genes in mice must be expressed in order for the pathogenic form of AD to manifest. However, it is challenging to apply findings from animal models to describe the underlying metabolic processes and pathogenic mutations linked to human AD [3]. By bridging the gap between present pre-clinical animal models and humans using novel in vitro models, it may be possible to identify promising therapeutic targets that can be examined in upcoming clinical trials [4]. Additionally, by assisting in the identification of the mechanism of action as well as any related dangers, in vitro testing can cut down on the time and expense of translation. Additionally, by assisting in the identification of the mechanism of action as well as any related dangers, in vitro testing can cut down on the time and expense of translation. In light of these facts, for the first time, we thoroughly reviewed and analysed all potential invitro models that may be used to examine the effectiveness of various medications for treating AD.

II. METHODOLOGY

We conducted a review based on scientific articles that addressed preclinical Alzheimer disease model. Science Direct, SciFinder, Google Scholar, MEDLINE, EMBASE, and Scopus were the search engines used to search for published articles (till 15, April 2023). For extracting information about the possible used invitro models, we used keywords such as "Alzheimer disease", "gene", "invitro model", "cell line", "molecular mechanism", and "mechanism of action", along with their corresponding MeSH terms and conjunctions OR/AND. We searched the available reports for scientific claims. All searches were limited to English. We excluded conference proceedings, gray literature, unpublished data, newspaper articles, preliminary reports without substantial proof of the claim, abstracts and full texts that could not be retrieved, and studies not relevant to this review.

Invitro models

In Stem Cell : It is essential to create human induced pluripotent stem cell (iPSC) models from sporadic AD (sAD) patients since doing so may aid in the study of the illness's aetiology and prospective treatment options. The complex character of the disease, however, still places limitations on the creation of sAD iPSC models. In a study, Sendai virus, which expresses Oct3/4, Sox2, c-Myc, and Klf4, was used to convert peripheral blood mononuclear cells (PBMCs) from a patient with sAD into induced pluripotent stem cells (iPSCs), which were then used to create a cell model of AD. Study indicate that the iPSC derived from PMBCs (PBMC-iPSC) had a normal karyotype and the capacity to differentiate into three embryonic layers evident from immunofluorescence staining, alkaline phosphatase staining, karyotype analysis, reverse transcription-polymerase chain reaction (RT-PCR), and teratoma formation in vitro. Also quantitative real-time polymerase chain reaction (qPCR) and immunofluorescence labelling indicated that PBMC-iPSCs successfully differentiated into brain cells. The AD cell model was successfully created in vitro based on the detection of beta-amyloid protein oligomer (AO), beta-amyloid protein 1-40 (A 1-40), and beta-amyloid protein 1-42 (A 1-42). In conclusion, utilising PBMC from a patient with sAD, this study successfully created an AD cell model with pathological hallmarks of beta-amyloid peptide deposition [5].

The most notable AD risk gene, APOE, whose ε4 allele increases risk in a dose-dependent way, has been identified by a genetic research. Research on AD in genetically human models has been made possible by the development of human induced pluripotent stem cells (hiPSCs), which can differentiate into a variety of brain cell types. Brain cell and tissue models made from hiPSCs investigate the role of APOE in causing AD pathogenesis. Such models have provided essential understanding of the cellular mechanisms and cell type-specific roles underpinning the dysregulated biological processes that start pathogenic cascades and spread neurodegeneration. Collectively, hiPSC-based models are expected to be a powerful tool for studying the underlying causes of AD, with significant potential for use in the development and testing of AD drugs [6].

In Neurospheroids : For in vitro illness modelling and medication screening, neurospheroids are frequently employed. However, the size variability of the neurospheroids mixes available restricts their applicability when used for fundamental mechanistic research on neurodegenerative illnesses or for the development of innovative therapies. Using human induced pluripotent stem cells and uniformly sized neurospheroids, a study constructs large-scale arrays of neurospheroids. The neurospheroids array was also proven to be a reliable and sensitive technique for screening chemicals over an extended period of time by this study. The neurospheroids made from stem cells exhibit significant neurite development and extend thick bundles of dendrites outward when suspended in three-dimensional extracellular matrix for several weeks. Additionally, study produced neurospheroids grown from genetically modified stem cells carrying mutations linked to familial Alzheimer's disease for eight weeks in our microarray system. It's interesting to note that they saw significant amyloid-β and phosphorylated tau, two important hallmark of Alzheimer's disease. The study's findings suggest that creating neurospheroid arrays in vitro is a useful technique for understanding complex neurodegenerative illnesses and hastening the development of new drugs [7].

Three dimensional chip : Researchers in a study described the creation of a microfluidic device based on three-dimensional (3D) neurospheroids that more accurately resembles the in vivo brain microenvironment by supplying a steady flow of fluid that can be seen in the brain's interstitial space. Concave microwell arrays were used to create uniform neurospheroids with cell-cell interactions and contacts in all directions, and an osmotic micropump system was used to maintain a modest interstitial level of flow. They used this platform to look into how flow affected the size of neurospheroid, neural network, and neural differentiation. In contrast to those that were cultured in static conditions, flow-cultured neurospheroids were larger and formed more durable and complex neural networks, suggesting that the interstitial level of slow and diffusion-dominant flow affects continuous nutrient, oxygen, and cytokine transport and removal of metabolic wastes. They also examined the toxicity of amyloid-β, which is frequently cited as the primary cause of Alzheimer's disease. In comparison to amyloid-β treatment under static conditions, amyloid therapy using an osmotic micropump dramatically decreased the survival of neurospheroids and significantly increased the breakdown of neural networks. They suggest using this 3D culture-based microfluidic chip as an in vitro brain model for neurodegenerative illness and high-throughput drug screening by include in vivo-like microenvironments [8].

Two dimensionsal neuronal culture : In 1907, conventional 2D cultures were made practicable [9]. Cells are directly plated on a rigid substrate (such as polystyrene or glass) in this type of model, which is typically covered with substrates that replicate the composition of the ECM, enhance cell adherence, and can support

desired cell behaviour like proliferation or differentiation [10]. For instance, common coating substrates for cell culture include laminin, poly-ornithine, poly-lysine, and fibronectin [11]. They facilitate cell attachment due to electrostatic attraction with the cell surface [12], coordinate synaptogenesis and synaptic activity, foster cell adhesion through integrin receptors, contribute to NSC differentiation through extracellular signal-regulated kinase (ERK) ERK signalling, foster cell attachment through integrin receptors, coordinate synaptogenesis and synaptic activity, and regulate neural cell migration and neurite outgrowth. Traditional 2D models are unquestionably important, especially because they offer a reasonably affordable and repeatable tool that can be used in conjunction with animal models, but they do not accurately represent the complexity and organisation of real brain tissue, limiting cell interaction to only side-by-side contact and lacking nutrient/oxygen diffusion and waste removal dynamics [13]. These modelling constraints may have an effect on cell morphology, cell survival, cell proliferation and differentiation, and consequently on disease pathways [14]. This sparked efforts to create more sophisticated platforms, including 3D models.

Three dimensional neuronal culture : There is mounting evidence that two-dimensional (2D) monolayer cultures, which are more commonly utilised, are inferior to three-dimensional (3D) in vitro cell culture platforms for simulating natural in vivo microenvironments. But current 3D culture models of AD rely on genetically modified cell lines that overexpress mutant genes or aggregate-based cultures with heterogeneities in composition, biological characteristics, and cell development stages. These drawbacks encourage research of substitute in vitro human neuronal AD models with improved repeatability and matrix consistency [15]. A study reported, three novel biomaterial-based scaffolding platforms created using tissue engineering techniques in order to achieve in vitro 3D neuronal cultivation. These include an electroconductive substrate made of graphene oxide hydrogel created by physical crosslinking, a hollow microfiber substrate made of synthetic polymers using core-shell electrospinning, and a nonwoven fibrillar substrate made of synthetic polymers using wet electrospinning. To ascertain their suitability for allowing long-term 3D neural culture, all three platforms were tested for cell encapsulation, distribution, viability, proliferation, neuronal differentiation, and neurite production.The best acceptable substrate in terms of design criteria comprising both physical and biological features is wet electrospun, non-woven poly(lactic-co-glycolic acid) (PLGA) microtopographic scaffolds, according to data from immunocytochemistry [16].

Human stem cell-derived neurons were supported by the highly porous fibrillar scaffolds for improved infiltration, uniform distribution, and long-term survival. Additionally, it was shown that the stiffness of the microfibre scaffold closely resembled the elasticity of genuine brain tissue, demonstrating its capacity to encourage true physiological responses in cellular phenotypes. Next, essential characteristics of 3D-cultured neural stem cells, such as proliferation and differentiation, were compared to 2D monolayer controls grown in Petri dishes. Within seven days of culture, the 3D fibrillar microenvironment greatly accelerated neuronal and glial differentiation while reducing cell proliferation. In order to replicate early-stage AD pathogenesis in vitro, the scaffolds were connected to neurons produced from familial AD (FAD) patient induced pluripotent stem cells (iPSCs). Between sick and age-matched controls, differentiated neurons in 3D PLGA scaffold-based culture showed a significant increase in pathogenic amyloid-beta 42 (A42) and phospho-tau (p-tau) levels. Furthermore, compared to similar 2D monolayer control cultures, the levels of spontaneous expression of these pathogenic markers in 3D culture were more pronounced. Together, the findings show for the first time how to effectively mimic and speed up early-stage AD pathogenesis by combining 3D synthetic polymer-based fibrillar scaffolds with human neuronal cultures produced from iPSCs [16].Additionally, it functions as a straightforward, standardisable, and straightforward in vitro platform, enabling very effective neuronal differentiation and noticeably quicker maturity than traditional monolayer cultures. The modelling of additional complex neurodegenerative disorders and the assessment of potential treatment candidates can both be done on this platform [16].

Cell culture : It is well recognised that the human neuroblastoma SH-SY5Y cell line is an appropriate cellular model for biochemical studies on AD. Additionally, SH-SY5Y cells were identified as neurosteroid-producing cells that expressed essential steroidogenic enzymes. In order to ascertain whether neurosteroidogenesis might be an endogenous mechanism involved in the defence against neurodegenerative diseases, an innovative method was developed using SH-SY5Y cells. Stable transfection of SH-SY5Y cells with essential AD proteins such as human native tau (hTau40), mutant tau (P301L), and wild-type amyloid precursor protein (APPwt) was the initial step. The second phase involved investigating neurosteroid synthesis in native SH-SY5Y cells as opposed to cells that had been stably transfected with hTau40, P301L, APPwt, or control vectors using pulse-chase studies, high-performance liquid chromatography (HPLC), and continuous flow sscintillation [17].

Biochemical studies demonstrated that hTau40 boosted neurosteroid synthesis from the precursor pregnenolone, despite microscopic investigations showing that AD protein overexpression had little impact on the morphology of SH-SY5Y cells. APPwt overexpression caused a selective effect depending on each stage of the neurosteroidogenic pathways, and the pathogenic P301L mutation inhibited the stimulatory activity of Tau on neurosteroidogenesis. The study's data demonstrate that genetic engineering in SH-SY5Y cells enabled identification of a considerable influence AD-associated peptides exerted on neurosteroid production. Therefore, AD pathogenic variables may cause neurodegeneration by reducing the natural production of neuroprotective neurosteroids in nerve cells. The technical technique adopted here seems to be a potential method for clarifying the pathophysiological pathways behind neurodegenerative disorders [17].

III. CONCLUSION

Alzheimer's disease is a fatal neurological condition for which there is no known cure. Numerous promising treatments for Alzheimer's disease in humans have fallen short of their efficacy in rat models. While there is still hope that earlier intervention with current therapies can improve results, it is becoming more and more obvious that novel strategies are required to comprehend and treat the pathogenesis of Alzheimer's disease. To research various facets of the disease, numerous cellular models are employed. The fundamental obstacles associated with employing this technology and the efforts being made to address them are covered in this discussion of important elements of AD pathophysiology and how these models have helped to a greater understanding of AD molecular pathways. Although complex environment of the human brain, which includes non-neuronal cells that could be very important in AD disease as well as the connections between neurons, cannot be accurately modelled by cellular models. However, they can be useful tools for understanding mechanistic pathways involved in drug discovery and AD investigations.

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