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Review Article

Integrating epigenetic modification and stem cell therapy strategies: A novel approach for advancing Alzheimer's disease treatment – A literature review

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Abstract

Alzheimer's disease (AD) is the most frequent form of dementia and represents an increasing global burden, particularly in countries like Indonesia, where the population has begun to age significantly. Current medications, including cholinesterase inhibitors and NMDA receptor antagonists, have modest effects on clinical symptoms in the early to middle stages, but there is no curative treatment available so far despite progress. Activating or repressing epigenetic modifications, including DNA methylation, histone modification and microRNA regulation, appears to play an important role in AD development. These alterations further enact transcriptional changes relevant to the signature AD pathologies of amyloid-β deposition, tau protein malfunctioning, neuroinflammation, and neuronal death. Here, we discuss the feasibility of targeting these epigenetic alterations as a new treatment strategy due to the reversibility of epigenetics and their ability to correct faulty gene expression. We also review the combined promise of stem cell therapies and epigenetic modulation in neurodegeneration, inflammation and cognitive decline. This combined approach may provide a multifaceted strategy to slow disease progression, replace lost neurons, and restore neural function. Despite challenges, including ethical, financial, and methodological barriers, ongoing research in epigenetic modulation and stem cell therapy holds promise for pioneering therapies in AD.

Keywords: Epigenetics, Alzheimer's disease, methylation, histone modification, stem cell therapy

Introduction

Alzheimer's disease (AD) is the most common spontaneous neurodegenerative pathology, which represents 60–70% of total dementia cases globally, according to 2022 data from the World Health Organization and ranks as the sixth leading cause of death worldwide [1]. The number of dementia cases in Indonesia will increase due to growth and aging population [2]. In 2016, more than 1 million people suffered from senility, and this number is projected to reach at least 2 million by 2030 [2]. No curative treatment is available for AD yet, and the existing pharmacopeia such as cholinesterase inhibitors (donepezil, rivastigmine, galantamine) and N-methyl-d-aspartate receptor (NMDAR) antagonists such as memantine remain efficacious solely during early to middle stages of symptomatology.

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Received: June 3, 2024 - Accepted: November 19, 2024 - Published online: December 2, 2024

According to many studies, a very important role in the development and progression of AD is attributed to epigenetic changes such as DNA methylation, histone modification or regulation by microribonucleic acid (miRNA) [3-6]. These changes, in turn, affect the expression of genes crucial for AD processes: Amyloid-β accumulation, tau pathology, neuroinflammation, synapse dysfunction and neuronal cell loss [3]. Epigenetic alterations offer a fresh perspective on therapeutic intervention because they are reversible and persistent [6-8]. Previous studies have indicated that epigenetic drugs such as DNA methyltransferase inhibitors and histone deacetylases might reverse the pathological gene expression profiles in AD.

Further research is required to address the knowledge gaps in utilizing epigenetic modification and stem cell therapy as innovative treatments for AD. These gaps are mostly due to the intricate genetic structure that underlies the disease and the limited experience in implementing these therapeutic techniques. Here, we summarize the common themes of what still need to be studied as these gaps: mechanisms, efficacy and translational potential of these approaches. Thus, the aim of this study was to overview the implications of alterations in levels of epigenetic modifications as a novel therapeutic strategy for AD. We summarize how the dysregulation nature of adult hippocampal NSCs by these pathways interplay with other mechanisms involved in different aspects of disease progression. With these facts in mind, the research is focused on addressing current limitations of AD treatments, such as stem cell therapy or exploring how epigenetic modifications could render new and improved treatment strategies. Additional studies will be crucial in uncovering groundbreaking therapeutic methods for AD.

Etiology and pathogenesis of Alzheimer's disease

Alzheimer's disease (AD), the most common form of dementia, is a chronic and progressive neurodegenerative disorder characterized by a slow decline in multiple cognitive functions, with memory impairment being one of the initial symptoms [9]. Two main elements in understanding disease progression are the extracellular accumulation of Aβ plaques as amyloid deposition between nerve cells and intraneuronal aggregation associated with NFTs generated from clumps tragic tau protein residues [10,11]. These pathogenic characteristics interfere with the transmission of signals between neurons, affect nutrient delivery to neurons, and ultimately kill off these cells, causing loss of brain mass. Starting with memory disturbances, further and already significant cognitive impairments, together with loss of independence, are encountered [10,11]. Knowledge of this neurocircuit is paramount to creating therapeutics that target the mechanisms responsible for initiating Alzheimer's disease before it becomes clinically symptomatic.

Several factors, including genetic predisposition, lifestyle and environmental features, contribute to the onset of AD [6]. Although genetic mutations have been implicated in the etiology of familial AD, most cases are sporadic and manifest through a complex interaction between hereditary risk factors, lifestyle determinants and environmental exposures [6] with several potential modifiable lifestyle-related leisure-time components, such as diet intake [12], physical activity levels and cognitive engagement could influence late-onset or predominantly sporadic AD development [2,6,12]. Research has repeatedly shown that a good, balanced diet can reduce genetic predisposition; also, it might take longer before the symptoms start to happen.

The complicated nature of the etiology and pathogenesis of AD, as presented in **Figure 1**, highlights the multiple factors involved in the development of the disease. The primary molecular pathways involved in AD mostly revolve around the atypical buildup of Aβ plaques and NFT [13-15]. The Aβ peptide is derived from amyloid precursor protein (APP), and its propensity to aggregate into plaques is a characteristic hallmark of AD [16]. These plaques interfere with the transmission of signals between cells and trigger immunological responses, resulting in neuronal damage. In AD, the tau protein is normally responsible for holding microtubules together in neurons by phosphorylation of amino acids. This abnormal phosphorylation leads to the formation of NFT that interferes with neuronal transport, resulting in a sequence of neurodegenerative events. The interaction between Aβ plaques and NFTs plays a central role in the progression of AD, significantly contributing to cognitive decline followed by memory deficits [13,17,18]. This knowledge is essential in the context of AD precision medicine.

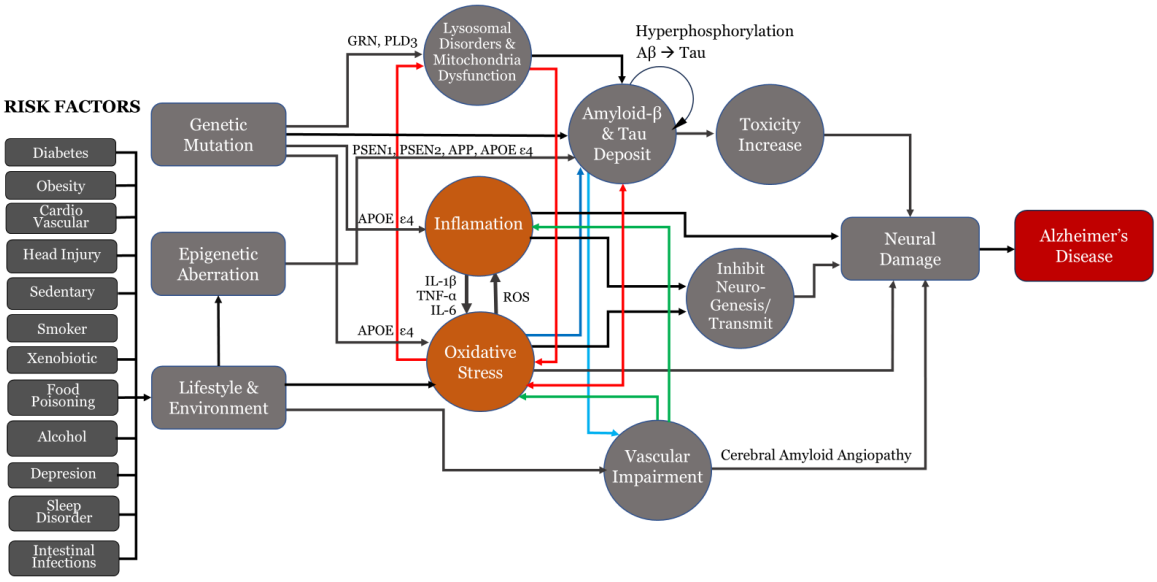

Figure 1. Etiology and pathogenesis of Alzheimer's disease.

Additionally, the occurrence of oxidative stress makes a point in favor of inflammation during AD pathogenesis [19]. This effect, which is linked to a higher production of reactive oxygen species (ROS), can lead to oxidative stress when the latter overcomes cell capabilities on its removal or repair. ROS can potentially cause damage to cellular components, including proteins, lipids, and DNA [20-23]. ROS and ROS-related derivatives trigger the activation of inflammatory pathways in AD within brain cells, including both neurons and glia, resulting in a "vicious cycle" between oxidative damage and inflammation. Oxidative stress plays an important role in the development of AD through its ability to increase amyloid-β peptide cytotoxicity and phosphorylated tau protein. This ultimately leads to the death of neurons and a decay in synaptic function [23]. Ultimately, this cycle exacerbates Aβ plaque and tau protein entropy, leading to cognitive dysfunction.

Microglia can be activated by Aβ plaques. This activation initially acts as a defense mechanism to get rid of Aβ. Nevertheless, chronic exposure to Aβ leads to microglia activation and becomes detrimental. Dysregulated microglial activity found in patients with AD exaggerates tau pathology, prompting the release of proinflammatory cytokines and ultimately leading to neuronal die-off surrendered [24-28]. Cytokines such as Tumor Necrosis Factor Alpha (TNFα), Interleukin-1β (IL-1β) and nitric oxide (NO) are released by activated microglia, which may either aggravate or mitigate neuroinflammation.

Dietary consumption of sugar may be associated with a higher risk for AD, possibly due to the synthesis of AGEs, as well as mechanisms underlying insulin resistance [29,30]. AGEs are harmful substances that develop in the bloodstream through chemical reactions between glucose, proteins and lipids (glycation), which do not require an enzyme intervention [30,31]. AGEs can also be generated in food, particularly high-fat, protein, and sugar-containing foods, as well as those being heated to high temperatures, such as through frying or baking [32,33]. AGEs are a contributing factor to the aging process as well as a direct causative agent for various pathologies, including diabetes, atherosclerosis, chronic kidney disease and AD.

Recent studies have shown that there is a unique type of cellular damage that occurs in the hippocampus during AD due to Cerebral Amyloid Angiopathy (CAA) [34-36]. CAA is developed by depositing amyloid on blood vessel walls in the brain to allow them to bleed easily [36,37]. Hence, CAA is potentially a cause of cerebral hemorrhages as well as dementia.

Interplay between reelin and epigenetics

The Reelin pathway is also involved in the development and maintenance of neuronal function (**Figure 2**). The Reelin pathway is one of the pathways that have been involved in AD

pathogenesis [38-41]. In addition, Reelin binds to the apolipoprotein E (ApoE), a serum lipoprotein vital for synaptic plasticity and neuron migration [43]. Impaired Reelin signaling, associated with oxidative stress, can result in the tau hyperphosphorylation and formation of NFTs seen in Alzheimer's disease [42-44]. This contribution to neuroplasticity is most pronounced in the human lineage, with an increasing capacity for associative learning required for advanced socio-cognitive behaviors. Oxidative stress, and possibly other factors, seems to lower the expression of genes associated with synaptic plasticity by accumulating epigenetic modifications [45]. This happens when proteins from the Reelin signaling system do not function properly after a decreased amount of Reelin associated with epigenetic alterations[44]. Given the range of immune functions already implicated in AD, it is conceivable that some alleles are predisposed under conditions (inflammation, toxins and oxidative stress, for example) that are etiologically relevant to AD susceptibility.

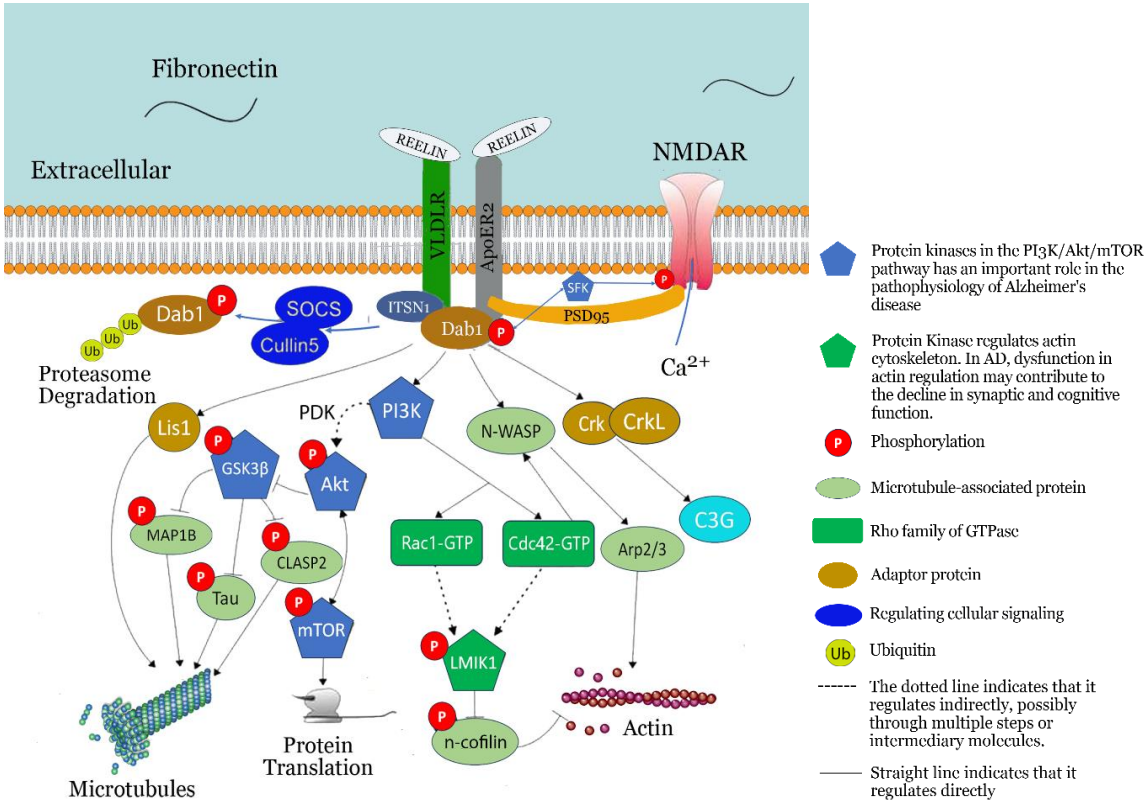

Figure 2. Reelin pathway - stabilization process, modified with permission from Santana and Marzolo [46].

In AD, feedforward effects of the Reelin pathway are abrogated, and amyloidogenic APP processing is activated, followed by Tau hyperphosphorylation to induce neurodegeneration. Protective effects on cognition can be elicited by normalizing Reelin-mediated signaling through treatment with Reelin or ApoER2 and may, therefore, represent a therapeutic potential for AD. In the following paragraphs, we will review the literature available to date on this extraordinary signaling cascade, known as the Reelin pathway, related to AD [46].

This cascade of molecular events starts with the binding of Reelin to its receptors and proceeds via different intracellular signaling molecules. Reelin acts by binding to its receptors, the lipoprotein tyrosine kinase receptor (LTR) proteins ApoER2 and VLDLR, located on the neuron surface. This leads to the phosphorylation of Disabled-1 (Dab1), an adaptor protein critical for intracellular signaling. Reelin binding to its receptors induces the phosphorylation of Dab1. This scaffold function of phosphorylated Dab1 mediates the recruitment and activation of a variety of effectors, such as PI3K or Src family kinases. Dab1 is an adaptor protein necessary for

transducing the Reelin signal from its receptors to intracellular targets in these and other cellular processes, such as neuronal migration or synapse formation.

PSD95 is a scaffolding protein that resides in the postsynaptic densities of neurons. It is essential for organizing synaptic signaling complexes. PSD95 binds to the receptors of Reelin and NMDA, thus regulating synaptic plasticity as well as stabilizing neuronal connections. ITSN1 is involved in endocytosis and actin cytoskeleton dynamics. ITSN1 links Reelin signaling to cytoskeletal rearrangements necessary for neuronal migration and spine morphogenesis. SOCS proteins are negative regulators of cytokine signaling. SOCS proteins can modulate Reelin signaling by interacting with components like Dab1, thereby providing feedback inhibition to fine-tune the pathway's activity. NMDAR is a type of glutamate receptor critical for synaptic plasticity and memory function. Reelin signaling enhances NMDAR activity, promoting synaptic strength and plasticity, which are essential for learning and memory.

Loading of AKT to the membrane occurs through the conversion of PIP2 to PIP3 by activated PI3K, mediated by phospho-dab1. Activation of PI3K is necessary for the activation of all signaling pathways that promote survival, growth and synaptic plasticity. Upon recruitment into the membrane via PIP3, mTORC2 and PDK1 both activate AKT. GSK3β is one of the key proteins phosphorylated by activated AKT, thereby inhibiting its role in growth arrest. GSK3β, a priming kinase involved in both anabolic and catabolic cell functions (e.g., metabolism, neuronal signaling), is inhibited by AKT phosphorylation, preventing deleterious effects on neuronal development and function. mTOR regulates the major signaling pathway, converting nutritional status into cell growth and protein synthesis. Downstream of AKT, mTOR is an important stimulator of protein synthesis and cell growth, which might allow it to meet the developmental as well as maintenance needs of neurons [38,39]. N-WASP is a molecule that aids in the remodeling of the actin-cytoskeleton. N-WASP is downstream of Dab1, and it has an important function in the regulation of cytoskeletal dynamics required for neuronal migration and neurite growth. This links extracellular signals from Reelin to the actin cytoskeleton reorganization that contributes towards modifying cell shape and motility, necessary for correct neuronal placement.

Reelin signaling influences microtubule stability and dynamics. The activation of MAPs such as CLASP2, which is a microtubule-binding protein responsible for regulating microtubule dynamics, allows this to occur [38,39]. Reelin signaling also controls the crosstalk between microtubules and actin filaments. This enables the activation of actin-binding proteins, such as CLASP2, which modulate actin cable dynamics [38,39]. The Arp2/3 complex is a central controller in the regulation of actin dynamics with important roles in both nucleation and branching of new filaments. Reelin signaling controls the Arp2/3 complex, an essential mediator of the Reelin pathway [39]. Actin-binding proteins like N-Cofilins can have an important role in regulating actin dynamics. The activity of N-Cofilins that are essential for the proper functioning of the Reelin pathway is controlled by phosphorylation through reelin signaling [38,39]. In addition, Reelin signaling promotes protein translation by inhibiting eIF4E. This occurs by triggering reactions of protein kinases, such as mTOR, to phosphorylate and activate eIF4E [38,39]. The mTOR pathway, which controls the activity of protein translation initiation factors like eIF4E, is implicated in coordinating stress and metabolic signaling to optimize cellular behavior [38,39]. Reelin signaling is also known to activate mTOR, which functions in protein synthesis and causes the phosphorylation of eIF4E for enhanced translation.

Reduced Reelin levels have been reported in AD, together with altered glycosylation [47,48]. Such dysregulation may impact epigenetic mechanisms that regulate the expression of genes required for preserving synaptic integrity and mediating neuroinflammation. Chronic inflammation itself (a property of AD) can also induce epigenetic changes that worsen the symptoms. As such, the anti-inflammatory effect of reelin may suggest its dysregulation contributes to an inflammatory environment that disrupts epigenetic regulation [49,50]. Reelin signaling may exert an epigenetic influence on gene expression related to cognitive function — a patron of epigenetic changes. Changes in Reelin levels, for example, could modulate transcriptional regulation of genes, causing synaptic plasticity through histone or DNA methylation [48,49]. The interaction of Reelin with epigenetic mechanisms could provide new therapeutic opportunities in AD where restoration of Reelin levels or enhancement of its signaling pathways may counteract the deleterious effects of AD-related epigenetic changes. The

relationship between the Reelin pathway and epigenetic mechanisms in AD is complex and multifaceted. Dysregulation of Reelin not only impacts synaptic health but also interacts with epigenetic modifications that may drive the progression of AD.

Epigenetic mechanisms in Alzheimer's disease

Developmentally, epigenetic pathways regulate cell differentiation and/or the transition from less specialized to more specialized types of cells. These pathways affect gene expression without directly changing the DNA sequence, typically through mechanisms such as chromatin remodeling (DNA methylation and histone modification) or the modulation of non-coding RNAs [51,52]. Such systems regulate gene expression by shuttling chromatin structure, affecting DNA accessibility to the transcription machinery.

In every cell, there is chromatin, which is composed of DNA wrapped around histone proteins as nucleosomes [53,54]. These chromatin accessibility changes are controlled by epigenetic mechanisms that either activate or suppress transcription [53]. Transcription occurs in euchromatin, an open form of chromatin that allows for gene expression, and transcriptionally silent heterochromatin. Hence, the bivalent state facilitates switching between silent and active states of transcription [53-55]. DNA-containing nucleosomes encode external chromatin accessibility, whereas a cadre of internal DNA changes or post-translational modifications on the globular domains of histone proteins within these nucleosomes determine whether these codes are executed. **Figure 3** presents some epigenetic alterations that influence gene transcription.

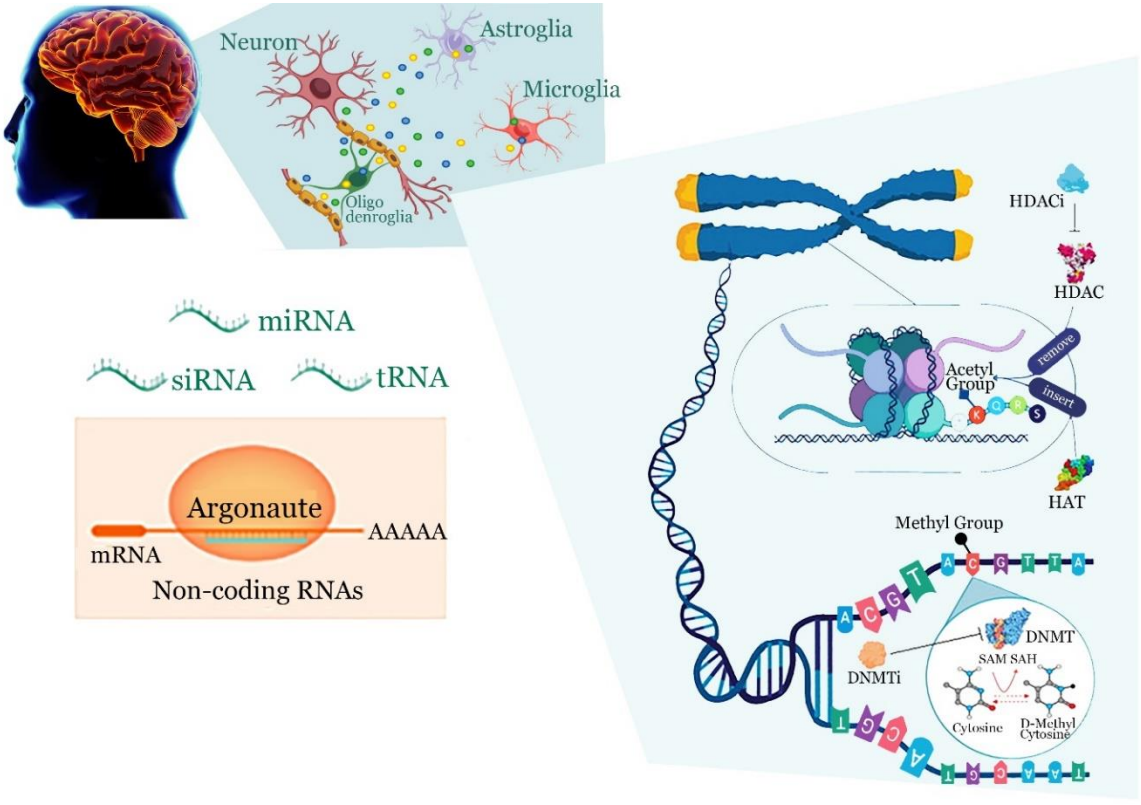

Figure 3. Epigenetic alterations (DNA methylation, histone modification, and microRNA) that influence gene transcription in Alzheimer's disease.

Regarding AD, over 20 epigenetic mechanisms have been discovered, primarily involving direct alterations to DNA (such as methylation), changes in chromatin structure (such as histone modifications), or modifications to mRNA-related processes, including ncRNA and miRNA [8]. Epigenetic markers in cell physiology include post-translational modifications of histone proteins and DNA methylation [56]. Chromatin remodeling enzymes and long non-coding RNAs (lncRNAs) also play crucial roles in the transcriptional control of epigenetics. These processes

often involve DNA methyltransferases (DMNTs) such as DNMT1, DNMT2, DNMT3, and recent reports on DMNT6 have now emerged [57]. Aging, associated with the accumulation of Aβ deposits, is a hallmark of AD and methylation at some cytosines (e.g., APP gene promoter region) [58,59]. Over-expression of the microtubule-associated protein tau (MAPT) can exacerbate brain pathology in animal models of AD with an epigenetic component to this process [58,59]. Many genes involved in AD (e.g., BIN1, HLADRB5, ABCA7, SORL1 and SLC24A4) have been shown to be specifically methylated at certain loci [60,61]. Methylation may be associated with increased Aβ levels by silencing the regulatory gene SORL1, which is involved in the generation of Aβ.

Histones, which give structural support to nuclear DNA and can be post-translationally modified by means of methylation or acetylation. The modification of histones, such as acetylation and deacetylation, mediated by enzymes like Histone Acetyltransferases (HAT) or Histone Deacetylases (HDAC), respectively, controls the compaction and distribution of the nucleosome wrapping DNA accessibility and regulating gene expression [62]. HDAC inhibitors such as valproic acid and sodium butyrate have demonstrated potential activities to ameliorate not only memory function in animal neurons [63], but also some neurodegenerative conditions, including AD. ncRNAs, including microRNAs (miRNAs), contribute to genetic silencing and regulation of gene expression [64]. They are predominantly present in the brain, where they contribute to neurodevelopment, neuronal migration, homeostasis, and plasticity.

Each type of ncRNA has distinct functions within the cell, and these functional connections are also complex [65]. MicroRNAs (miRNA) and Small Interfering RNAs (siRNAs) are involved in RNA interference pathways. tRNAs play a fundamental role in protein synthesis, whereas poly A (AAAAA) is one of the most important regulatory signals controlling gene expression and mRNA stability [65,66]. Understanding these interactions comprehensively is necessary for full understanding of cellular regulatory networks. Argonaute proteins are key to the RNA-induced silencing complex (RISC) and are essential for gene silencing, including siRNA and miRNA regulation.

DNA methylation

DNA methylation is an essential epigenetic mark formed by the addition of methyl groups to cytosine bases, usually at CpG sites, resulting in gene silencing. This process is carried out by DNMT enzymes, which can be further divided into de novo and maintenance categories. DNMT3a and DNMT3b are already known as de novo DNMTs (newly synthesized) - whereas the rest are mainly maintenance DNMTs (such as DNMT1) that maintain existing methylated islands [67]. Methylation is the most important role in stem cells differentiation, as genes required for pluripotency are silenced due to the methylation of their promoters and specialized cell function gene become demethylated so that they can be activated.

DNA methylation regulates the proliferation, differentiation, and maturation of neural stem cells, particularly in the hippocampus [51,68,69]. Altered DNA methylation pattern is a feature of AD, notably in the hippocampal and other AD-affected brain regions [4-6]. These altered forms of regulatory sequences could disturb gene regulation and drive disease progression. For example, changes in the methylation status of specific genes, such as APOE, have been identified to be associated with AD. APOE can lead to lowering their activity resulting during the lifespan and increasing risk for late-onset AD [70-73].

Importantly, DNA methylation is reversible through the action of Ten-Eleven Translocation (TET) enzymes that oxidize 5-methylcytosine (5mC) to other forms [74]. This may lead to the creation of euchromatin and stimulate active transcription.

Histon modification

Histones, the proteins involved in chromatin structure, are modified by post-translational modifications (PTMs) that influence their binding to DNA and nuclear factors. These modifications, such as methylation, acetylation, phosphorylation, and ubiquitination, are changes that alter gene expression in different ways [75]. For example, acetylation generates an open chromatin conformation that is conducive to transcriptional activation, while methylation can activate or repress gene expression depending on the site of modification as well as which specific amino acid residue is being modified [76].

The adult brain maintains a high level of plasticity due to the presence in it, differentiating with age yet reducing their numbers to be mature neural stem cells (NSCs) because of chromatin modifications. Restrictions in modifications like acetylation and methylation are important for maintaining pluripotency or directing differentiation [76-78]. This protein acts in the transcription of neuronal pro-genes due to their activity, including those that help keep stem cells and promote neurogenesis, exhibiting a fine-tuning role by carrying out specific targeted adjustments. Certain alterations help or hinder the activity of genes that are necessary for stem cell self-renewal as well as neuronal development.

One example is the change in histone acetylation, which has been traditionally associated with the activation of gene transcription and that becomes somehow altered during AD. For example, decreased histone acetylation has been related to impaired cognitive performance and memory loss in AD models [6,40,79]. HAT and HDACs (histone deacetylase) are involved in the addition or removal of acetylation on histones, respectively. Variations in HDAC activity have been suggested to be implicated in AD. This observation provides some targets for potential therapeutics to modulate gene expression pertaining to cognitive decline [6,79,80]. The exact same alteration of histone methylation can thus either repress or activate gene expression, depending on its combinatorial state and downstream location [6,40]. Altered histone methylation patterns associated with AD have also been reported in conjunction with disrupted synaptic plasticity and neuronal dysfunction.

Therapy for epigenetic in Alzheimer's disease

Considering the role of histone changes in the development of AD pathology, numerous treatment approaches have been suggested, such as HDAC inhibitors, histone methyltransferase (HMT) and demethylase inhibitors. HDAC inhibitors have demonstrated encouraging outcomes in preclinical investigations, enhancing cognitive performance in animal models of AD [6,79,80]. In fact, boosting memory and learning in mouse models of AD has been achieved through the administration of HDAC inhibitors that target gene expression programs impoverished in most cases [81-83].

These data might warrant the use of histone methyltransferase (HMT) and demethylase inhibitors in order to correct these identified aberrant methylation patterns in AD [84]. Although research on this topic is less advanced than that for HDAC inhibitors, they hold promise in the development of new AD therapeutics [7,40]. For the complex modifications and regulatory networks of histones, these treatment strategies may be more effective when combined with other therapeutic methods to tackle multiple facets of AD pathology. HDAC6 is a major target in the context of AD, as it has been correlated with amyloid and tau pathologies. Studies in animal models of AD have proven the benefit of HDAC6 inhibition to improve cognitive deficits and reduce pathological features. Effects of HDAC6 Inhibitor WT161 on Modulating APP Secretases and Aβ Deposition [85,86]. Additionally, recent findings highlight a novel small molecule inhibitor of HDAC6 that significantly reduces AD neuropathology by enhancing tubulin acetylation and regulating inflammatory responses.

DNA methyltransferase (DNMT) inhibitors, such as azacitidine and decitabine, are mainly used in cancer treatment to reverse abnormal patterns of DNA methylation. Such agents have shown promise in neurodegenerative diseases, including AD, potentially through epigenetic changes that modify pathologic mechanisms [87,88]. DNMT inhibitors are known to alter DNA methylation, and this may restore gene expression profiles characteristic of neurons, improving properties associated with normal neuronal function [88] and survival to ameliorate some part of the underlying neurodegenerative mechanisms.

Non-coding RNA

Recently, the emerging role of non-coding RNAs, including microRNA (miRNAs) and long non-coding RNAs (lncRNAs), in gene modulation during cell development has attracted significant attention. RNA molecules can direct chromatin-modifying complexes to specific sites in the genome, resulting in changes to histones and DNA methylation [51,89]. For example, specific miRNAs have been found to predominantly target transcription factors and other regulatory proteins that control cellular differentiation, making them bona fide regulators of the expression of genes involved in its regulation.

Non-coding RNAs, including miRNAs and lncRNAs, also participate in regulating adult NSCs and AD. In NSCs, multiple facets of stem cell fate are under the control of miRNAs, modulating from division to specialization and responsiveness to downstream signals [90,91]. These constitute short RNAs that can regulate the expression of genes in these processes by targeting specifically mRNA transcripts for degradation or inhibition of their translation.

In the global analysis of miRNAs, alterations in expression profiles are common findings during AD pathology, indicating a disruption in normal regulation by miRNAs with specific species being over-expressed as well as under-expressed within the brains of people suffering from AD. It is likely that these changes could change the way many of the genes involved in Aβ processing, tau hyperphosphorylation, as well as neuroinflammation are expressed and regulated. Taken together, these processes are important contributors to the pathogenesis of AD [90,91]. In addition, miRNAs were postulated to serve as potential biomarker tools for disease and therapeutic intervention targets.

This group of miRNAs, including miRNA-29a, miRNA-29b, and miRNA-29c, is known to regulate the expression of the enzyme BACE1, which plays a role in the formation of β-amyloid peptides [92]. Decreased expression of miRNA-29 has been associated with increased β-amyloid production, which is a hallmark of AD. miRNA-153 directly targets and reduces the expression of amyloid precursor protein (APP), thereby reducing β-amyloid production. Research shows that increased expression of miRNA-153 can decrease β-amyloid accumulation in cellular models [93]. miRNA-146a is involved in the regulation of inflammatory responses in the brain [94]. Increased expression of miRNA-146a has been observed in the brains of AD patients and is believed to play a role in modulating neuroinflammation associated with disease progression.

Integration of epigenetic mechanisms

Stem cell therapy combined with epigenetic modification may be a promising strategy for the treatment of AD. This strategy is also designed to address complex disease pathology more effectively than current medications. Current Alzheimer's disease treatments approved for use in mild to moderate stages comprise cholinesterase inhibitors (e.g., galantamine, rivastigmine and donepezil) that help alleviate symptoms by preventing the breakdown of acetylcholine, an important neurotransmitter brain organizer ward memory and thought [95]. Also, Lecanemab is FDA approved targeting beta-amyloid to reduce amyloid plaques, showing a benefit in slowing the cognitive decline and reduction of brain levels, which might have led to the Horne hypothesis [96]. However, it comes with risks, though; as an immunoregulatory gene therapy process, part of the protocol is to monitor side effects like brain swelling after receiving a large dose. Other treatments include sedatives, anxiolytics, anticonvulsants and antipsychotics to control symptoms of sleep disturbance, restlessness or wandering at night, agitation, aggression, and hallucinations [95]. Their side effects are severe, including a higher risk of death in certain older people with dementia.

The benefit of the combined approach is its potential to be more effective at treating Alzheimer's disease by preventing or reducing much of the underlying pathology. Restoration of stem cell therapy, whereby lost neurons are replaced or regenerated, and resetting epigenetic alterations due to genetic and epigenetic alterations in AD defense, offers a novel insight [63,97]. It involves dealing with both the context and connection of disease, giving a broader strategy for therapeutic management. Stem cell transplantation can replace lost neurons, and epigenetic modifications may repair gene expression patterns to normal function of cells, which in turn ameliorates the course of disease and outcome [98].

However, if we could do this, it would completely devastate the current cognitive performance of AD individuals—inducing lost neurons to repair and correct epigenetic dysregulation while at the same time lowering neuroinflammation. There is a near certainty that extra neural power or transplanted cells can be biomechanistic on all possible complexities for complementing tasks. However, more studies are needed to fully understand the involved mechanisms and translate these findings of therapeutic use in a clinical setting.

The potential benefits of utilizing stem cell therapy in conjunction with epigenetic alteration for the treatment of Alzheimer's disease

Increased neurogenesis and neuron replacement

There is potential to replace lost or damaged neurons in AD brain using stem cell therapy, thus alleviating AD-impairing cognitive functions [99-101]. By pre-differentiating, purifying and transplanting stem cells that have been epigenetically altered to increase the number of surviving grafts or which express factors promoting differentiation into neurons [8,101], it may be possible to enhance the efficacy of neuronal replacement strategies.

Epigenetically altered stem cell therapy for AD encompasses multiple pathways that contribute to its possible therapeutic advantages. Stem cells that have been transformed by epigenetic changes could differentiate into neurons [102]. These neurons can then merge with the existing brain tissue, fix damaged neural pathways, and replace neurons that have been destroyed. It is important for cognitive rehabilitation and AD progress delay. Neurotrophic factors have also been increased where BDNF and Fibroblast Growth Factors have been released by epigenetically modified stem cells. It enhances cell survival, raises dendritic spine density and strengthens cognitive performance. This is advantageous for the survival of the transplanted cells and, more importantly, restoration of cognitive function because epigenetic changes can decrease the levels of Aβ [102,103]. Furthermore, epigenetically induced alterations decrease levels of pro-inflammatory species; hence, an inflammatory signal to attenuate neuroinflammation is an important hallmark in the pathogenesis of AD after transplantation with differentiated stem cells.

Correction of epigenetic dysregulation

Epigenetic changes in AD have been reported, including alternations in DNA methylation and histone acetylation, which control gene expression and lead to abnormal protein production that could contribute to disease pathology [8,104]. Specifically, hypermethylation of promoters of neuroprotective genes and hypoacetylation of histones can result in the downregulation of essential neuronal functions and synaptic plasticity. These epigenetic alterations can both increase amyloid-beta plaques and neurofibrillary tangles formation, as well as directly modulate inflammatory responses or oxidative stress signaling pathways, promoting AD progression [105]. These modifications can be in the form of changes to DNA methylation and histone acetylation, which leads to a different gene expression pattern that has also been linked with disease pathology. They are important and involved in synaptic plasticity, along with neuroinflammation and oxidative stress, which contribute to major pathological events during AD [8,100,104] . This would correct the epigenetic dysregulation by restoring normal gene expression patterns and cellular functions, thus reversing disease progression or ameliorating its effects. The method of correction has been discussed above in therapy for epigenetic alterations in Alzheimer's disease.

Improved survival and function of transplanted cells

DNA methylation and histone modifications in transplanted stem cells; these epigenetic changes could allow for promoting survival, expansion of undifferentiated state or lineage re-routing into neurons [8,99,100]. This, in turn, promises to enhance the success of stem cell therapy towards AD treatment, as it will leverage that a greater fraction of transplanted cells will survive and contribute to neural repair and function.

Expression of the pro-survival genes Hif1a, Aktl, Bcl-2 or Bcl-xl have demonstrated a beneficial effect with human neural stem cell (hNSC) transplantation [105]. Histone modifications determine cell fate, and the increased H3K4me3/H3K27me3 ratio at the promoter of PPARγ2 resulted in reduced expression [106]. Such epigenetic modifications are critical for increasing the survival, integration and functionality of a greater fraction of transplanted cells, likely supporting neural repair and cognitive improvement in AD.

Nerve inflammation reduction

The development of AD is also associated with chronic neuroinflammation effects, leading to nerve damage and progression into the disease state [99,107]. Mesenchymal stem cells (MSCs),

in particular, have been demonstrated to inhibit inflammation in several studies [99,106]. Modification of these cells to be anti-inflammatory in AD may inhibit neuroinflammation, prevent neuronal degeneration and preserve higher cognition.

Targeted delivery of neurotrophic factors

Stem cells could be genetically engineered, for example, to overexpress neurotrophic factors like brain-derived neurotrophic factor (BDNF). This protein supports the survival and functioning of neurons [99,108,109]. Epigenetic modifications may regulate the expression of these factors in transplanted cells and improve their therapeutic efficacy for AD, partly owing to neuronal homeostasis (cell survival, synapse formation, synaptic plasticity).

Addressing multiple pathological aspects simultaneously

Stem cell therapy and epigenetic modification in combination may simultaneously solve most pathological aspects of AD. Specifically, this complex may combat neuronal loss, epigenetic dysregulation, neuroinflammation, and impaired neurogenesis [8,101,107]. Therefore, this solution may become more sophisticated than curing just one aspect of the disease. Epigenetic modifications have great therapeutic potential to alter the epigenetic status of cells in other ways and be used to prevent AD. Among the possible techniques, DNMT inhibitors can stop the enzyme from adding methyl groups to DNA to restore normal gene expression patterns [109]. Histone modification medication can influence histone modification, which works with DNA methylation to regulate gene expression and may have a therapeutic value [8,109], as well as nutrigenomic strategy in which dietary nutrients or bioactive compounds, such as folic acid and vitamin B12 [110], which are critical for DNA methylation during the one-carbon cycle and may help in DNA methylation concerning AD.

The link between stem cell therapy and epigenetic modification in Alzheimer's disease

Stem cell therapy and epigenetic modification are closely linked in the context of AD. Epigenetic mechanisms play a crucial role in determining the fate of stem cells and their ability to differentiate into mature cells, which is essential for treating neurodegenerative diseases like AD [102]. Stem cell therapy is a promising strategy in which diaphysis neuron replacement and the influence on the microenvironment of the brain are included. This offers the potential to differentiate them into target cell types, like neurons and glial cells, for these grafted stem cells can restore neural function and reduce AD pathology. Stem cells are able to affect epigenetic marks in the brain, which may be useful in AD amelioration [111,112]. For example, neural cells derived from stem cell-mediated therapy have the competence to reprogram epigenetic modifications in AD brain, aiding reversal of a subset or all pathological conditions associated with cognitive impairment.

The potential mechanisms underlying the efficacy observed following stem cell therapy for treating AD include neurogenesis and differentiation, immunomodulation with eventual reduction in inflammation, as well as epigenetic regulation. This positive impact can be seen clearly in the brain, as these cells together trans-differentiate into neurons, which then replace damaged or lost neurons to restore cognitive function. It is a highly regulated process that requires the execution of intricate epigenetic modifications to ensure normal neural function and connectivity. Moreover, stem cells can regulate immune responses and inhibit the inflammatory and oxidative stress observed in AD pathologies [111,112]. This may alter epigenetic marks, which consequently maintain a hospitable and healthier brain landscape.

Additionally, stem cells are known to produce numerous factors that modify the cellular microenvironment by altering DNA methylation or gene expression in adjacent cells. Mesenchymal stem cell-secreted factors can modulate the epigenetic status of neurons and glial cells within the brain with attendant improvements in function and regeneration [102]. It is known that there are multiple cytokines and growth factors secreted by stem cells, particularly mesenchymal stem cells (MSCs) [103,113]. These secreted factors can alter DNA methylation and gene expression in neighboring cells to modulate the cellular environment. These interactions are vital to maintain cellular homeostasis and support tissue regeneration [106]. DNA methylation

plays a central role in the regulation of gene expression, most notably and extensively studied for its implications in cellular differentiation and function.

AD is associated with a chronic inflammatory process. Stem cells, particularly mesenchymal stem cells, have been shown to have anti-inflammatory properties [114]. MSCs secrete bioactive molecules that modulate the epigenetic status of immune cells in the brain, thereby reducing inflammation. MSCs, through trans-differentiation, can become neurons or other types of cells necessary to replace damaged tissue in the brain [114]. During this process of differentiation, epigenetic modifications are used (e.g., DNA methylation) whereby molecules attach to the genetic material and thus activate or deactivate certain genes that form new nerve cells.

Although stem cell therapy shows great promise, the hurdles remain high, with several problems to be overcome before it can effectively be translated into clinical applications. This includes but is not limited to cell sources and methods of delivery, as well as long-term safety and efficacy requirements. More research is still needed to unravel the endogenous signals that stem cells use for conditioning these different epigenetic marks in AD. For anything more elaborate, the relationship between stem cell therapy and epigenetic modification in Alzheimer's requires a better understanding [5,61,112]. Because stem cells possess the potential to differentiate into neurons, attenuate immune response, and potentially provide a means for altering epigenetics as well, all therapeutic interventions that have been directly linked with reversals of some disease-related changes in AD.

Challenges in combination of stem cell therapy and epigenetic modification

Understanding and controlling epigenetic mechanisms

A primary problem is the fact that epigenetic regulation processes are complex. Gene expression is regulated by epigenetic modifications, such as DNA methylation, histone modifications, and chromatin remodeling [115]. Control of these modifications in stem cells so that they are not detrimental when the stem cell is transplanted to an AD patient and behave like a neuron as well as be exploited appropriately for neurodegeneration prevention [116].

Although histone modification shows promise as a therapeutic approach for AD, there are still significant obstacles that need to be addressed: (1) several histone-modifying enzymes participate in several physiological processes, exhibiting specificity and potential side effects. Consequently, in order to prevent negative consequences, medications that aim at these enzymes must possess a high level of specificity to avoid unintended effects on other targets [117]; (2) progressing from preclinical models to successful human treatments requires overcoming substantial challenges, such as enhancing drug delivery to the brain and proving safety and effectiveness in humans [7,117]; (3) investigating histone modification is a crucial field of study for comprehending and potentially addressing AD [117].

Therapeutic approaches targeting these gene expression changes are under development at preclinical and clinical stages. More studies are needed to better understand the complex epigenetic landscape of AD and improve these therapies for maximum therapeutic efficacy with minimum adverse effects.

Ensuring safety and efficacy

Other than the scramble to determine whether stem cells could act alone in this case, it has not been completely verified that a fix for Alzheimer's is coming from changing our genes. There is a possibility that transplanted stem cells could divide out of control and form tumors [99,118]. Equally critical are studies on the long-term survival and engraftment of these cells in the brain, whether they properly integrate into existing neural circuitry, and how well such connections form [99,101]. In stem cell therapy, however, one of the biggest challenges is how to keep these cells from growing wildly and causing cancer.

Technological and methodological limitations

Current technologies and methods may not be adequate to fully utilize the potential of combining stem cell therapy with epigenetic modification [116]. Moreover, the differentiation of these cells

into the specific types of neurons required to treat AD requires precise control, which is difficult to achieve [116,119]. This detail of the brain's microenvironment and demands for particular signaling pathways that are necessary to instruct a differentiation program are considerable hurdles. Moreover, the long-term stability and incorporation of these engineered cells into physiological circuits are still questioned, in addition to their safety [120]. Such barriers will require advanced tools and methodologies to be able to treat AD more effectively.

Regulatory and ethical issues

The ethical implications of stem cell use must be considered. Moreover, the legal situation for this type of advanced therapy is often quite difficult and can differ significantly between regions, even within one country, which might slow down research as well as application [118]. Hardline regulations and multiple clinical trials are needed to pass cell-based therapies as safe, legitimate tools for human use. Besides, therapeutic misconception (the notion that study patients will derive direct medical benefit from trial participation) also requires appropriate informed consent [121] and clear communication to minimize misunderstanding.

The inhibition of certain signaling pathways can be taken as a strategy to prevent the survival and metastasis of cancer cells. However, until now, it continues to show side effects, just like chemotherapeutic agents. Thus, we need multi-regulated preclinical studies for toxicity and tumorigenicity followed by various phases of clinical trials to prove safety, which are necessary before larger-scale application. These regulations are good and necessary for patient protection. However, it also has the effect of delaying the development and approval of new therapies [121]. It is an important and still unfulfilled challenge in the history of stem cell research for Alzheimer's disease treatment combining therapy progress with ethics, which must be weighed together with safety.

Lack of comprehensive models

A major hurdle is the lack of comprehensive, physiologically relevant models that accurately mimic the complexity of AD pathology. This limitation makes it difficult to predict how stem cell therapies, modified by epigenetic changes, will work in human patients based on preclinical models [119]. Challenges in the stem cells and epigenetic modifications research for AD have always been an attractive but tough nut to crack, especially with respect to comprehensive animal models [99,122]. AD is a multifactorial and complex disorder related to genetics, environment, and lifestyle [122]. That is evidenced by amyloid-beta plaques, tau tangles, neuroinflammation, and neuronal loss [8,122]. As a result, it has been appreciated that there are manifold pathological features which will be extremely complicated to recapitulate faithfully in animal models [99,122]. The key features of AD pathology (in the sporadic form occurring late-onset) are human-specific and are not seen as part of a naturally occurring process or disease state for any co-existing diseases [122]. For instance, although some mouse models can be engineered to develop amyloid plaques in a gene-specific process, they often lack the neurofibrillary tangles and accompanying neurodegeneration that typifies human AD.

Although great strides in 3D cultures of neurons and organoids have brought us close to replicating the brain environment of mammals, such models still do not comprehensively account for all aspects of AD pathology found in mammalian hosts [119]. Further, such models are often associated with challenges, including between-batch variability, incomplete recapitulation of disease progression and nutrient diffusion problems in large organoids that cause necrosis at their centers [119]. Thus, the number of new treatments developed to assess their safety and efficacy has overwhelmed these models' predictive potential. Better models are needed to gain insight into the mechanisms of disease and for therapeutic development.

Functional integration and recovery

The challenge, however, is not only to ensure that stem cells transplanted into the brain survive but also integrate with the existing architecture of pluses and minuses such as they were wired together before. The cells must make synaptic functions and respond correctly to neurotransmitters in order to restore cognitive function after AD [99,101]. The critical feature of

successful therapy is the fact that these are integrated with the host brain tissue, and they function in harmony with their surrounding nervous system.

Financial and resource intensity

The development and implementation of treatments involving stem cell therapy and epigenetic modification require large financial and resource investments. Research, development, and clinical trials are expensive and time-consuming, which can be a barrier to progress [99,101]. This financial burden can be a significant barrier to progress, particularly for small research institutions and startups without extensive funding.

Stem cell culture, particularly induced pluripotent stem cells (iPSCs), needs expensive equipment and reagents, along with devoted skills. Establishing the differentiation of these cells into disease-relevant neuronal subtypes for a complex disorder like Alzheimer's is very difficult and time-consuming, often requiring many months to finely tune [123]. Sterile conditions, high-quality culture media and growth factors, as well as other reagents, are expensive to generate [123].

Also, the cost is even higher through epigenetic research. High-throughput sequencing technologies used for the analysis of DNA methylation patterns or histone modifications often come with massive investments in terms of costs (sequencing platforms and consumables) but also computing power [5,8]. Anyone who has run or analyzed modern next-generation sequencing (NGS) experiments knows that the resulting massive amounts of complex data are resource-intensive to manage and analyze due to the substantial computational endeavors as well as storage capacities [5,8]. This branch of science requires the use of specialized laboratory facilities with modern technology to study both stem cells and epigenetic factors. These resources include grade B clean rooms for stem cell culturing, high-security storage of genetic materials, state-of-the-art equipment used in molecular biology techniques, and highly trained workers, all contributing to the total cost.

This is frequently followed by in vivo validation using animal models, which necessitates keeping the transgenic animal colonies and incurs additional financial costs compounded with logistical hassles [123]. Obtaining grants from governmental bodies, non-profit organizations and private foundations funds for Alzheimer's research is very competitive. Applying for grants is time-consuming, difficult, and there is no certainty about whether funding will be secured. AD is such a slowly developing condition, it could be many years before the effects of any treatments can be measured. Therefore, it requires long-term funding that is difficult to secure.

Furthermore, the complex nature of Alzheimer's disease demands interdisciplinary collaboration among multiple scientific disciplines, such as neurology, genetics, molecular biology and bioinformatics [123]. Tissue has to be collected, processed, sorted and shipped across the globe in order for a local laboratory to do useful experiments as part of their studies, yet these resources are not simply free grants that can be allocated without consequence; this is a time-consuming, costly and logistically challenging undertaking (including tissue collection) which may only end up providing negative results or limited novel insights, adding little value, if any.

In conclusion, the research is expensive and resource-intensive, with regulatory and ethical issues. Strict regulatory and ethical guidelines need to be followed, even more so when working with patient-derived samples or modified genetics, requiring additional oversight and resources.

Conclusions

Even though epigenetic modification-based therapies are promising, they remain a subject of continued exploration and experimentation. Further clinical studies are, of course, required to evaluate their usefulness and safety in the context of AD. This review is designed to provide readers with the more recent advances in studies that aim for better understanding of epigenetic patterns occurring during AD and develop drugs able to effectively target these disrupted pathways.

Animal studies suggest that the existence of adult stem cells in specific areas within organ tissues offers immense promise because they frequently reproduce to create mature cell types when therapeutic intervention is urgently needed. In addition to replacing lost cells, stem cell

therapies could even engage the cellular milieu within the damaged brain in ways that elicit protective and regenerative responses through epigenetic means. Further investigations are needed to refine stem cell delivery methods in the context of AD and understand completely how their use impacts epigenetic regulation. The future research domain in AD therapy is to synergize stem cell therapy with epigenetic interventions.

Ethics approval

Not required.

Acknowledgments

None to declare.

Competing interests

All the authors declare that there are no conflicts of interest.

Funding

This study received no external funding.

Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

Widaja E, Pawitan JA. Integrating epigenetic modification and stem cell therapy strategies: A novel approach for advancing Alzheimer's disease treatment – A literature review. Narra J 2024; 4 (3): e935 - http://doi.org/10.52225/narra.v4i3.935.

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