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Review

Stem cell-free therapy for healthy brain aging: Mechanisms, challenges, and prospects

Jahnavy Madhukar Joshi [1], Shweta Verma [1], Raviraja Neelavar Seetharam [*], Abhishek Kumar Singh [*]

Department of Biotherapeutics Research, Manipal Academy of Higher Education, Manipal, India

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ABSTRACT

The stem cell secretome, which includes bioactive molecules and extracellular vesicles (EVs), has been reported to have neuroprotective effects in various neurological conditions. Current research indicates that secretome constituents, particularly EVs, can regulate pathways related to aging hallmarks and are therapeutic targets for brain aging. EVs can traverse the blood-brain barrier (BBB) and transfer neuroprotective cargoes such as proteins, peptides, miRNAs, and lipids to aged neural tissue. By acting on inflammation, apoptosis, mitochondrial damage, and cellular senescence, the secretome can restore neural homeostasis and induce neurogenesis and angiogenesis. While stem cell therapy is hindered by the risk of tumorigenicity and immune rejection, secretome and EV-based acellular therapies are safer and possibly more targeted choices. The content and delivery optimization of such vesicles to influence significant regulatory pathways of aging is currently of interest. This review highlights the dual importance of mechanistic insights and translational perspectives and discusses our current understanding of how the secretome, along with EVs, regulates hallmarks of brain aging with practical aspects of clinical application. The present review provides a novel and comprehensive analysis of current knowledge on stem cell-derived secretome and EVs as targeted modulators for healthy brain aging, with a critical evaluation of translational hurdles and clinical implementation.

1. Introduction

Aging is an imminently complex process characterized by structural, functional, and physiological decline. There are more than 300 aging theories proposed to date. Among all the theories, oxidative stress, mitochondrial function, and senescence have garnered increasing attention. Cellular and molecular levels of aging can be characterized by accumulated damage, altered gene expression, loss of proteostasis, stem cell exhaustion, and metabolic alterations. It is driven by numerous pathways of sustained oxidative stress associated with reactive oxygen species (ROS) overproduction [1]. Aging affects three levels: molecular, cellular, and systemic alterations. According to the National Council on Aging, approximately 92 % of aged individuals have an age-related ailment, whereas 77 % exhibit two or more. The most common conditions observed are neurological diseases, heart disease, diabetes, ischemic stroke, degenerative joint diseases, skin and hair deterioration, and even cancer.

The hallmarks of aging as defined by Lopez-Otin et al. include telomere erosion, genome instability, changes in epigenetic makeup, dysregulation of the proteome, mitochondrial dysfunction, cell death, nutrient-sensing deprivation, depletion of stem cells, and improper intercellular communication (Fig. 1). All these factors contribute to accelerating neurodegenerative symptoms [2]. In Alzheimer's disease (AD), the accumulation of p-tau and amyloid-β plaques is the main cause. The disease progression of AD is caused mainly by inadequate telomere shortening, epigenetic modifications, dysfunctional mitochondrial function, and senescence of astrocytes [3]. Current AD therapies focus primarily on the inhibition of acetylcholine esterase; however, these treatments cannot manage AD disease progression. Aging pathways, such as neuroinflammation, mitochondrial stress, and proteostasis, are well known to contribute to Parkinson's disease (PD). The risk of PD is also known to increase due to defects in DNA repair, alterations in epigenetic signatures, insufficient nutrient sensing, and cell death [4]. In addition to these prevalent diseases, other

* Corresponding authors.
E-mail addresses: raviraja.ns@manipal.edu (R.N. Seetharam), abhishek.singh@manipal.edu (A.K. Singh).
[1] These authors contributed equally.

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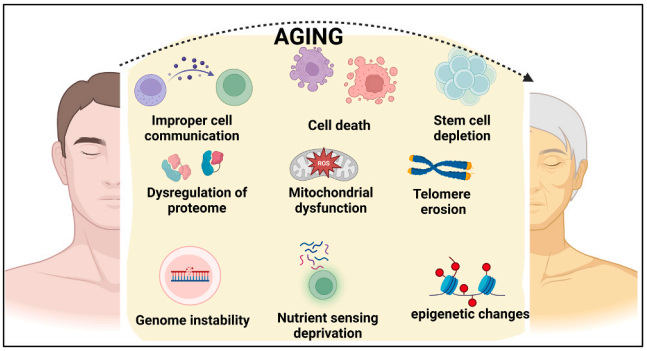

Fig. 1. Major hallmarks of aging.

neurodegenerative diseases, such as ataxia telangiectasia, Huntington's disease (HD), spinal muscular atrophy, motor neuron disease, and amyotrophic lateral sclerosis (ALS), have been linked to cell senescence and neuroinflammation [4]. Therapies used to address the effects of aging include the inhibition of cell death pathways, the triggering of mitophagy, and drugs that increase the level of NAD^+, which is known to increase the extent of the DNA repair process. In addition, inflammation is a driver of neurodegenerative diseases, and anti-inflammatory drugs are also being applied to help alleviate inflammation in these diseases. The abovementioned contributors are now being targeted, and exploring these avenues may lead to more effective therapies.

The current strategies used to delay the process of brain aging and treat neurodegeneration include calorie restriction, good sleep quality, regular exercise, and lifestyle changes [5]. For example, the correlation between caloric restriction, decreased plaque formation, and increased autophagy has been studied in an *in vivo* AD model [6]. In the elderly population, compromised sleep quality has been linked to an increased risk of heart disease. However, lifestyle changes may prove to be inadequate for forestalling age-related conditions.

There is a need to identify new therapies for brain aging, as age-related neurodegeneration is complex. Stem cells and their derivatives are being widely investigated in current research. Although stem cell therapies hold great promise, their clinical application remains limited due to challenges such as immune rejection, injection site complications, and variability in therapeutic outcomes. One way to resolve these issues is to utilize the stem cell secretome, which is a heterogeneous blend of proteins, free nucleic acids, and EVs. These bioactive molecules might provide a safer, more homogeneous alternative to direct stem cell transplantation and related disadvantages. This review discusses the therapeutic value of the stem cell secretome and EVs in targeting brain aging and the underlying molecular mechanisms leading to neurodegeneration.

2. Changes associated with brain aging

2.1. Blood-brain barrier (BBB)

The BBB is a selectively permeable interface between the brain and the bloodstream that tightly regulates the exchange of substances and maintains homeostasis in the central nervous system. BBB is compromised during aging, which leads to neurodegenerative diseases. Transport mechanisms across the BBB include passive diffusion, paracellular transport, active efflux transport, carrier-mediated transport, receptor-mediated transport, absorptive-mediated transport, or ion transport [7]. The BBB is composed of a neurovascular unit containing various cell types, such as endothelial cells, astrocytes, neurons, vascular smooth muscle cells, and pericytes, all of which perform various functions. In a healthy BBB, there is restricted paracellular diffusion, and a high degree of transendothelial electrical resistance is maintained [8].

A major cause of BBB breakdown is endothelial cell degradation and decreased expression of tight junction proteins [9]. These endothelial cells, in turn, contribute to an increase in leukocyte adhesion molecules, which leads to the migration of $CD4^+$ lymphocytes to the brain. The interactions between endothelial cells and migrated immune cells may also elevate proinflammatory cytokines and ROS production. This enhanced inflammation increases BBB disruption [10]. However, *in vivo,* cognitive decline follows sustained inflammation rather than being solely attributed to BBB disruption [11]. Inflammation triggers a cascade of alterations, such as glial cell activation, immune cell infiltration, disruption of BBB integrity by changes in paracellular and transcellular transport, and changes in tight junction protein expression [12]. Aging in mice is associated with increased IgG infiltration into the brain tissue, diminished levels of occludin, and a decline in pericyte coverage along the vasculature [13]. A change in the function of the GLUT1 transporter leads to a decreased uptake of glucose via the BBB [7]. Although these changes are aspects of healthy brain aging, they are often amplified and detrimental in neurodegenerative disorders.

2.2. Microglial polarization and neuroinflammation

Within the CNS, microglia are the predominant immune cell type and a part of the innate immune system, accounting for 16 % of the parenchymal cellular content of the brain [14]. These cells act by countering damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) via the secretion of neurotrophic factors and phagocytosis. The biomarkers used to identify microglia are Iba1, CXCR1, CD200R1, EMR1, CD45, CD11b, and CD172a, which can help distinguish them from other resident cell types [15]. Resting microglia are highly motile with filopodia-like projections, which allow for continuous immune surveillance. Increased levels of DAMPs and PAMPs trigger a more robust and engaged phenotype in microglia [16]. The secretion of chemokines such as CCL12, CXCL10, CCL4, CCL5, CCL3, CCL2, and CCL1 by these activated microglia contributes to neuroinflammation. Although microglia can be classified broadly into resting M0, activated proinflammatory M1, and activated anti-inflammatory M2 phenotypes, transcriptomic data suggest a more versatile nature of microglial phenotypes, such as activated response microglia, lipid-accumulating microglia, damage-associated microglia, and neurodegenerative-phenotype microglia, which cannot be bracketed within the confines of traditional microglial divisions [17]. During age-related neuroinflammation, microglia exhibit downregulation of homeostatic genes and increased expression of apolipoprotein E (Apo E) [18].

The ApoE-mediated expression of various genes coordinates various metabolic pathways, mainly concerning lipids, and induces the phagocytic actions of neurodegenerative-phenotype microglia. CNS resident microglia are meningeal macrophages, choroid plexus macrophages, and perivascular macrophages, which perform various functions ranging from defense against pathogen attack to suppression of endothelial damage during neuroinflammation and protection of BBB integrity [19]. The importance of microglia in neuroinflammation has been demonstrated by a study showing perivascular microglia decline, impairing leukocyte migration, angiogenic signaling pathways, BBB stability, and neural function [20].

2.3. Neurogenesis and vascularization

Neural stem cells (NSCs) give rise to numerous neurons during neurogenesis in the embryonic stage; however, this capacity decreases as aging progresses. A decrease in neurogenesis has been linked to decreased expression of fibroblast growth factor (FGF), epidermal growth factor (EGF), and insulin-like growth factor-1 (IGF-1), and external supplementation with EGF and FGF has been shown to restore NSC proliferation [21–24]. Growth differentiation factor-11 (GDF-11) facilitates cerebrovascular integrity and restores neurogenesis in aged mice [25].

Proteostasis is essential in managing the proliferation and differentiation of NSCs. In the adult brain, resting NSCs need to switch to activated NSCs (aNSCs), allowing self-sustenance or division into transit-amplifying progenitors (TAPs) to facilitate neurogenesis. A study demonstrated that aNSCs have effective proteasomal degradation compared with quiescent NSCs (qNSCs), which have protein aggregates stored in large lysosomes. The enhancement of the lysosomal pathway by pharmacological agents can restore its activation capacity [26]. The age-related reduction in neurogenesis can also be attributed to mitochondrial dysfunction. Aging is associated with a decrease in mitochondrial abundance and oxygen consumption, despite stable mitochondrial DNA mutation rates in neural progenitor cells, as observed *in vitro* [27]. Hypoxia-inducible factor-1α (HIF-1α) is upregulated under hypoxia and is known to facilitate NSCs' proliferation. It regulates adult neurogenesis by inducing the proliferation and differentiation of NSCs and progenitor cells via the Wnt/β-catenin pathway [28]. A decrease in the proliferative capacity of NSCs has also been linked to bone morphogenetic protein-5 (BMP-5) and IGF [29].

3. Role of the stem cell secretome and EVs in age-related neurodegenerative disorders

Progress in the field of neurological disorders has led to progress in stem cell-based therapies. Ongoing research has demonstrated that the therapeutic effects of stem cells are driven by their secreted bioactive factors, which include the secretome and EVs, rather than their ability to replace damaged cells directly. This has diverted the focus toward cell-free approaches that harness the neuroprotective, regenerative, and immunomodulatory effects of stem cell-derived products without the complexities associated with cell transplantation. Such strategies are being explored as safer and more practical in addressing neurodegenerative and neuroinflammatory diseases [30,31] (Fig. 2).

The secretome refers to a complete array of biomolecules secreted by cells in a cell's microenvironment. The secretome includes soluble proteins such as growth factors, cytokines, chemokines, proteases, extracellular matrix components, signaling peptides, lipids, and EVs [32]. Growth factors, such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), vascular endothelial growth factor (VEGF), and hepatic growth factor (HGF), promote cell survival, proliferation, and tissue regeneration. Cytokines and chemokines, such as interleukins, tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and monocyte chemoattractant protein-1 (MCP-1), regulate the immune response and inflammation. The anti-inflammatory molecules present in the secretome include transforming growth factor-beta (TGF-β) and prostaglandin E2 (PGE2) [33].

EVs are small spherical secretions that are surrounded by a phospholipid bilayer membrane and are involved in intracellular communication, as they can act as cargo for various biomolecules, such as proteins, lipids, and nucleic acids, across the body and influence target cell activity [34]. EVs are categorized primarily based on their size and biogenesis into exosomes from size 30 nm to 150 nm originating from the endosomal system as intraluminal vesicles inside multivesicular bodies released via exocytosis; micro vesicles sized 100 nm to 1000 nm buds directly from the plasma membrane; apoptotic bodies from 500 nm to 2000 nm released during programmed cell death [34]. Distinct biogenesis pathways confer EVs with unique molecular cargos and surface markers, influencing their biological roles and therapeutic potential. Once released, EVs facilitate intercellular communication by delivering bioactive cargo directly into recipient cells through mechanisms such as receptor-mediated binding, membrane fusion, or endocytosis, thereby modulating intracellular signaling pathways [35]. The main components of EVs are proteins, lipids, and nucleic acids. Generally, the proteins present in the plasma membrane and cytosol are found on EVs, whereas proteins from the Golgi apparatus, endoplasmic reticulum, nucleus, and mitochondria are not found actively on EVs. Metabolic enzymes, cytoskeleton proteins, heat shock proteins, annexins, tetraspanins, etc., are some of the proteins commonly found in EVs.

The specific functions of MSC-derived EVs can be divided into three groups: common proteins, enzymes (generally involved in glycolysis), and signaling molecules (such as cytokines, growth factors, ILs, and

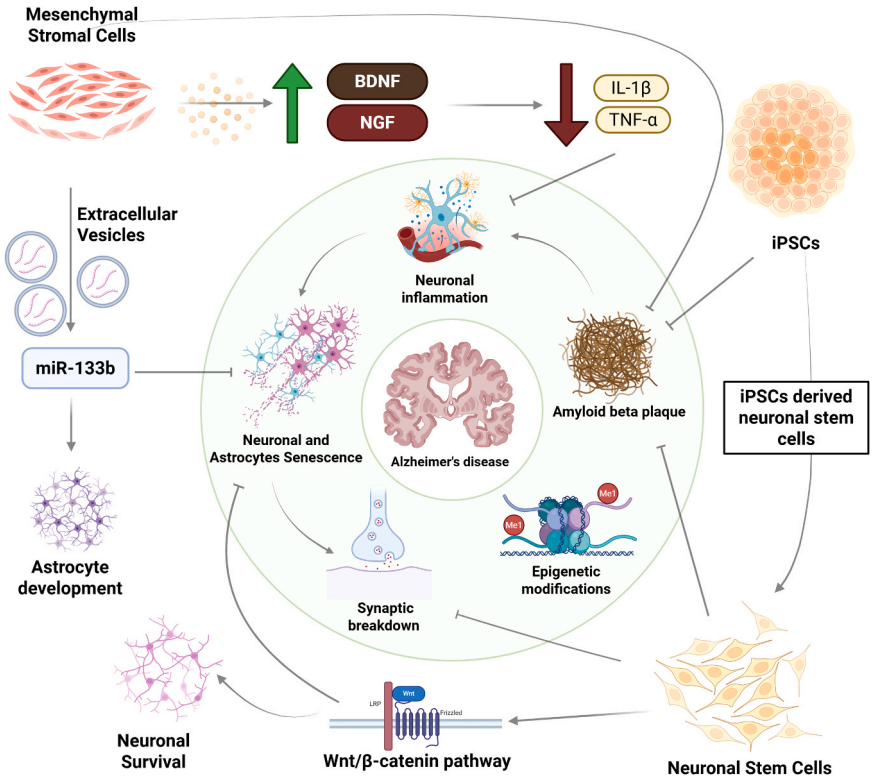

Fig. 2. Systemic representation of the mechanism by which the stem cell-derived secretome modulates Alzheimer's disease.

chemokines) [36]. MSC-EVs commonly contain proteins such as CD9, CD63, CD82, CD81, HSP70, MHC-I, and MHC-II, along with cell motility and structural proteins, including tubulins, myosin, and actin [37,38].

EVs are lipid-rich, and the presence of a type of lipid depends upon the source cell and the microenvironment around the EV. To pass through a biological membrane, escape from phagocytosis in the reticuloendothelial system, and protect against carried information, lipids are essential for EVs. Phospholipids, cholesterol, diglycerides, phosphatidylserine, phosphatidylcholine, phosphatidylinositol, polyglycerol, and phosphatidylethanolamine are some commonly found lipids in EVs.

EVs contain nucleic acids such as coding RNA, noncoding RNA, and DNA fragments. The presence of DNA fragments is common in apoptotic bodies, but their function is still not clear, whereas cRNAs and ncRNAs are abundant in exosomes and microvesicles. Two types of ncRNAs, which are small noncoding RNAs and long noncoding RNAs (lncRNAs), are smaller than 200 nucleotides in size and are longer than 200 nucleotides. lncRNAs are found in the largest proportion of genes, but their functions, other than tumorigenesis, are still not fully known. On the other hand, various miRNAs, mitochondrial RNAs, piwiRNAs (pRNAs), snRNAs, snoRNAs, tRNAs, Y-RNAs, and vault RNAs are some of the sncRNAs that have various therapeutic effects [35,39].

A notable advantage of EVs is their ability to cross the BBB, which becomes increasingly permeable and dysfunctional during aging and neurodegenerative disease progression. EVs traverse the BBB via mechanisms such as receptor-mediated transcytosis, enabling the delivery of therapeutic cargoes such as neuroprotective proteins, miRNA, and mitochondrial components directly to neural tissues. In aged brains, EVs can modulate hallmarks of aging, including neuroinflammation, mitochondrial dysfunction, oxidative stress, and cellular senescence, by attenuating inflammation through the transfer of anti-inflammatory cytokines and miRNAs. By promoting neurogenesis and synaptic plasticity by delivering growth factors and miRNA clusters such as miR-133b and miR-17–92, they enhance mitochondrial function and reduce oxidative damage via mitochondrial RNAs and antioxidant proteins [40]. EVs contribute to the regulation of microglial phenotype by reducing the proliferation of the detrimental M1 phenotype.

3.1. Alzheimer's disease

The disease progression of AD is caused mainly by inadequate telomere shortening, epigenetic modifications, dysfunctional mitochondrial function, and senescence of astrocytes [3,41] (Fig. 3). Current AD therapies focus primarily on the inhibition of acetylcholine esterase; however, these treatments cannot manage AD disease progression. Cell death resulting from aging cannot be reversed; hence, growth factors and EVs in the secretome may help facilitate this parameter. The bovine UC-MSC-derived secretome upregulated brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) while simultaneously downregulating inflammatory factors such as IL-1β and TNF-α, and increased neuronal protection in a rat AD model [42]. Few studies have indicated that MSC-derived EVs contribute to neurite development, possibly by delivering miR-133b to neurons *in vivo* and *in vitro* [43]. Evidence has shown that hUCB-MSCs can counteract the synapse loss that is induced by Aβ42 *in vivo*, highlighting their potential to shield against thrombospondin-1-induced synaptic breakdown [44]. The

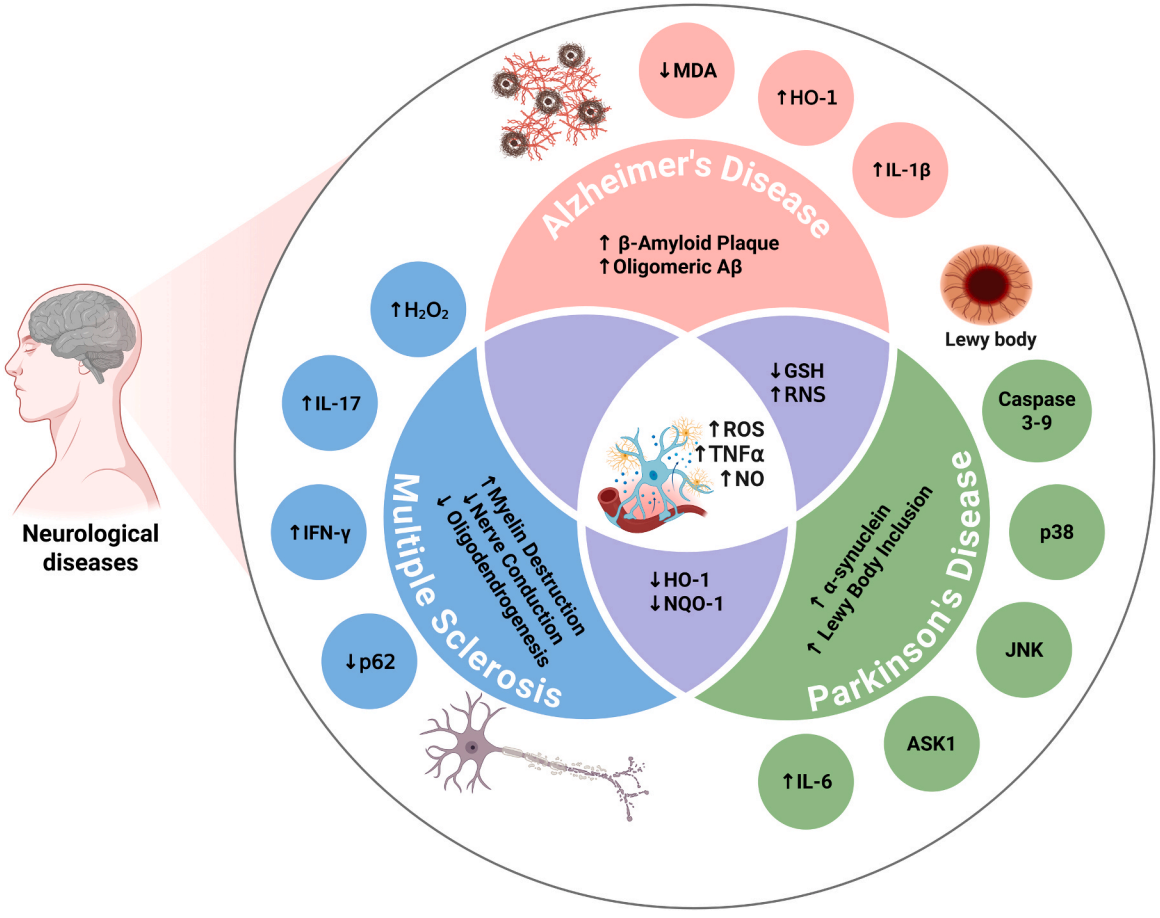

Fig. 3. Schematic representation of cytokines and growth factors involved in the pathophysiology of neurological disorders. [**Abbreviations:** IL-6: Interleukin-6; ASK1: Apoptosis signal-regulating kinase 1; JNK: c-Jun N-terminal kinase; IL-1β: Interleukin-1β; HO-1: Hemeoxygenase-1; MDA: malondialdehyde; H_2O_2: Hydrogen peroxide; IL-17: Interleukin-17; IFN-γ: Interferon-γ; ROS: Reactive oxygen species; TNF-α: Tumor necrosis factor-α; NO: Nitric oxide].

paracrine action of MSCs was observed in a study in which intranasal administration of the MSC secretome to an APP/PS1 mouse model improved memory and amyloid plaque burden by affecting cytokine expression and microglial activation [45].

The NSC secretome improved memory retention and mitigated Aβ plaque deposition in an AD mouse model via activation of the Wnt/β-catenin signaling pathway, indicating its ability to support neuronal survival and function and alleviate AD symptoms [46]. In a recent study, intranasal delivery of iPSC-derived cortical neural stem cells improved cognitive and spatial memory impairments in 5xFAD mice, with associated augmented cortical neuronal differentiation and diminished amyloidosis, and metabolomic analysis revealed various components that may help in the restoration of cognitive ability [47]. A subsequent study revealed that MSC-derived exosomes increased subventricular zone neurogenesis and improved β-amyloid 1–42-induced cognitive impairment, with outcomes equal to those of parent cells [48]. Further evidence has indicated that MSC exosome therapy can restore cognitive performance and mitigate Aβ deposition in a mouse model. Furthermore, exosomes regulate microglial activation, resulting in inhibited neuroinflammatory reactions with an increase in proinflammatory cytokines and an accompanying decrease in anti-inflammatory cytokines within systemic circulation as well as brain tissue [49]. Interestingly, a modified form of MSC exosomes containing a central nervous system-specific rabies viral glycoprotein (RVG) peptide represents an advanced method for the targeted delivery of exosomes to the hippocampus and cortex and was found to enhance cognitive performance in AD mice, reduce Aβ deposition, and restore inflammatory cytokines [50]. Secretome-derived therapies, such as exosomes and EVs, are promising options for the treatment of AD, with effects such as a reduction in the amyloid plaque burden, antagonism of reactive gliosis, and increased neuronal density. These findings are indicators of the underlying processes of neuroprotection, and neuroinflammation modulation, which are central events in AD pathology.

3.2. Parkinson's disease

Aging pathways, such as neuroinflammation, mitochondrial stress, and proteostasis, are well known to contribute to PD. The risk of PD is also known to increase due to defects in DNA repair, alterations in epigenetic signatures, insufficient nutrient sensing, and cell death [4]. A previous study investigated the effects of various culture conditions on the functionality of the MSC secretome in a *C. elegans* PD model, in which secretome differed in their molecular composition and expression levels of various bioactive factors, including interleukins (IL-4, IL-6, and IL-13), matrix metalloproteinases (MMP-2 and MMP-3), tumor necrosis factor-beta (TNF-β), osteopontin, nerve growth factor beta (NGFβ), granulocyte colony-stimulating factor (G-CSF), and heparin-binding EGF-like growth factor (HB-EGF), in spinner and vertical wheel bioreactors [51]. In a rat model of PD, both the whole stem cell secretome and the secretome vesicular fraction were able to rescue symptoms and promote the differentiation of neural progenitor cells *in vitro* [52,53]. In another study, PD animals were treated with BM-MSC secretome injections. Nine weeks following treatment, control animals treated with the A53T α-synuclein viral vector or vehicle exhibited substantial dopaminergic cell loss in the substantia nigra pars compacta (SNpc) and denervation in the striatum (STR). In comparison, secretome treatment significantly decreased α-syn in the SNpc and provided neuroprotection to dopaminergic neurons in the SNpc and STR [54].

The therapeutic efficacy of the stem cell secretome needs to be further explored, and a clinical trial is underway that explores the safety effect of the AT-MSC secretome for multiple system atrophy (NCT04876326). MSC-derived exosomes lack immunoregulatory components and can cross the BBB. SHED-derived exosomes have been shown to protect dopaminergic neurons from 6-OHDA-induced apoptosis, suggesting that SHED-derived EVs have potential as regenerative therapies for PD [55]. Human umbilical cord MSC-derived exosomes have been shown to downregulate SH-SY5Y apoptosis *in vitro* and the ability to cross the BBB, reduce dopaminergic neuron loss, alleviate apomorphine-induced rotational symmetry, and increase dopamine levels in the striatum *in vivo* [56]. Another study focused on miRNA-106 b-enriched hUC-MSC-EVs that suppressed neuronal apoptosis and enhanced autophagy in a PD mouse model [57]. Another study by Zhang et al. reported that hUC-MSC-EV treatment led to improved behavior, elevated striatal dopamine levels, reduced neuronal damage, and suppressed excessive microglial proliferation [58]. Human NSC-derived EVs (hNSC-EVs) effectively reduce intracellular ROS levels and inhibit apoptosis-related pathways. These EVs contain specific miRNAs, such as hsa-miR-182–5p, hsa-miR-183–5p, and hsa-let-7, which are known for enhancing cell differentiation, neurotrophic support, and immune regulation [59].

3.3. Multiple sclerosis

Multiple sclerosis is an autoimmune neuroinflammatory and neurodegenerative disorder that results in progressive neurological, cognitive, and physical disability [60] (Fig. 3). Recombinant myelin oligodendrocyte glycoprotein (MOG) fully activates B lymphocytes, which play a major role in multiple sclerosis pathology in the EAE model. The administration of the MSC secretome in the model was able to delay the onset of disease and reduce demyelination, B-cell infiltration, and microglial activation in rMOG-induced mice but not in myelin oligodendrocyte glycoprotein peptide (MOG)-induced experimental autoimmune encephalomyelitis (EAE) [61]. The human exfoliated deciduous teeth stem cell-derived secretome (SHED-CM) reduces demethylation, axonal damage, immune cell infiltration, and the expression of proinflammatory cytokines in the spinal cord of the EAE model. This improved the phenotypic shift from the M1 to the M2 anti-inflammatory state. The key component identified in SHED-CM was the ectodomain of sialic acid-binding immunoglobulin-like lectin-9 [62]. The adipose-derived mesenchymal stromal cell secretome inhibits splenocyte proliferation and reduces the levels of IFN-γ and IL-17 in MOG-induced splenocytes, along with the upregulation of IL-4 and T regulatory cells [63]. WJ-MSC-derived secretome administration attenuated inflammatory cell infiltration and increased the expression of proinflammatory markers, leading to improvements in remyelination and neurological scores. WJMSC-conditioned media contain BDNF, GDNF, and capillary neurotropic factors [64].

Exosomes derived from bone marrow MSCs altered inflammation and demyelination through the regulation of microglial polarization. Exosomes increase M2 polarization and M2-related cytokine levels [65]. IPSC-EVs reverse microglial senescence and alter polarization. iPSC-EV therapy prevents the proinflammatory activation of microglia and decreases cellular senescence indicators, such as P16, P21, P53, SA-β-gal, and γ-H2AX, and differentiates into an anti-inflammatory microglial phenotype. These treatments reduce neuronal damage and improve stroke outcomes. AKT phosphorylation and Rictor expression are restored in microglia after treatment with iPSC-EVs. This pathway controls inflammation and senescence [66–68].

3.4. Other neurological disorders

Stem cell-derived cell-free therapies are also being investigated for a variety of other neurological disorders, such as HD, multiple sclerosis, ALS, and epilepsy. In all these diseases, the stem cell secretome and EVs have been shown to have the ability to manipulate inflammation, prevent neuron loss, and facilitate neural repair.

Vascular dementia (VD), a leading cause of cognitive dysfunction in older adults, is underpinned by a diverse range of pathological mechanisms, including neuronal loss, impaired synaptic connectivity, cerebrovascular abnormalities, sustained neuroinflammation, and increased oxidative stress [69]. MSC-EVs can promote neuronal axonal development. The overexpression of certain miRNAs, such as miR-23a, miR-200,

miR-133b, miR-17–92, and miR-132–3p, improves brain gene expression and myelination [70]. EVs facilitate axon extension by transferring miRNAs from the neuronal cell body to the axon. A study by Young et al. emphasized the function of the miR-17—92 cluster in neuroplasticity and proposed its application in the treatment of central nervous system damage [71]. Stem cell therapies in HD are aimed at repairing neurons lost or damaged due to pathogenic CAG repeats. Stem cells or their derivatives transplantation has been promising in animal models of HD, although clinical and preclinical development is in the initial stages and requires more studies. HD is defined by the degeneration of striatal GABAergic medium spiny neurons (MSNs). NSCs with a low risk for teratoma formation could be valuable sources for HD therapy if the grafted MSNs form proper axonal projections and receive proper synaptic inputs [72,73]. Various stem cell types, such as ESCs, NSCs, and MSCs, are promising for HD treatment because of their different characteristics. Specifically, ESCs have the ability to give rise to neuronal lineages and are therefore potential candidates for replacing dying neurons and restoring neural function in HD; however, they have ethical limitations. NSCs have the potential to release neuroprotective molecules that increase the survival of existing neurons. Although few stem cell therapy studies are in the clinical trial phase, the stem cell secretome has not yet been explored for HD.

In comparison to stem cell therapy, secretome and EV-based therapies provide several benefits by reducing the risk of tumorigenicity and immune rejection owing to their acellular nature. Cell-free therapy has an improved safety profile that allows repeated dosing with comparatively fewer complications. Moreover, they can be used for targeted CNS delivery due to their capability to cross the BBB. The protection of EV cargo by enzymatic degradation in circulation enhances the therapeutic stability and half-life of biotherapeutics. In contrast, their large-scale production, storage, and standardization remain a challenge in cell-free therapy. Despite all these advantages, other challenges like heterogeneity in EV populations, quantifying effective doses, ensuring reproducibility, and optimization of administration routes require further research for their clinical translation.

4. Mechanistic insights into secretome function and its therapeutic potential

By targeting various cell signaling pathways, secretome components ameliorate the effects of aging, most notably by regulating the autophagy process (Fig. 4). Autophagy is a process of removing dysfunctional proteins from the cell through lysosome-dependent mechanisms. Its dual role in neurodegenerative diseases involves protective mechanisms, such as the clearance of harmful protein accumulations, as well as detrimental actions, such as promoting apoptosis or insufficiently degrading damaged proteins. In DNA repair-compromised mice, hyperactivation of mechanistic target of rapamycin (mTOR) impairs muscle-derived stem cells, which are restored by autophagy stimulation upon treatment with rapamycin [74]. Additionally, a reduction in mTORC1 signaling was observed in mice with an extended life span, along with improved age-associated HSC function compared with wild-type mice [75]. The mTOR pathway is reportedly induced by HGF and EGF growth factors, which are present in the secretome and hence may contribute to age-associated manifestations. Interactions between VEGF and NRF2 are reported to involve a positive feedback loop that stimulates angiogenic pathways [76]. The role of NRF2 in inflammatory cascades is well known, as is the interaction between IL6 and NRF2,

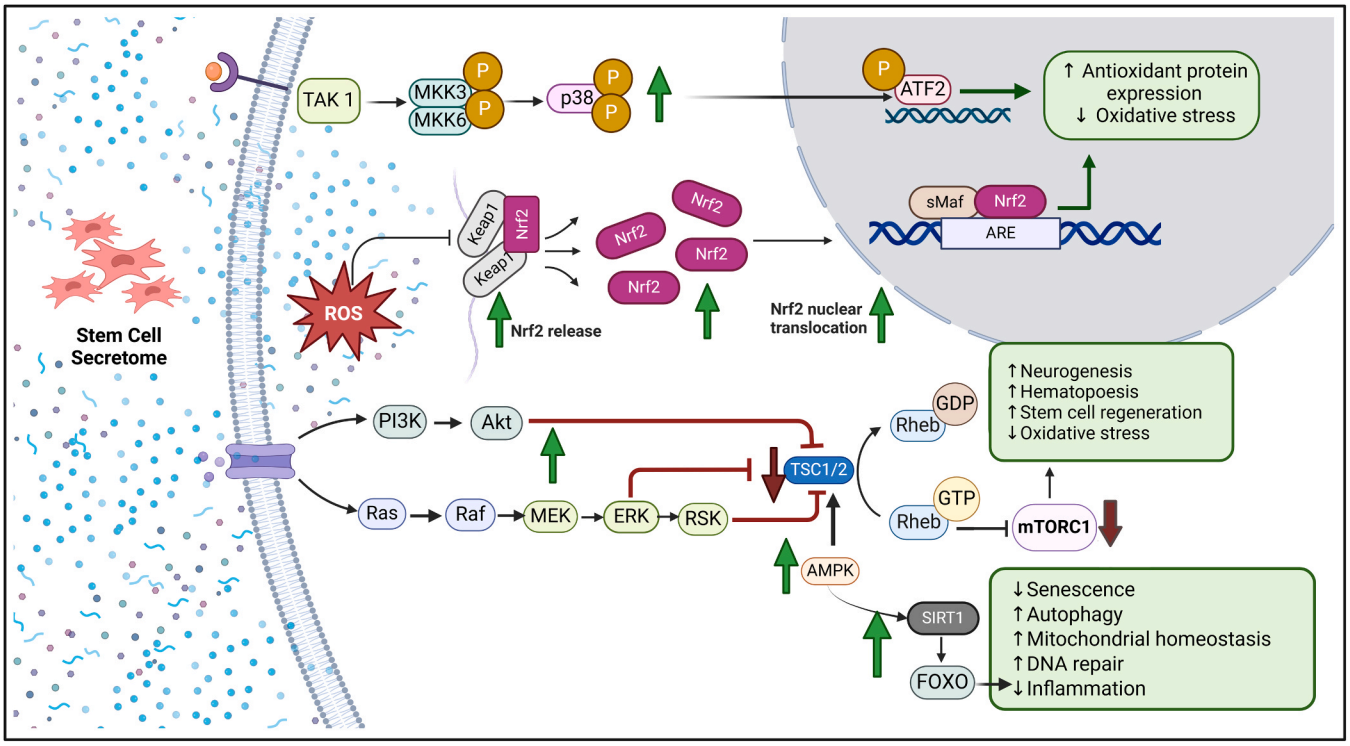

Fig. 4. Systemic mechanism by which the stem cell-derived secretome modulates the Nrf2, AMPK, PI3K/Akt, sirtuin, mTOR, and MAPK pathways to maintain aging disorders. Stem cell secretome components enhance the phosphorylation of p38 and Nrf2 release; upregulate the expression of Akt, AMPK, and SIRT1; and downregulate the mTOR pathway, which enhances antioxidative protein expression, neurogenesis, mitochondrial hemostasis, and DNA repair and downregulates the oxidative stress and senescence necessary for antiaging. **Abbreviations:** P: Phosphorylation; Nrf2: Nuclear factor erythroid-related factor 2; ARE: Antioxidant response element; GDP: Guanosine diphosphate; GTP: Guanosine triphosphate; MEK: mitogen-activated protein kinase; FOXO: Forkhead box O; SIRT1: Sirtuin 1; mTORC1: mammalian target of rapamycin complex 1; AMPK: adenosine 5'-monophosphate-activated protein kinase; Rheb: Ras homolog enriched in the brain; TSC1/2: Tuberous sclerosis complex 1/2; RSK: ribosomal s6 kinase; ERK: Extracellular signal-regulated kinase; Raf: rapidly accelerated fibrosarcoma; Ras: Rat Sarcoma; PI3K: Phosphoinositide 3-kinase; sMaf: small musculoaponeurotic fibrosarcoma; ATF2: activating transcription factor 2; TAK: Tat-associated kinase; ROS: Reactive Oxygen species.

where NRF2 activation leads to IL6/IL1 suppression, ultimately leading to the prevention of hyperglycemia [77]. In the context of neuronal injury and oxidative stress, the AKT pathway is associated with protection and survival at the cellular level. Stem cell secretome components are reported to stimulate the PI3K/AKT pathway to induce angiogenesis, suggesting therapeutic applications in age-related disorders [78]. In addition, the reduced expression of SIRT1 leads to increased DNA damage and aging; on the other hand, it delays senescence.

Collectively, SIRT1, SIRT3, SIRT4, and SIRT5 regulate age-related endothelial functions by mediating caloric restriction, apoptosis, and autophagy to maintain metabolic balance [79]. The growth factors in the stem cell secretome are reported to increase the activity of sirtuin 1, which can affect cellular processes during aging via the deacetylation of histone and nonhistone proteins [80]. Continuous stimulation with TGF-β1 enhances TAK1 activation for persistent activation of the p38 MAPK pathway, which is a clinical target for neuroinflammation. Thioredoxin, a major antioxidant protein, is sorted into exosomes, released into the mesenchymal stem cell (MSC) secretome, and known to initiate the p38 MAPK pathway [81]. Hence, the secretome exerts its effects by engaging various signaling pathways. Through these mechanisms, the stem cell secretome can support neuroprotection, angiogenesis, immunomodulation, and overall tissue homeostasis.

The therapeutic effects of secretome may potentially be benefited by the use of various combinatorial approaches instead of only secretome. The secretome can be combined with pharmacological small molecules or naturally derived compounds such as melatonin, polysaccharides, flavonoids, polyphenols, and aldehydes, and this combination may act synergistically to enhance therapeutic outcomes. [82–84]. Evidence from a study indicates that the BM-MSC secretome in combination with stiripentol which is a stress response inhibitor, effectively reversed stroke-induced neuropathological damage and behavioural changes associated with it [85]. Another study has reported that secretome in combination with metformin and irinotecan, a topoisomerase 1 inhibitor, showed higher potential against colorectal cancer cells *in vitro* [86]. An interesting study reported that combining dental MSC-derived secretome with Yemeni Sidr honey enhanced osteoblast and fibroblast proliferation and migration *in vitro* [87]. The combination of secretome and hyaluronic acid in one study showed that this combination was successful in mitigating the symptoms of psoriasis [88].

In addition, CRISPR-Cas9 technology has opened new horizons to tailor the output of the cells; an example of such has been reported, wherein overexpression of stromal cell-derived factor-1 (SDF-1) in MSCs was reported to improve vascular conditions and tumor growth [89,90]. In neurological diseases like PD, CRISPR-Cas9 technology has been utilized in various measures, from designing adeno-associated virus (AAV) to remove the PD-associated A53T-SNCA gene to developing sRAGE-MSCs to prevent neuronal death in the PD disease model [91]. Alongside these approaches, the secretome and EVs of stem cells can be modified by exposure to various external stimuli, a process recognized as licensing, priming, or preconditioning. This method allows the tailoring of the secretome and EV cargo, and the composition can vary based on the culture conditions [33]. Priming with inflammatory proteins, hypoxia, 3D environment, or small molecules has been known to alter the secretome composition [92]. An example of this can be noted in a study where tamoxifen-exposed MSCs showed greater immunoregulatory properties as seen through RNA sequencing [93]. Genetic manipulation of cells is another way to control the secretory profile of stem cells, such as by overexpression of certain proteins. This is illustrated by research where EVs were tailored to carry miR-146a thereby enhancing their anti-inflammatory potential [94]. Moreover, genetic modification can be used to introduce surface ligands or targeting peptides on EV membranes, enabling selective delivery to targeted tissues. At present, the potential of secretome and EVs in this respect remains untapped, and more research is needed to develop viable therapies.

5. Clinical translation of the stem cell secretome and EVs

Although the preclinical promise of stem cell secretome and EVs for neurological and age-related disorders is robust, published clinical outcomes remain scarce, with most results stemming from early-phase or ongoing trials. A preclinical to clinical translational study demonstrated that intranasal delivery of hiPSC-derived neural stem cell EVs in late middle-aged mice attenuated neuroinflammation and oxidative stress while enhancing cognitive function. These benefits were mechanistically linked to suppressed activation of the NLRP3 inflammasome, p38/MAPK, and interferon-1 signaling pathways, all related to human brain aging and neurodegeneration [95]. Additionally, systematic and meta-analyses highlight that MSC-derived secretome and EVs exhibit neuroprotective, anti-inflammatory, and neuroregenerative effects across animal models of AD, PD, ALS, and multiple sclerosis, suggesting translational potential; however, published phase II and III clinical trial results in humans remain pending [96,97]. Some of the ongoing and recruiting clinical trials have been listed in Table 1. Similarly, preliminary clinical reports from secretome-based therapies in AD reported cognitive improvements reflected in MoCA and ADAS-cog scores and reductions in markers of neurodegeneration, through effects on amyloid-β or tau deposition remain inconclusive pending validation in larger controlled studies [98].

Despite significant therapeutic potential, there are multiple translational challenges that need to be overcome before clinical adoption. Standardization and batch consistency are critical concerns, as donor variability, cell culture conditions, and purification approaches contribute to significant heterogeneity in EV and secretome composition, complicating reproducibility and regulatory approval [99]. Next limitation comes in scalability and GMP compliance, with efforts ongoing to establish reproducible, large-scale manufacturing pipelines that ensure GMP-grade quality, precise control over production, purification, and formulation steps, which is essential for regulatory acceptance [100]. Another barrier is dosing, safety, and pharmacokinetics, as optimal dosing regimens, delivery routes, and frequency of administration remain undefined. While vigorous study on pharmacokinetics and toxicological studies is still needed, early clinical trials indicate a favorable safety profile compared to cell-based therapies with no reports of tumorigenicity or major immune reactions [101]. Regulatory and quality control frameworks for cell-free therapeutics are still evolving, with unresolved requirements surrounding potency assays, cargo characterization, and functional endpoints [102,103]. Comprehensive safety evaluation, particularly for repeated and long-term dosing as well as immunogenicity and tumorigenicity assessments, remains mandatory for clinical programs. Cost and market access will also significantly influence translation as large-scale production implementation requires careful consideration of pricing, cost effectiveness, and health economic impact to ensure equitable accessibility [104,105].

6. GMP manufacturing requirements

As per the WHO and the National Guidelines for Stem Cell Research by ICMR and DBT, the manufacturing and commercialization of cell-free products require adherence to GMP (Good Manufacturing Practices) and GCP (Good Clinical Practices) standards. That includes multiple processes involving facility, environmental controls, aseptic processing, documentation, product characterization, regulatory approval, labeling, batch-to-batch QC, and post-market surveillance. For the production of cell-free products, the preparations should be initiated in cleanroom facilities with controlled environment, advanced heating, ventilation, and air conditioning (HVAC) systems, and regular validation to prevent contamination.

The manufacturing of products, a well-specified workflow (SOPs), and in-process controls must be managed. These include the control of source cells, cell culture, expansion, harvesting, and isolation of secretome/EVs, etc. The protocol, method, and systems used in the

Table 1
Clinical trials on stem cell-derived secretomes and exosomes as therapeutics for neurological disorders.

S. No.	NCT Number	Study Title	Conditions	Interventions	Age	Phases
1	NCT04388982	The safety and efficacy evaluation of allogenic adipose MSC-Exos in patients with Alzheimer's disease	Alzheimer Disease	MSCs-Exos administered for nasal drip in low, mild, and high dosages	Adult, Older adult	Phases 1 and 2
2	NCT06632470	Clinical utility and safety of human umbilical cord mesenchymal stem cell secretome in moderate neurocognitive impairment (dementia)	Dementia, Moderate Dementia	Secretome injection	Adult, Older adult	Phase 1
3	NCT06598202	Exploring nasal drop therapy with small extracellular vesicles for ALS	Amyotrophic Lateral Sclerosis	Exosomes derived from human umbilical cord blood mesenchymal stem cells for nasal drops	Adult, Older adult	Phases 1 and 2
4	NCT06551649	Human amniotic mesenchymal cell secretome for neurodegeneration and neuroinflammation	Amyotrophic Lateral Sclerosis, Multiple Sclerosis	Venous blood draw	Adult	N/A
5	NCT06607900	Huc-msc-sev−001 nasal drops for neurodegenerative diseases	Alzheimer's Disease, Parkinson's Disease, Lewy Body Dementia, Multiple System Atrophy, Fronto-temporal Dementia	hUC-MSC-sEV−001 nasal drops	Adult, Older adult	Phase 1

production should be optimized to maintain reproducibility and consistency of products. Rigorous sterility of the GMP Lab must be maintained by regular environmental monitoring, and viability and exclusion of microbial growth every 15 days. Documentation is another requirement for GMP that will include the documentation/ tracing at each step from the source of raw materials, cells/ biological materials, analysis of contents, size, and concentration of secretome/EVs. Validation and documentation of the potency, purity, and stability of batch-to-batch products as mandated by regulatory guidelines is necessary. After preparation, the packaging and labelling should also follow GMP regulations for advanced therapy medicinal products [106]. Key information includes product identity, donor and traceability data, expiration date, lot/batch code, safety warnings, handling and storage guidelines, as well as detailed transport documentation. For marketing meetings and approvals from regulatory authorities such as the FDA, EMA, and national agencies are required. Adherence to authorized pharmaceutical supply chain standards is essential for product distribution, enabling complete traceability from production to patient use [107,108].

7. Current challenges and future directions of cell-free therapy

Despite promising results regarding cell-free therapies, the application of secretome and EVs remains limited due to a lack of standardized protocols for preparation, characterization, and large-scale manufacturing. The inconsistent potency and efficacy of secretome across batches can be attributed to the variations in cell source, isolation techniques, and culture conditions [109]. In the case of EVs, the use of different isolation methods like ultracentrifugation, precipitation, and chromatography has shown varied EV purity and yield [110]. The regulatory agencies require a stringent, reproducible process and characterization of the secretome before it can be approved as a product. Currently, there are no established standards to evaluate secretome or EVs. The *in vitro* functional and potency assays, which are used to evaluate the secretome to check for their batch-to-batch consistency, do not capture the complete *in vivo* functionality. The current advances in metabolomics, proteomics, and lipidomics may aid in defining the molecular signatures and correlating them with various biological processes of these heterogeneous cell-free products. Incorporation of aging biomarkers to track the effect of therapy is also essential to track patient-specific outcomes and long-term responses.

The complexity of the secretome due to its lipid, protein, and nucleic acid composition makes the dose, duration, and outcome measures of cell-free therapy tricky to determine. A low dose may fail to elicit a therapeutic effect, while excessively high concentrations of secretome or EVs may induce unwanted immune reactions, side effects, or disruption of metabolic pathways. The duration of dose administration is critical in the case of acute and chronic disorders. In the former case, early intervention can be beneficial, whereas in the latter case, repeated dosing may prove to be better. Understanding the disease pathophysiology and aligning therapy delivery with the disease stage will significantly influence clinical outcomes. Clear ethical guidelines, production in good manufacturing practice certified facilities, attention to dosing strategies, along with rigorous clinical validation, are necessary to develop cell-free therapies from stem cells.

Monitoring the progression of aging using circulating biomarkers may provide valuable insights into disease progression and patient progression. Biomarkers for aging can be classified broadly as molecular and cellular, image-based, clinical measure-based, and composite aging biomarkers [95]. One of the most widely studied biomarkers associated with aging is telomere length, as human somatic cells experience a limited replicative lifespan due to progressive telomere shortening, increasing their vulnerability to mutations [96]. Hence, with aging, the telomere length shortens. In addition, methylation and other epigenetic modifications associated with DNA can serve as a biomarker since the majority of DNA methylation modifications take place at CpG sites, which have been strongly linked to aging [97]. Along with the molecular changes, cellular biophysical characteristics may serve as valuable biomarkers associated with aging [98]. EVs originating from neural cells, including microglia, astrocytes, and neurons, contain characteristic cargo that may serve as valuable biomarkers for different neural degenerative disorders.

8. Concluding remarks

Addressing aging at physiological and pathological levels has numerous challenges. Utilization of the stem cell secretome holds immense promise against the effects of aging. By modulating different pathways, the stem cell secretome can aid in combating oxidative stress, mitochondrial dysfunction, stem cell rejuvenation, and mitigating DNA damage. Secretome components, such as NRF2, P38 MAPK, and PI3K AKT, can activate various pathways in tandem. The anti-inflammatory properties of the stem cell secretome add to its therapeutic benefits and can target various aging-related diseases. Secretome components aid in maintaining the ECM of the skin, hence providing a youthful appearance, and hair follicle development results in hair regeneration. These components can act beyond dermatological applications, and many clinical trials are currently ongoing (Table 1). Recently, intranasal administration of an iPSC-derived cortical neural stem cell secretome was shown to decrease Aβ plaque aggregation and restore cognitive ability through nerve function restoration in a 5 ×FAD mouse model of AD. These results underscore their potential applications in relieving neurodegenerative disorders related to aging.

By modulating major aging-related signaling pathways associated with oxidative stress, cell rejuvenation, and inflammation, the stem cell secretome can provide an additional intervention for joint degeneration, cardiovascular diseases, cellular senescence, and neurological disorders. Both the iPSC and MSC secretomes have advantages; the former has more regenerative properties due to its pluripotent nature, and the latter is known to have better anti-inflammatory properties. The site of administration and the organ to be targeted play major roles in considering the secretome of EVs as a therapy. For injectables or topical administration routes, quantity plays a crucial role since a larger number of EVs are needed. The isolation and purification of EVs and exosomes is a tedious and expensive task. However, in aging diseases such as neurodegenerative diseases, EVs are preferred since they are known to cross the BBB and have no immunogenic response. Understanding how the stem cell secretome acts on various signaling pathways and ultimately affects the aging phenotype can help harness it for aging-related pathologies. Integrating these therapies in a noninvasive way will be a new way to improve the quality of life of the geriatric population.

CRediT authorship contribution statement

Shweta Verma: Writing – original draft, Visualization, Data curation. **Jahnavy Madhukar Joshi:** Writing – original draft, Visualization, Data curation. **Abhishek Kumar Singh:** Writing – review & editing, Validation, Investigation, Formal analysis, Conceptualization. **Raviraja Neelavar Seetharam:** Writing – review & editing, Resources, Investigation, Formal analysis.

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The authors report that there are no competing interests to declare.

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Data availability

No data was used for the research described in the article.

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