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A systematic review of secretome-based therapies for Alzheimer's disease: Bridging the preclinical and clinical gap

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SUPPLEMENTARY MATERIAL

Table S1. Characteristics of Animal Studies

No.	Types of Intervention	Author	Year	Animal model	Age/ BW	Sex	Sample size	Exposure	Grouping
1	Secretome	Kim *et al.*	2018	5XFAD mice	6 mo	NR	9	-	1 = CTRL: MEMα-administrated 5XFAD 2 = MSC: hUCB-MSC-administered 5XFAD
2		Santamaria *et al.*	2021	WT & APP/PS1 mice	12 & 22 mo	male	129	-	1 = 12-month-old APP/PS1 mice received one IV injection of either PBS, MSC-CS or MSC-UCS 2 = 12-month-old APP/PS1 mice and age-matched WT received one IV injection of PBS or MSC-CS 3 = 12-two-month-old APP/PS1 mice received one IV injection of MSC-CS 4 = 22-month-old APP/PS1 and age-matched WT underwent a repeated IN treatment regimen 5 = 22-month-old and 25-month-old APP/PS1 and age-matched WT mice underwent a repeated IN treatment regimen with MSC-CS
3		Hijroudi *et al.*	2022	BALB/c mice	8 wk	male	36	Aβ1-42 was injected into the ICV space using stereotaxic surgery	1 = Control 2 = AD + vehicle 3 = AD + NSCs-CM
4		Mo *et al.*	2023	WT & 5XFAD mice	11 wk	male	24 (5xFAD mice)	-	1 = WT group 2 = 5×FAD AD mouse model 3 = CNSC-SE-treated 5×FAD mice 4 = MSC-treated 5×FAD mice
5	Exosomes	Ding *et al.*	2018	APP/PS1 mice	7 mo	male	36	-	1 = Control 2 = hucMSC-exosomes
7		Micci *et al.*	2019	C57BL/6 J and Nestin-δ-HSV-TK mice	6 - 8 wk	male and female	20	NR	1 = PBS 2 = Exosomes 3 = PBS + Aβo 4 = Exosomes+Aβo
8		Cui *et al.*	2019	APP/PS1 mice	7 mo	NR	39	-	1 = PBS (AD) 2 = exosomes derived from MSCs (MSC-Exo) 3 = RVG-conjugated MSC-Exo (MSC-RVG-Exo)
9		Reza-Zaldivar *et al.*	2019	C57BL/6 mice	7 - 8 wk	NR	48	Aβ aggregates (Aβ1–42) were administered in the dentate gyrus bilaterally in 14 days	1 = Control 2 = AD 3 = exosomes 4 = MSC

10		Chen *et al.*	2021	WT and J20 mouse model of AD	9 mo	NR	24	-	1 = WT-PBS 2 = WT-Exosomes 3 = Tg-PBS 4 = Tg-Exosomes
11		Poltavtsesa *et al.*	2021	NMRI mice	6 mo	male	23	-	1 = Sham operated + Saline 2 = Sham operated + Exosomes 3 = Olfactory bulbectomized + Saline 4 = Olfactory bulbectomized + Exosomes
12		Zhdanova *et al.*	2021	NMRI mice	6 mo	male	23	-	1 = Sham operated + Saline 2 = Sham operated + Exosomes 3 = Olfactory bulbectomized + Saline 4 = Olfactory bulbectomized + Exosomes
13		Liu H *et al.*	2022	APP/PS1 mice	2 mo	male	40	-	1 = PBS (AD) 2 = exosomes derived from ADSCs (Exo) 3 = hypoxia-pretreated ADSCs (HExo) 4 = circ-Epc1- expressing ADSCs (circ-Epc1-Exo)
14		Liu S *et al.*	2022	C57BL/6 mice	4 wk	male	24	STZ was injected into the lateral ventricle of mice by the autosampler in the STZ group (dose of 0.3 mg/kg, speed of 0.5 μL/min, the volume of 1 μL/side, and the STZ was dissolved in ACSF and prepared for current use).	1 = Control 2 = model group 3 = exosomal lateral ventricle injection (Lv) group 4 = exosomal caudal vein injection (Cv) group
15		Sheykhhasan *et al.*	2022	Wistar rats	250-300 g	N/R	40	To induce AD model, dissolution of STZ was performed in 0.9% saline solution. Then, STZ was maintained at − 20◦C before use. STZ (3 mg/kg, twice) was injected ICV using a Hamilton syringe after perforation of the recent site.	1 = Control 2 = AD 3 = coQ10 4 = Exo 5 = Exo+coQ10

16		Hou *et al.*	2023	WT & 5XFAD mice	female	4 mo	24	-	1 = 5 ×FAD group 2 = MSCs-exo group 3 = co-housed group 4 = MSCs-exo + Abx group
17		Pourhadi *et al.*	2023	Wistar rats	250-300 g	male	8-10/group	STZ or normal saline was injected directly into the intracerebral ventricle (ICV) using a 10 μl Hamilton syringe (gauge 30) with the polyethylene tube (AP − 0.8, ML 1.5, DV − 3.5). STZ (3 mg/kg) or the vehicle was administered at a rate of 1 μl/min.	1 = Sham (PBS), 2 = STZ 3 = STZ+Exosomes 0.7 4 = STZ+Exosomes 7 5 = STZ+Exosomes 70
18		Li *et al.*	2024	WT and SKO-AD mice	9 mo/25-25 g	male	75	-	WT group, SKO-AD-Veh group, AD-Veh group, SKO-AD-ex group, and AD-ex group
19	Mico-vesicles	Elia *et al.*	2019	APP/PS1 mice	3 mo	male	11	-	1 = Control 2 = MSC-EVs
20		Losurdo *et al.*	2020	triple-transgenic AD mice	7 mo	female	8	-	1 = Control 2 = MSC-EVs
21		Cone *et al.*	2021	5XFAD and C57BL/6J mice	6 wk	male and female	56	-	1 = NT - Saline 2 = NT - EV 3 = AD - Saline 4 = AD = EV
22		Zhdanova *et al.*	2022	NMRI mice	6 mo	male	6	-	1 = Control 2 = cytochalasin B–induced membrane vesicles (CIMVs) of MSCs

Table S2. In vivo study results

No.	Author	Year	Outcome Measures	Results	Mechanism of action

1	Kim *et al.*	2018	Synaptic Density Markers, TSP-1 Secretion	• Significant increase in synaptic markers SYP and PSD-95 throughout the brain. • hUCB-MSCs can rescue synaptic density loss induced by Aβ42 peptide in vivo	• The protective effect of hUCB-MSCs against synaptic dysfunction (mediated by TSP-1) • hUCB-MSCs increases the expression of NLGN1 and α2δ-1
2	Santamaria *et al.*	2021	NORT, Amyloid Plaques, Microglial Activation, Astrogliosis, Cytokine Levels	• Memory recovery in 12-month-old APP/PS1 mice was observed 7 days post a single IV injection of MSC-CS, but the improvement was not sustained beyond 14 days. • A 30% reduction in hippocampal and cortical amyloid plaques was noted following the same treatment (decreased amyloidosis). • Microglial activation was significantly reduced, evidenced by decreased IBA1 positivity and CD68-marked area • The treatment did not affect astrogliosis. • Levels of cytokines IL-1β and TNF were not significantly altered	• MSC-CS mimics the neuroreparative effects of MSCs through paracrine action, releasing bioactive components in response to the AD brain environment. • Decrease in amyloidosis • Reduce neuroinflammation through decreasing microglial activation
3	Hijroudi *et al.*	2022	Passive Avoidance Test and MWM test, RT-PCR, ELISA, Western blot, Double-IF Staining (BrdU/Nestin and BrdU/NeuN co-expressing cells), Nissl staining	• Improved memory retention in AD mice treated with NSCs-CM, by increased step-through latency compared to untreated group. • Reduced escape latency and increased time spent in the target quadrant for AD mice treated with NSCs-CM • There was a significant increase in the expression levels of PI3K, Akt, MAPK, ERK, Wnt3a, β-Catenin, and GSK3β genes in NSCs-CM group compared to untreated AD mice • NSCs-CM increased levels of BDNF and NGF • Reduction in Aβ plaque formation in the brains of AD mice treated with NSCs-CM • Increased cells co-expressing BrdU/Nestin and BrdU/NeuN in NSCs-CM group. • Decreased neurotoxicity and cell death in the hippocampus of NSCs-CM group.	• NSCs ability to form neurospheres and express the stem cell marker nestin • NSCs-CM modulated the Wnt/β-catenin signaling pathway (neuroprotection and neurogenesis) • Support neuronal survival and function based on levels of BDNF and NGF • Reduce Aβ plaque formation • Improved neural tissue integrity
4	Mo *et al.*	2023	Neural markers (NEUN, vGLUT, and MAP2), growth factors (BDNF, GDNF, and VEGF), IF staining, Multielectrode array recording, RT-PCR, Western blot	• Intranasal delivery of iPSC-derived CNSC-SE improved spatial memory and cognitive impairments in 5xFAD mice. • CNSC-SE-treated group showed significant improvement in behavioral performance in the Barnes maze, with increased time in the target zone and reduced error rate compared to AD group. • CNSC-SE treatment resulted in a similar pattern of movement to WT mice, with fewer erroneous entries. • Significant decrease in APP in CNSC-SE-5xFAD mouse brains compared to the AD group.	• iPSC-derived CNSC-SE promoted cortical neuron differentiation in vitro • CNSC-SE increased neuronal network activity and action potential bursts • CNSC-SE reduced amyloidosis and neuro-inflammatory proteins in 5xFAD mouse brain (anti-amyloid and anti-inflammatory effects)

5	Ding *et al.*	2018	Behavior Test Modified MWM test, IF Staining, Quantitative RT-PCR, ELISA, Western Blot	• Mice treated with hucMSC-exosomes showed improved performance in the MWM test with shorter mean escape latency compared to control. • Reduced microglial activation and increased alternative activation in the hucMSC-exosome group. • Decreased Aβ plaques in the cortex and hippocampus of treated mice	• Change microglial activation states and reduced inflammation • Decrease in Aβ40 and Aβ42 levels implicated in plaque formation and AD pathology • The presence of exosome markers CD63 and CD9 indicated successful isolation and potential delivery of therapeutic contents
6	Micci *et al.*	2019	Electrophysiological Assessments, NORT, Synaptosomes Preparation	• Significant percentage changes from the initial average baseline fEPSP slope • Mice study demonstrated the ability to discriminate between familiar and novel objects • Significant reduction in Aβ oligomer binding to hippocampal synaptosomes treated with NSC-exo compared to those treated with PBS or MN-exo	• Synaptic plasticity indicates changes in synaptic strength following the conditioning stimulus • Protective effect of NSC-exo against Aβ oligomer-induced synaptic vulnerability
7	Cui *et al.*	2019	MWM test, Thioflavin-S staining, ELISA, IF staining	• MSC-RVG-Exo treatment reducted amyloid plaque deposition in both cortex and hippocampus compared to MSC-Exo treatment. • MSC-RVG-Exo treatment resulted in lower concentrations of soluble Aβ40 and Aβ42, and insoluble Aβ40 and Aβ42 in the brain. • MSC-RVG-Exo treatment significantly attenuated the expression of GFAP (reduced astrocyte activation) • MSC-RVG-Exo treatment improved spatial learning and memory in APP/PS1 mice	• Exosomes derived from MSCs were tagged with RVG peptide to target the CNS. • The RVG modification enhanced the engraftment of exosomes in the cortex and hippocampus. • Targeted exosomes facilitated the clearance of Aβ plaques and reduced astrocyte activation (improve cognitive function)
8	Reza-Zaldivar *et al.*	2019	MWM test, NORT, IF staining (DCX and PSA-NCAM markers)	• Both exosome and MSC treatments reduced cognitive impairment in AD mouse model • Exosome treatment stimulated neurogenesis in the SVZ. Similar effects were observed with MSC treatment.	• MSCs and Exosomes mediate effects through paracrine activity. • Exosomes promote neurogenesis and reduce cognitive impairments, which may internalize and degrade Aβ oligomers, secrete antioxidant enzymes, anti-inflammatory cytokines, and neurotrophic factors.

9	Chen *et al.*	2021	Glucose Metabolism, NORT, Amyloid Plaque, Astrocyte Activation, Neuronal Memory and Synapse-Related Genes	• MSC-exosomes treatment resulted in a significant increase in [18F] FDG uptake in both the whole brain and specific brain regions • Significant improvement in long-term recognition memory following MSC-exosomes treatment • MSC-exosomes regulated the phase of neurons and astrocytes in the brain of AD mice	• Decreased the expression of Aβ in a human neural cell culture model with familial AD mutations • Restored the expression of neuronal memory/ synaptic plasticity-related genes • Modulated the phase of neurons and astrocytes in the brain • Exosomal miR-29a upregulated memory/ synaptic plasticity-related genes by HDAC4
10	Zhdanova *et al.*	2021	MWM test and localization of exosomes	• Improved performance in the MWM, with animals spending more time and making more visits to the target sector, indicating enhanced spatial memory. • Labeled exosomes were found in the hippocampus and neocortex 4 hours after intranasal administration, areas crucial for learning and memory and affected by AD.	• Exosomes expressed typical markers CD9, CD63, and CD81, which demonstrate high therapeutic efficacy. • Intranasal administration allows direct delivery to the brain, bypassing the BBB. • Exosomes facilitate intercellular communication by transferring bioactive compounds to target cells.
11	Poltavtsesa *et al.*	2021	Spatial memory and localization of exosomes	• Exosomes prevented spatial memory deterioration in OBE model. • Significant differences showed in factor detection between control and treated groups. • Fluorescently labeled exosomes were found in the brain tissue after IV administration • Exosomes localized in the hippocampus and neocortex.	• Penetrate the BBB and reach the hippocampus and temporal cortex (learning and memory) • The therapeutic effect is likely due to the transfer of proteins, nucleotides, amino and fatty acids, mRNA, and microRNA from exosomes to recipient cells, facilitating intercellular communication. • Exosomes may exert their effects without the need for immunological compatibility with the recipient tissue, unlike MMSCs.

12	Liu H *et al.*	2022	MWM test, RT-PCR, Luciferase reporter assays, IHC, IF, ELISA	• Mice treated with circ-Epc1-containing ADSC exosomes showed reduced escape latency and increased platform crossing numbers in the spatial probe test • Circ-Epc1-containing ADSC exosomes decreased neuronal damage. • TUNEL staining in brain tissue sections indicated a decrease in neuronal apoptosis in mice treated with circ-Epc1-containing ADSC exosomes. • The presence of circ-Epc1 in ADSC exosomes facilitated the shift of microglial polarization from M1 to M2 in the hippocampus • Decreased IL-1β, IL-6, and TNF-α levels cytokines	• High-throughput sequencing identified circEpc1 as a crucial component in hypoxia-pretreated ADSC exosomes for improving cognitive functions. • Luciferase reporter assays revealed TREM2 and miR-770-3p as downstream targets of circ-Epc1. • Overexpressing miR-770-3p or downregulating TREM2 reversed the effects of circ-Epc1 on M2 microglial polarization during lipopolysaccharide treatment, indicating their role in the modulation of microglial phenotypic transformations and inflammatory cytokine expressions.
13	Liu S *et al.*	2022	OFT, EPM test, NORT, TST, Nissl staining, IF staining, Western blot, RT-PCR	• BMSC-exos improved AD-like behaviors • Positive correlations were observed between the duration and distance in the center in the OFT and the preference of the novel object in the NOR. • Reduced glial cell activation, detected new neurons and measured the positive area of Aβ1−42 in the hippocampus. • Reduced expression of IL-1β, IL-6, TNF-α, Aβ1−42, and p-Tau • Upregulated protein expression of synapse-related proteins and BDNF	• BMSC-exos are associated with the regulation of glial activation and the associated neuroinflammation, as well as BDNF-related neuropathological changes in the hippocampus. • The reduction in the expression of inflammatory cytokines such as IL-1β, IL-6, and TNF-α, along with the decrease in Aβ1−42 and p-Tau, suggests a reduction of the neuroinflammatory response. • Potential restoration of synaptic function and promotion of neuronal survival and plasticity.
14	Sheykhhasan *et al.*	2022	MWM test, passive avoidance task, Nissl staining, ELISA, IHC	• CoQ10-loaded exosomes derived from ADSCs-Exo significantly improved memory impairment induced by STZ in rats • Treatment with CoQ10-loaded ADSCs-Exo led to an increase in BDNF expression in STZ-induced rats, compared to groups treated with CoQ10 or exosomes alone • CoQ10-loaded ADSCs-Exo group showed the highest cell density and SOX2 gene expression	• CoQ10 helps in reducing the expression of pro-inflammatory cytokines and improving mitochondrial function • Exosomes derived from ADSCs contribute to the therapeutic effects of CoQ10 by facilitate drug delivery to the brain, overcome the BBB, and potentially carry other beneficial molecules. • CoQ10-loaded ADSCs-Exo treatment not only protects existing neurons but also promotes the growth and differentiation of

					new neurons and synapses.
15	Hou *et al.*	2023	CFC test, MWM test, HE staining, Nissl staining, IHC, ELISA, GFAP staining	• Plaque deposition was reduced in the MSCs-exo group and increased in the co-housed group compared with the 5xFAD mice • The MSCs-exo + Abx group had lower plaque deposition than the MSCs-exo treated group. • Aβ1-40 and Aβ1-42 levels in the hippocampus and serum of mice reduced after MSCs-exo treatment compared with the 5xFAD group. • Co-housing increased Aβ1-40 and Aβ1-42 levels, whereas these levels reduced after Antibiotics treatment compared with MSCs-exo treatment • AD gut microbiota removed MSCs-exo therapeutic effect. • Antibiotics improved MSCs-exo efficacy by treating disordered gut microbiota and metabolites.	• MSCs-exo treats AD by promoting Aβ degradation, modulating immune responses, protecting neurology. • Promoting axonal growth, and improving cognitive impairment. • Gut microbiota dysbiosis may limit MSCs-exo therapy. • Antibiotics enhance therapy by modulating gut microbiota and metabolites.
16	Pourhadi *et al.*	2023	MWM test, Congo Red Staining, IF staining, Western blot, MTT assay, flow cytometry, AO/PI staining	• STZ-induced rats showed learning deficits in the MWM test, which were dose-dependently reduced by Exosomes treatments. • Amyloid plaque deposition was observed in the hippocampal regions in AD-model rats • There were changes in neuronal marker expression due to Exosomes treatment. • Confirmed protein expression alterations in response to treatments. • Cell viability was improved in Exosomes-treated groups compared to the glutamate group. • Increased live cell percentages and decreased apoptotic cell percentages in Exosomes groups. • Improved cell viability in 3D culture conditions with Exosomes treatment.	• Improve cell viability and counteract the cytotoxic effects of L-glutamate. • Reduce amyloid plaque deposition in the hippocampus. • Influence the expression of neuronal markers (neuroregeneration or synaptic plasticity) • The dose-dependent effects of EXOs on learning and memory suggest a potential therapeutic action in sporadic AD models
17	Li *et al.*	2024	MWM test, immunostaining, whole brain imaging, Fluorescence signal intensity analysis, Western Blot, ELISA	• NSC-derived exosomes led to reduced escape latencies and increased time spent in the target quadrant • Enhanced expression of mitochondrial biogenesis-related proteins in multiple brain regions • Increased fluorescence signal intensity for mitochondrial biogenesis-related proteins in selected brain regions • Increased levels of SIRT1 and mitochondrial biogenesis-related proteins in the brains of mice treated with NSC-derived exosomes • Significant decrease in the ratio of soluble Aβ42 to Aβ40 was observed in the SKO-AD groups, indicating a slight positive effect on Aβ levels	• Increased SIRT1 levels and enhanced the production of mitochondrial biogenesis-related factors. • Inhibited astrocyte activation but did not suppress amyloid-beta production. • Activate of the SIRT1-PGC1α signaling pathway and increase synthesis of NRF1 and COXIV (improved mitochondrial biogenesis) • Restored abnormal protein distribution in the brain, indicating promotion of mitochondrial biogenesis.

18	Elia *et al.*	2019	Aβ Plaque and Dystropic Neurites	• Administering BM-MSC-EVs at 3 and 5 months of age in APP/PS1 mice (before and just as clinical signs start to appear) effectively reduced pathological signatures of AD. • Quantitative analysis showed a significant reduction in plaque area, solidity, and density, as well as a decrease in dystrophic neurite occurrence.	• BM-MSC-EVs carry Neprilysin, a β-amyloid degrading enzyme, suggesting a direct action on Aβ degradation. • The EVs inherit protective, anti-inflammatory, and neurotrophic properties from their parental BM-MSCs
19	Losurdo *et al.*	2020	ELISA, Western Blot, IF staining, Analysis of microglia activation, Golgi-Cox staining, Dendritic spine analysis	• Decrease in microglia activation following the treatment with MSC-derived EVs. • Intranasal administration of MSC-derived EVs resulted in an increase in dendritic spine density in the hippocampal CA1 pyramidal neuron, entorhinal cortex, and prefrontal cortex neurons of EV-treated mice compared to control mice.	• MSC-derived EVs reached the brain, reducing microglia activation and increasing dendritic spine density. • This suggests a shift of microglia toward an anti-inflammatory phenotype, contributing immunomodulatory and neuroprotective effects
20	Cone *et al.*	2021	Cognitive Performance, Aβ plaque, GFAP and Aβ Colocalization, Behavioral Assays, IHC	• 5XFAD mice treated with hMSC-EV showed significantly improved performance in cognitive tests compared to control. • Reduction of Aβ plaque load was observed in the hippocampus of EV-treated mice compared to control. • There was less colocalization between GFAP and Aβ plaques in the brains of EV-treated mice compared to control. • hMSC-EV group performed better in behavioral assays assessing learning and memory than control. • EV-treated mice showed lower Aβ plaque load and reduced colocalization of GFAP and Aβ plaques in the hippocampus	• MSC-derived EVs exhibit similar immunoprotective and immunomodulatory abilities • EVs play a role in cell-to-cell communication, carrying a diverse molecular payload • EVs can cross most barriers, including the BBB
21	Zhdanova *et al.*	2022	localization of vesicles	• Four hours after intranasal administration, vesicles containing the fluorescent protein RFP were observed in the hippocampus and neocortex of OBX mice	• Microvesicles act as nanocontainers for targeted delivery of biologically active compounds or drugs to brain regions affected by neurodegeneration. • Intranasal delivery provides a non-invasive route to the CNS, bypassing the BBB