

Early Adult Exosomes Reverse Aging-Associated Endothelial Transcriptional Networks in Alzheimer’s

Brian E. Packard, Pierre F. Roux, Stephen C. L’Heureux, William C. Zamboni — HealthSpan Research LLC

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Serum exosomes from early adult donors (18-25 years) reverse the aging transcriptional signature that late-adult donors (65-72 years) exosomes impose on human brain microvasculature endothelial cells, restoring mitochondrial and ribosomal programs and suppressing inflammatory and junctional remodeling linked to blood-brain barrier breakdown in Alzheimer’s disease.

INTRODUCTION

Neurovascular dysfunction and blood–brain barrier (BBB) breakdown are early events in Alzheimer’s disease (AD), preceding amyloid-β deposition and tau pathology. Endothelial impairment, reduced cerebral blood flow, nutrient and oxygen supply while BBB leakage permit influx of plasma proteins, microbes and immune cells, which collectively amplify neuroinflammation.

Circulating exosomes (30–150 nm extracellular vesicles) are systemic mediators of vascular health, carrying proteins, lipids, and microRNAs that regulate endothelial metabolism, oxidative stress, and junctional stability (Fig.1).

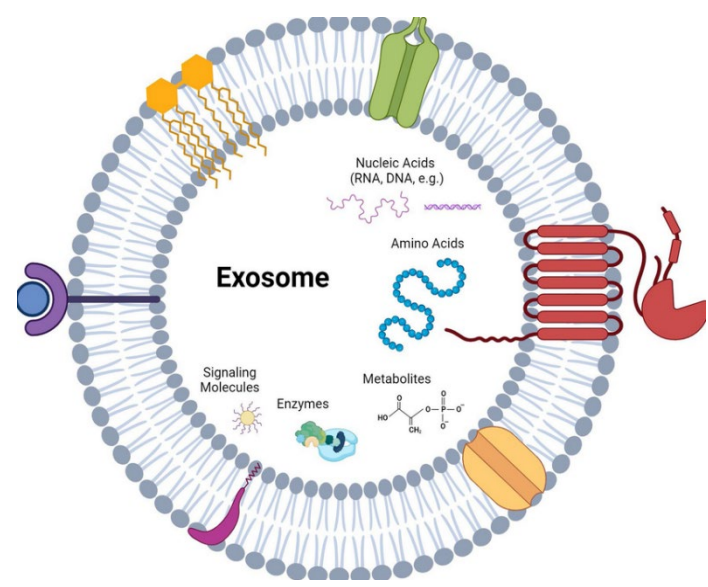


Fig.1 Structure of exosomes

Exosomes from late-adult donors are enriched in pro-inflammatory cytokines, senescence-associated microRNAs, and oxidized lipids that propagate endothelial dysfunction. Exosomes from early-adult donors deliver regenerative cargo, including miR-126 and miR-21, that enhance mitochondrial function and reinforce tight junctions. This study characterizes how serum exosomes from early and late adult human donors reprogram the transcriptome of primary human brain microvascular endothelial cells (HBMECs), and whether young exosomes reverse the effects of late adult-donor exosomes.

METHODS

Exosome Isolation
Serum from healthy donors (n=3 per cohort) grouped as young-adult (18–25 yr) and late-adult (65–72 yr) was purchased from BioIVT. Exosomes were affinity-purified from 20 mL serum using Norgen Maxi kit; concentration and size confirmed by nanoparticle tracking analysis (Malvern, Nanosight).

Cell culture.
HBMECs from a 38-year-old male donor (Neuromics) were cultured to confluence. Four conditions in biological triplicate: 1) untreated control; 2) early-adult exosomes; 3) late-adult exosomes; and 4) sequential late-then-early, each at 1×10^{12} exosomes/mL for 24 h.

Total RNA Isolation
Total RNA, including small RNAs, was extracted from HBECs using TRIzol reagent, and integrity was confirmed by Bioanalyzer, with all samples exhibiting RIN values of 8 or higher

Transcriptomics
Polyadenylated RNA libraries were prepared using the TruSeq RNA Library Prep Kit (Illumina). Libraries were sequenced on Illumina NovaSeq 6000 ($\geq 20M$ paired-end reads). Reads aligned to GRCh38 (STAR); differential expression by edgeR ($FDR < 0.05$, $|\log_2 FC| > 1$). DEGs partitioned by hierarchical clustering; KEGG and Hallmark enrichment by hypergeometric testing.

RESULTS

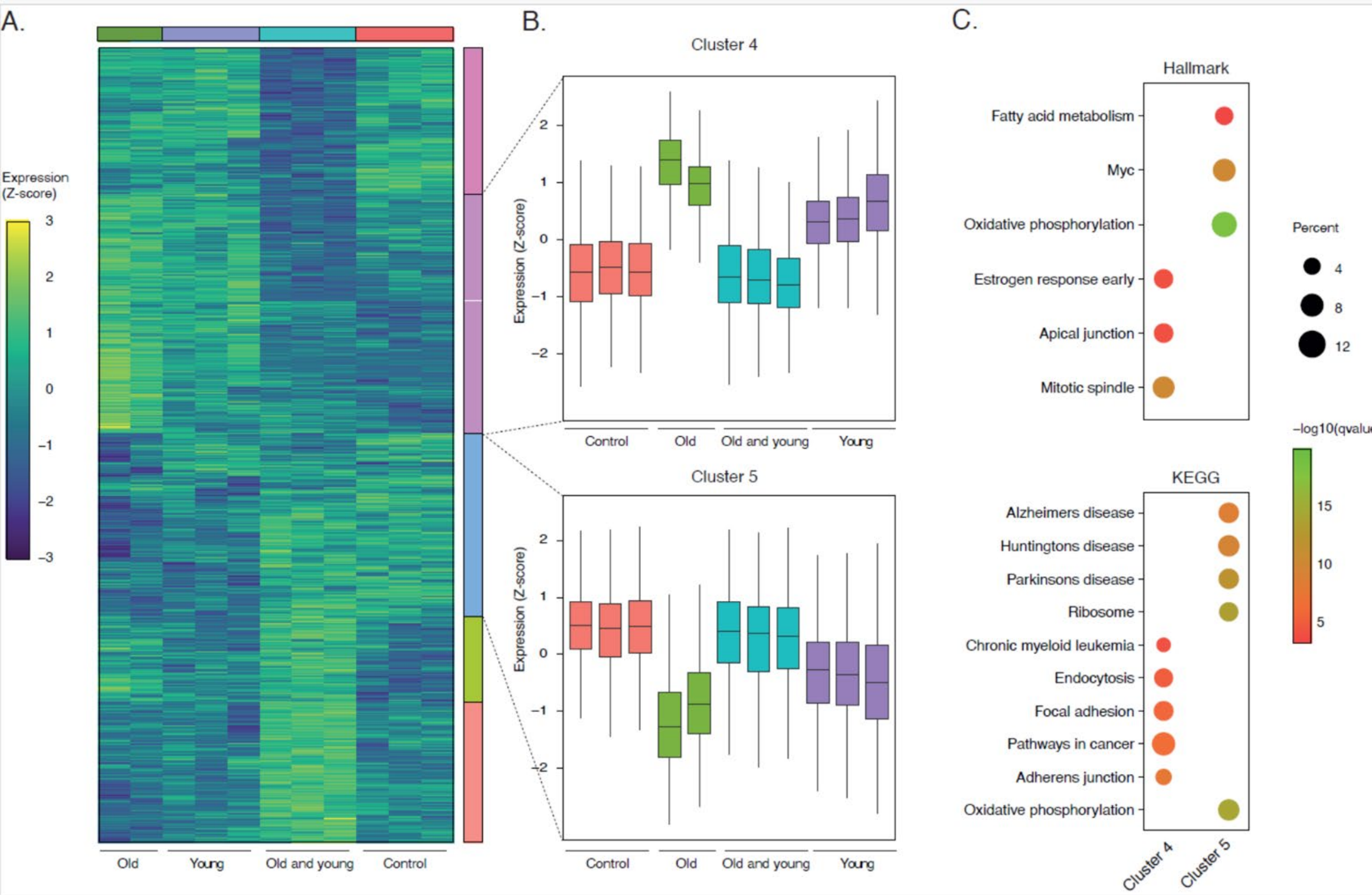
Analysis
5,432 genes met differential expression criteria across conditions, resolving into five clusters. Clusters 4 and 5 showed reciprocal donor-age patterns (Fig. 2).

Late-adult exosomes suppress mitochondrial and translational programs.
Cluster 5 genes for oxidative phosphorylation (NDUFS3, COX4I1, ATP5F1A), fatty acid metabolism, and ribosomal biogenesis were downregulated by old exosomes. (Fig.2)

Late-adult exosomes drive inflammation and junctional remodeling.
Cluster 4 inflammatory (MMP9, ICAM1, VEGFB), junctional (CDH1, COL4A1), and proliferative (EGFR, MTOR) genes were induced — an inflammaging signature.

Early-adult exosomes reverse age-related dysfunction.
Early-adult exosomes maintained or restored mitochondrial and ribosomal expression toward baseline. Sequential late-then-early treatment broadly reversed late-induced dysfunction and attenuated inflammatory and junctional stress.

Fig. 2. Global transcriptomic reprogramming of HBMECs by serum exosomes from early-adult versus older donors. (A) Heatmap of 5,432 DEGs across control, young, old, and old-then-young conditions, partitioned into five clusters. (B) Cluster 4 (induced by old) and Cluster 5 (suppressed by old) expression, both reverting toward baseline with young and old-then-young treatment. (C) KEGG and Hallmark pathway enrichment for Clusters 4 and 5.



CONCLUSIONS

This study demonstrates that serum-derived exosomes from late-adult human donors (65-72 years) elicit transcriptional changes in primary HBMECs consistent with mitochondrial suppression, translational decline, inflammatory activation, and disorganized junctional signaling, hallmarks of cerebrovascular aging and contributors to AD pathogenesis. Crucially, serum-derived exosomes from early-adult donors (18–25 years) reversed these deleterious transcriptomic profiles, restoring gene expression associated with oxidative phosphorylation, ribosomal biogenesis, tight junction assembly, and anti-inflammatory signaling. These findings underscore that age critically influences exosomal cargo and that this differential cargo in turn determines the function of human brain endothelial cells. Accordingly, exosome-based therapeutics may offer a non-cellular, scalable strategy to counteract neurovascular decline in aging and AD. To advance toward clinical applications, future work should prioritize functional validation, comprehensive exosome characterization and standardization, and rigorous in vivo pharmacology, efficacy, and safety assessments

REFERENCES

Full reference list and complete manuscript are available online:

