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Research Article

The Multi-Omics Profiling of Early-Passage Mesenchymal Stem Cell Exosomes Reveals Potential Targets for Attenuating Diabetic Inflammatory Pathophysiology

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Abstract

Background: Mesenchymal Stem Cells (MSC) derived exosomes are emerging as promising, cell-free therapeutic agents for diabetes. However, the precise protein, metabolite, and lipid contents of exosomes remain ill characterized and their potential for modulating diabetes pathophysiology remains underexplored.

Methods: We performed an exploratory multi-omics analysis of the proteome, lipidome, and metabolome of exosomes derived from early-stage pass i) bone marrow and umbilical cord derived MSCs and ii) conditioned media from early- and late-MSCs. Mass spectrometry was used to characterize exosomal cargo. Integrative analyses were conducted using Multi-Omics Factor Analysis (MOFA), and the results were compared with publicly available datasets related to Type 1 Diabetes (T1D) to identify inversely correlated pathways to identify inversely correlated pathways that may represent candidate targets for therapeutic intervention in T1D.

Results: Multi-Omics Factor Analysis (MOFA) pinpointed Factor 1 as the principal axis of variation, contrasting an anti-inflammatory, homeostatic exosome signature from early-passage MSCs with a pro-inflammatory Senescence-Associated Secretory Phenotype (SASP) profile in late-passage secretomes. Early passage exosomes also contained cytoprotective factors, including heat-shock proteins and antioxidant enzymes, which may counteract β-cell apoptosis and oxidative stress. In contrast, conditioned media from late-passage MSCs exhibited classical SASP features, marked by increased expression of inflammatory mediators. MOFA further revealed that early-passage exosomes shared latent factors inversely correlated with diabetes-related gene signatures, and pathway enrichment analyses indicated that these vesicles could restore immune and metabolic homeostasis disrupted in Type I diabetes (T1D).

Conclusions: Our findings underscore the importance of MSC passage stage in influencing exosomal content and function. Additionally, early-passage MSC-derived exosomes exhibit potent anti-inflammatory and cytoprotective molecules that may mitigate β-cell injury, immune dysregulation, and metabolic dysfunction in diabetes. These data provide a multi-omics rationale for considering early-passage MSC-derived exosomes as a promising, scalable option in the development of diabetes therapies.

Keywords: Mesenchymal Stem Cells, Exosomes, Type 1 Diabetes, Multi-Omics, Proteomics, Lipidomics, Metabolomics, MOFA, Senescence

Abbreviations: E-cad, E-cadherin; GLP-1R, glucagon-like peptide-1 receptor; GLUT-2/4, glucose transporter-2/4; IGF-1, insulin-like growth factor-1; LRP5/6, low-density-lipoprotein-receptor-related protein-5/6; MSC, mesenchymal stem cell; PDX1, pancreatic and duodenal homeobox 1; ROS, reactive oxygen species; TNFR, tumor-necrosis-factor receptor; VEGF, vascular endothelial growth factor

Background

Mesenchymal Stem/Stromal Cells have shown promise for treating T1D due to their regenerative and immunomodulatory capabilities [1]. Emerging evidence suggests that the therapeutic effects of MSCs are largely mediated by their secretome, particularly with bioactive molecules housed within extracellular vesicles or exosomes, rather than by direct cell engraftment [1,2]. MSC-derived exosomes have been proposed as a novel cell-free therapy for diabetes and its complications, with studies showing that they can be as effective as or even superior to MSC transplantation [1]. For example, MSC-derived exosomes have been shown to alleviate peripheral insulin resistance and preserve pancreatic β-cell function in diabetic models [1]. They can reduce hyperglycemia and autoimmune responses in T1D models by increasing regulatory T cells and protecting islet cells. These findings underscore the therapeutic potential of MSC-derived exosomes in diabetes, through mechanisms of immunomodulation, tissue regeneration, and metabolic regulation.

A critical but often overlooked factor in MSC-based therapies is the effect of repeated cell culture passages and concomitant cellular aging on therapeutic potency. Repeated in-vitro expansion of MSCs (higher passage number) is known to induce replicative senescence and "inflammatory drift" in phenotype [3]. Consecutive passaging shifts MSCs from a pro-regenerative, anti-inflammatory state toward a pro-inflammatory state, accompanied by a Senescence-Associated Secretory Phenotype (SASP) rich in inflammatory cytokines [3,4]. Senescent MSCs secrete altered exosomes that can carry pro-inflammatory factors, contributing to the adverse effects of aging [4,5]. Thus, exosomes from late-passage or older MSCs may have diminished therapeutic efficacy and potentially even prove counterproductive, as they may increase the SASP milieu [5]. By contrast, early-passage MSCs (young, minimally expanded cells) are expected to produce exosomes enriched in regenerative and immunosuppressive cargo, with fewer deleterious SASP components.

Given these considerations, we hypothesize that early-passage MSC-derived exosomes possess molecular components with potential to modulate pathological pathways associated with diabetes, compared to exosomes from late-passage MSCs. To date, a comprehensive multi-omics characterization of MSC exosome cargo (including proteomics, metabolomics, lipidomics) in the context of diabetes has not been reported. Moreover, it remains unclear how the bioactive molecules housed within exosomes overlap with or inversely correlate to the pathways known to be dysregulated in diabetic tissues. Identifying such relationships could reveal whether exosomes may naturally carry factors that counteract the inflammatory, apoptotic, and metabolic dysregulations in diabetes.

a) In this study, we performed an in-depth multi-omics profiling of exosomes from early and late-passage MSCs and compare their cargo to molecular signatures of diabetes from public datasets. Specifically, we characterized the proteins, metabolites, and lipids contained in MSC-derived exosomes using mass spectrometry;

b) Determined how the exosome contents align with or oppose the altered pathways in diabetes by integrating our data with public transcriptomic and metabolomic datasets of diabetic patients; and

c) Contrasted the profile of early-passage exosomes with that of late-passage MSC-derived exosomes known in the literature as we hypothesize that aging- or passage number-related changes should be measurable in the exosome cargo. In this study, we employed bioinformatics tools for multi-omics integration (MOFA) and pathway evaluation for downstream analysis. Our findings help to inform whether early-passage MSC-derived exosomes are appropriately equipped to counteract diabetes-associated molecular dysfunction and thus provide rationale for their use as a regenerative therapy for diabetes.

Methods

Exosome Isolation and Multi-Omics Data Generation

MSC Culture and Exosome Collection: Human MSCs were obtained from a young, healthy donor source (e.g. umbilical cord tissue) and expanded in vitro under standard conditions. Early-passage MSCs (passage 3) were used for exosome isolation. Exosomes were collected from serum-free conditioned medium by sequential ultracentrifugation and size-exclusion chromatography to ensure high purity. To obtain a comparative reference, we also collected layered omic data from late-passage MSC-derived exosomes (passage >10) reported in literature, to represent the senescent MSC secretome. For example, previously published studies have characterized Extra Cellular Vehicles (EVs) from MSCs at passages 2-5 versus 9-12 [6]; those data were used for qualitative comparison of exosome content changes with passaging.

Multi-Omics Profiling

To comprehensively characterize the molecular cargo of exosomes, we employed three complementary omics platforms: proteomics, metabolomics, and lipidomics.

a) Proteomics: Exosomes were lysed and proteins analyzed using high-resolution mass spectrometry on Sapient Bioanalytic' Bruker timsTOF Pro platform, which utilizes trapped ion mobility spectrometry for enhanced separation and label-free quantification. Peptide identification and quantification were conducted using proprietary Signal Finder™ processing algorithms, enabling a comprehensive proteomic profile of the exosomal contents.

b) Metabolomics: Exosomal metabolites were extracted via organic solvent extraction and profiled using Sapient' s rapid liquid chromatography coupled with high-resolution ion mobility mass spectrometry (rLC-IM-MS). This platform allows for the simultaneous detection of over 15,000 small molecule biomarkers per sample, encompassing a broad spectrum of metabolite classes, including amino acids, central carbon compounds, nucleotides, organic amines, and neurotransmitters. Sapient leverages an in-house library of over 13,000 chemical reference standards and a spectral

database containing more than 6 million MS/MS spectra, facilitating high-confidence metabolite identification.

c) **Lipidomics:** To specifically profile lipid content, we performed untargeted lipidomics analysis using Sapient's high-throughput rLC-IM-MS platform. This approach enables the identification and quantification of thousands of lipid species across various classes, including glycerophospholipids, sphingolipids, sterol esters, cardiolipins, and fatty acid esters of hydroxy fatty acids (FAHFAs). The platform's extensive reference library and advanced spectral matching capabilities ensure accurate lipid identification, providing a detailed lipidomic landscape of the exosomal cargo [7]. Each dataset (proteome, metabolome, lipidome) underwent quality control and preprocessing. To focus on biologically relevant signals, we filtered for consistently detected molecules across replicate exosome preparations (n = 3 replicates).

Reference Public Datasets for Comparative Analysis

To contextualize our exosomal findings, we incorporated several publicly available transcriptomic and metabolomic datasets relevant to Type 1 and Type 2 Diabetes. We first retrieved a whole blood gene expression dataset from GEO Series GSE123658, which profiled transcriptomic signatures in individuals with Type 1 Diabetes (T1D) and matched healthy volunteers [8]. This RNA-seq dataset includes samples from 43 healthy controls and 39 T1D patients, with RNA extracted from PAXgene tubes and processed to remove abundant globin transcripts prior to sequencing. Libraries were prepared using the TruSeq Stranded mRNA kit and sequenced using paired-end 2×75 bp protocols on Illumina NextSeq 500 or HiSeq 4000 platforms. Gene expression normalization accounted for sex and batch effects. This dataset provides a systems-level view of immune and inflammatory gene dysregulation in T1D and was leveraged to compare against differentially expressed RNAs identified in MSC-derived exosomes.

For metabolomic comparisons, we utilized two publicly available datasets focused on Type 1 and Type 2 Diabetes. The T2D dataset, reported by *Ma et al. (2025, PMCID: PMC11880315)*, investigated serum metabolites in patients with T2D versus healthy controls. The T1D dataset (DOI: 10.25584/2405140) analyzed urinary metabolites from individuals with T1D compared to control participants [9]. To enable cross-comparisons, we identified and focused on metabolites common to both datasets. For the T1D dataset, raw intensities were normalized by Total-Ion-Current (TIC), $\log_2$-transformed, and analyzed using the Mann-Whitney U test. Resulting p-values were corrected using the Benjamini-Hochberg (BH) method, with $q < 0.05$ considered significant. For the T2D dataset, published statistics were directly extracted. A comparative dot plot was generated using ggplot2, highlighting metabolite-level differences across the two conditions [10,11]. All external datasets were harmonized to match gene/protein identifiers and metabolite naming conventions used in our exosome dataset. Where necessary, batch correction methods were applied to mitigate inter-study variation. This integrative approach enabled a robust comparison of MSC exosomal cargo with diabetes-associated molecular features.

Multi-Omics Data Integration and Analysis

Given the heterogeneity of molecular cargo under consideration in this, we employed integrative bioinformatics tools to derive a systems-level understanding of the exosome profiles. To perform unsupervised integration of the exosomal omics layers, we applied Multi-Omics Factor Analysis (MOFA), a latent variable model designed to uncover shared and modality-specific sources of variation across high-dimensional multi-modal datasets [12]. Input matrices from proteomic, metabolomic, and lipidomic profiling were fed into the MOFA2 framework. The resulting latent factors revealed underlying biological axes of co-regulation, allowing us to identify composite patterns in exosome composition.

Pathway and network analyses were conducted to functionally annotate the omics features. For proteomics and transcriptomics, pathway enrichment was performed using cluster Profiler in R, with ontology terms and Reactome pathways retrieved for significantly altered exosomal components [13,14]. We also interrogated datasets related to Type 1 and Type 2 Diabetes to extract known disease-enriched pathways, such as insulin signaling, JAK/STAT–IL-6 signaling, TNF/NF-κB axis, and oxidative stress responses. Particular attention was paid to pathways that exhibited inverse enrichment patterns between exosomal cargo and diabetic patient data. Protein–protein interaction (PPI) networks of exosomal proteins were mapped using STRING, and these were compared with networks of diabetes-associated proteins to identify potential points of intersection or antagonism.

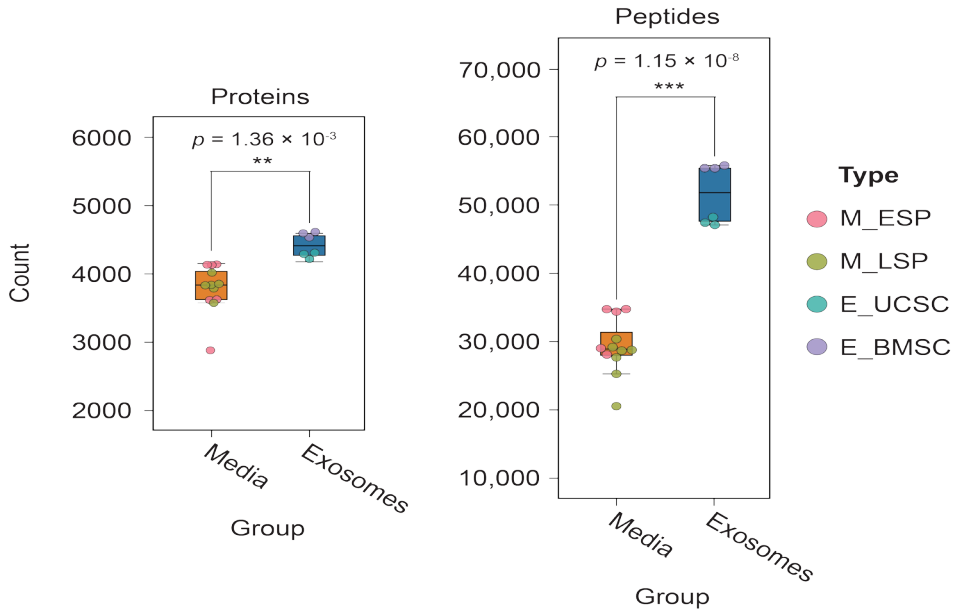
For statistical analysis, we conducted differential expression testing where applicable, such as comparing exosomal RNA abundance to cellular or control baselines. Moderated t-tests or non-parametric tests were applied depending on sample distribution and size, with adjusted p-values < 0.05 considered significant after correction for multiple testing. For MOFA-derived latent factors, the proportion of variance explained was used to assess the biological relevance of each factor. Correlation analyses between exosomal components and disease features (e.g., transcriptomic or metabolomic readouts from public diabetes datasets) were performed using Pearson or Spearman coefficients, with statistical significance used to support inferences of inverse or concordant associations.

All analyses were performed using R version 4.4.2 and Python version 3.12.7. Key packages included MOFA2 for latent factor modeling, lima and DESeq2 for differential expression analysis, and cluster Profiler for enrichment analysis. Figures were generated using ggplot2 in R and matplotlib and seaborn in Python.

Results

Early-Passage MSC Exosomes Are Enriched for ECM-Interactive, Cytoprotective, and Vesicle-Trafficking Proteins. Early Passage MSC Exosomes Are Enriched for ECM Interactive, Cytoprotective, and Vesicle Trafficking Proteins Mass spectrometry successfully

identified 4,554 protein groups with more than one peptide match across all groups in the study (Supplementary Figure 1a). Early-passage MSC-derived exosomes contained a rich protein cargo of 2,403 differentially expressed proteins at the FDR threshold of < 0.05 in the MSC-derived exosomes (comparison) to early-stage pass media (reference) comparison (Figure 1e). Proteomic analysis of MSC-derived exosomes confirmed significant enrichment of classical exosomal markers, including the tetraspanins CD9, CD63, and CD81, along with Alix (PDCD6IP), validating the identity and purity of the vesicle preparations. Beyond these canonical markers, the upregulated proteins in early-passage exosomes revealed a diverse and functionally rich cargo reflective of active roles in extracellular remodeling, signaling, metabolism, and cytoprotection (Supplementary Figure 1a) (Figure 1).



Supplementary Figure 1a

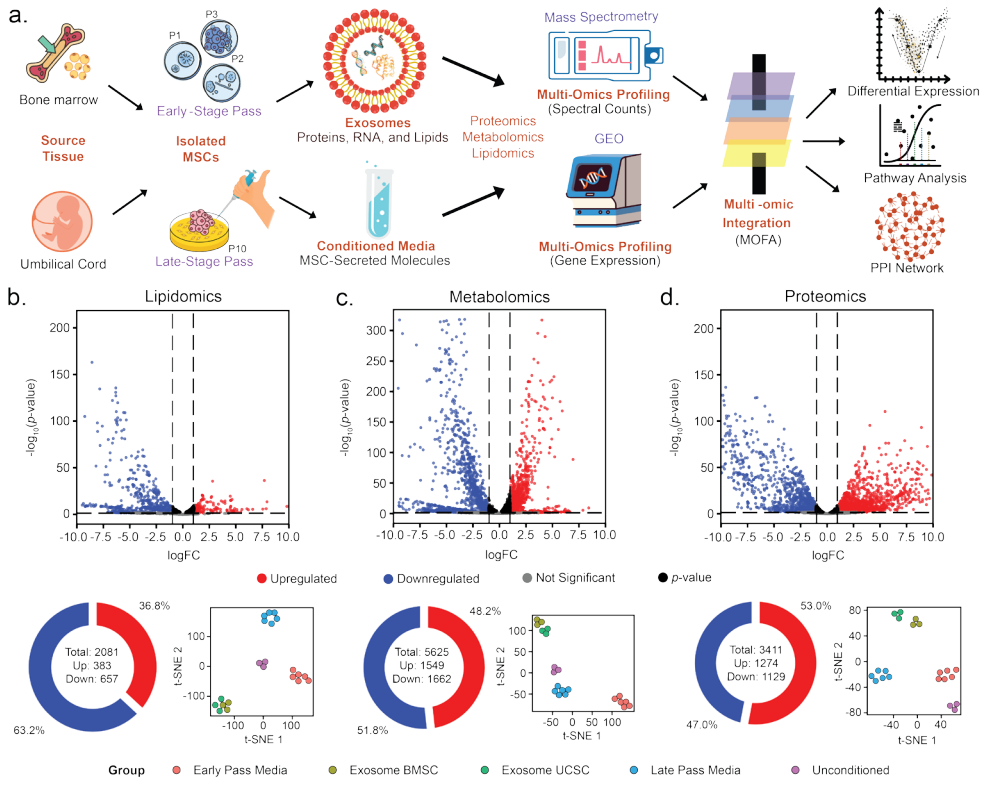


Figure 1: Experimental Workflow and Multi-Omics Profiling of MSC-Derived Exosomes and Conditioned Media.

Figure 1: (a) Schematic overview of the experimental design and multi-omics workflow. Mesenchymal stem cells (MSCs) were isolated from source tissues (bone marrow and umbilical cord) and cultured through early-stage (P1-P3) and late-stage (P10) passages. Exosomes (containing proteins, RNA, and lipids) were isolated from conditioned media, and MSC-Secreted Molecules in the conditioned media were also analyzed. These samples, along with cellular Gene Expression Data (from GEO), underwent multi-omics profiling. Proteomic, metabolomic, and lipidomic data were generated using mass spectrometry. The resulting multi-omics datasets were integrated using Multi-Omics Factor Analysis (MOFA) and subjected to downstream analyses including differential expression, pathway analysis, and Protein-Protein Interaction (PPI) network construction.

(b-d) Differential abundance analysis of features in MSC-derived samples. Volcano plots display differentially abundant features from (b) lipidomics, (c) metabolomics, and (d) proteomics. Each point represents a feature, with the x-axis indicating log Fold Change (logFC) and the y-axis representing -$\log_{10}$(p-value). Features significantly upregulated are shown in red, downregulated in blue, and non-significant features in grey. Adjacent pie charts illustrate the total number of features identified in each omics layer, with proportions of upregulated and downregulated features indicated. Corresponding t-SNE plots depict sample clustering based on their respective omics profiles, with sample groups color-coded as: Early Pass Media (pink/red), Exosome BMSC (Bone Marrow MSC-derived exosomes, olive green), Exosome UCSC (Umbilical Cord MSC-derived exosomes, teal), Late Pass Media (light blue), and Unconditioned media (purple).

Notably, STX12 and GBF1, central regulators of endosome to Golgi traffic, ranked among the most abundant vesicle associated proteins, highlighting an active endosomal sorting machinery in MSC-derived exosomes (Figure 2). The marked presence of adhesion molecules CD99 and CD166, both implicated in leukocyte trans-endothelial migration, suggests an inherent tropism of these vesicles for vascular or immune microenvironments. Enrichment of proteasome subunits (PSMB1, PSMD11) together with the E3 ligase UBE3A points to a cargo capable of modulating proteostasis and ubiquitin mediated signaling in target cells. Additionally, detection of actin capping protein CAPZA2 and Septin 8 indicates that exosomes convey cytoskeletal regulators that could remodel actin dynamics after uptake. This differential abundance analysis depicts MSC derived exosomes as nanocarriers enriched in proteins that

a) orchestrate vesicle trafficking and fusion,

b) direct vesicle adhesion to inflamed tissues, and

c) fine tune proteostasis and cytoskeletal reorganization in recipient cells (Figure 2).

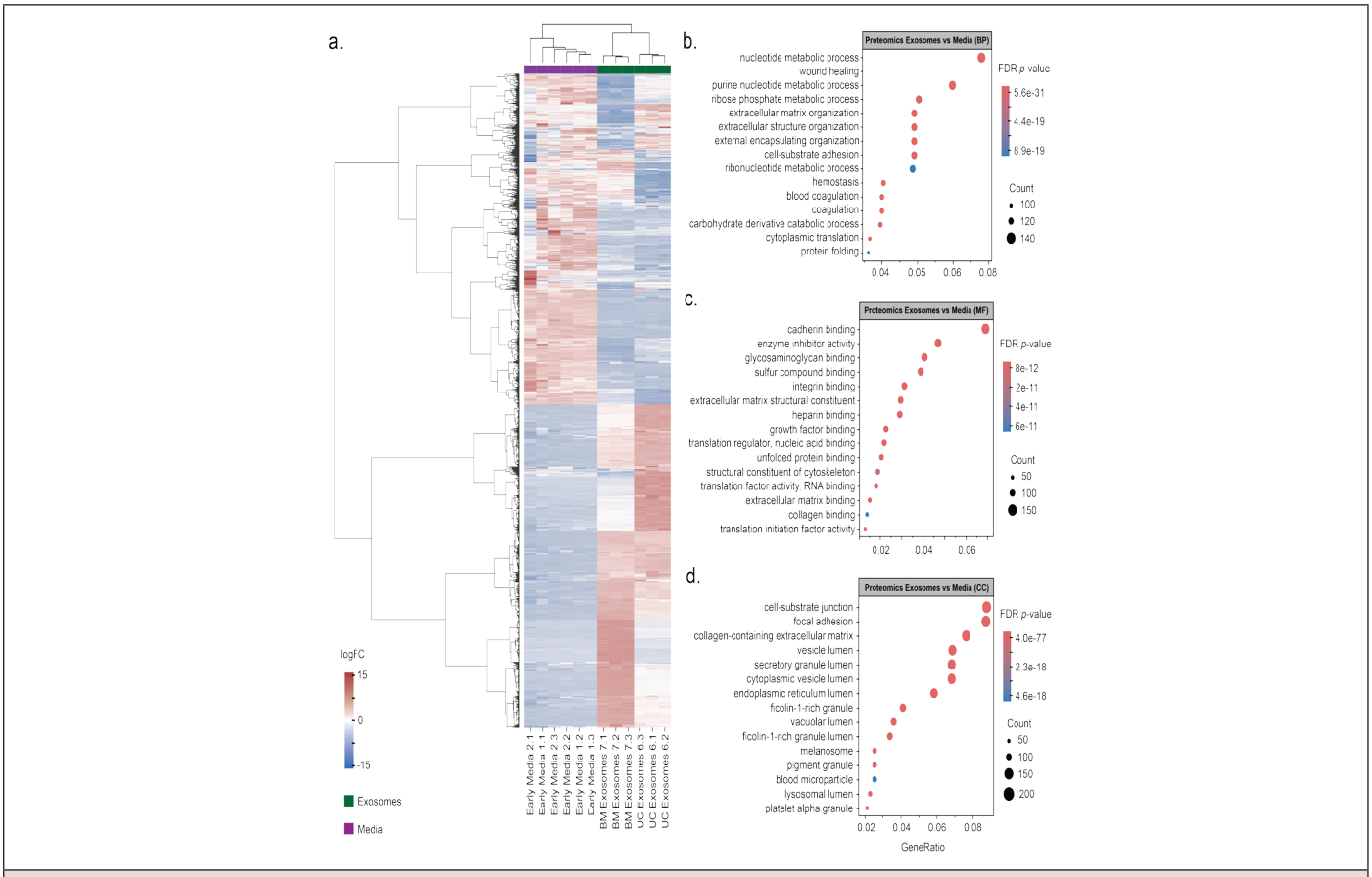


Figure 2: Proteomic analysis reveals distinct protein profiles and enriched functional pathways in exosomes compared to media controls.

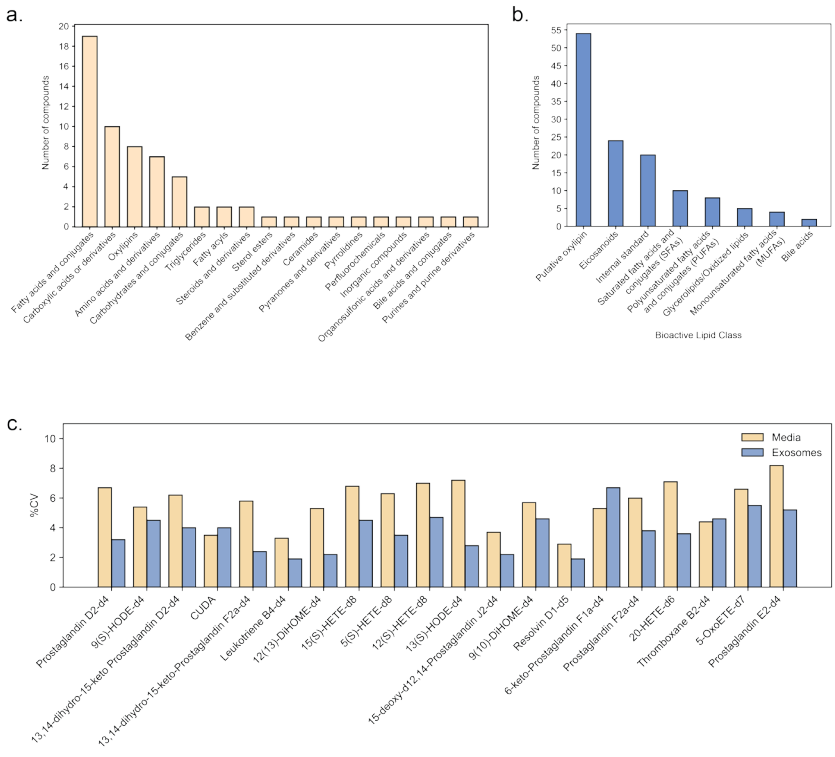
Figure 2: (a) Hierarchical clustering heatmap of differentially expressed proteins (False Discovery Rate [FDR] < 0.05) between exosome samples (n=6, blue bar) and media (baseline) control samples (n=6, red bar). Each row represents an individual protein, and each column represents an individual sample. Protein expression levels are indicated by color intensity, with red signifying higher relative abundance and blue signifying lower relative abundance, scaled per protein. Dendrograms display the clustering of samples (top) and proteins (left).

(b-d) Gene Ontology (GO) enrichment analysis of proteins significantly differentially abundant between exosome and media samples. Dot plots display the top enriched GO terms for (b) Biological Process (BP), (c) Molecular Function (MF), (d) Cellular Component (CC)

In these plots, the x-axis represents the Gene Ratio (the ratio of the number of differentially expressed genes associated with a term to the total number of differentially expressed genes in the input list). The size of each dot corresponds to the count of proteins associated with the respective GO term. The color intensity reflects the FDR-adjusted p-value, with warmer colors (e.g., red) indicating higher statistical significance (lower p-values).

Gene Ontology enrichment analysis of the exosomal proteome (Figure 2a-c) underscores Extracellular Matrix (ECM) remodeling and cell adhesion and intracellular luminal trafficking as key themes influencing biological processes. Within the Biological Process domain, MSC derived exosomes were markedly enriched for extracellular matrix organization, cell substrate adhesion, and sequential terms describing wound healing and hemostasis/coagulation. These pathways mirror the high abundance of collagens, laminins, fibronectin and integrins we detected by LC MS/MS, and together suggest that the vesicles are primed to orchestrate matrix deposition and platelet-endothelium cross talk at sites of tissue injury. Unexpectedly, enzymes involved in nucleotide and ribose phosphate metabolic processes were also overrepresented. This may serve as preliminary signal for MSC-derived exosomes to deliver purine handling capacity to metabolically stressed recipient cells.

The Molecular Function and Cellular Component views reinforce these observations. Proteins with cadherin, integrin, glycosaminoglycan, and growth factor binding activities dominated the MF list, alongside structural constituents of the cytoskeleton and translation machinery, implying the vesicles can couple adhesion cues with local protein synthesis control in target cells. Cellular Component terms clustered strongly to cell substrate junctions and focal adhesions, but also to luminal compartments (vesicle lumen, secretory/platelet α granule lumen, lysosomal lumen), consistent with an endosomal origin and with a role in modulating secretory and degradative pathways after uptake. Pathway analysis supports the hypothesis of MSC-derived exosomes as nano carriers enriched in ECM interactive and hemostatic modules, equipped to remodel injured microenvironments while simultaneously delivering metabolic and translational support to neighboring cells.



Supplementary Figure 2

MOFA Factor 1 Defines a Pro-inflammatory SASP Axis Opposed by an Anti-inflammatory, Homeostatic Exosome Signature Untargeted profiling yielded a deep but sparsely annotated chemical space. Of 2,085 bioactive lipid features detected across all samples, only 127 (6 %) could be confidently annotated to lipid subclasses, and of 5,629 metabolite features, only 65 (1 %) had definitive structure assignments, despite high-accuracy m/z measurements for the entire feature set (Supplementary Figure 2). The paucity of structural labels precluded conventional pathway- or ontology-based enrichment analyses and would have biased any interpretation toward a small, well-characterized fraction of the data. To capitalize on the full quantitative information while circumventing annotation gaps, we therefore integrated the lipidomic, metabolomic and proteomic matrices using Multi-Omics Factor Analysis (MOFA). MOFA models latent factors that capture shared variance across data layers irrespective of annotation status, allowing both identified and unidentified m/z features to contribute to biologically meaningful signatures. The resulting factors provide a systems-level view of coordinated molecular changes driven by MSC passage stage.

Factor 1 accounted for the largest proportion of variance across the integrated lipidomic, metabolomic and proteomic layers and cleanly separated samples according to passage stage (Figure 3a). Samples with high positive Factor 1 scores corresponded to late-passage MSCs, whereas high negative scores mapped to early-passage exosomes (Figure 3b). Examination of the protein loadings revealed that the directionality of Factor 1 captures an inflammatory gradient that mirrors the Senescence-Associated Secretory Phenotype (SASP). (Figures 3c-e) capture the top absolute loadings for factor 1 across the proteome, metabolome, and lipidome (Figure 3).

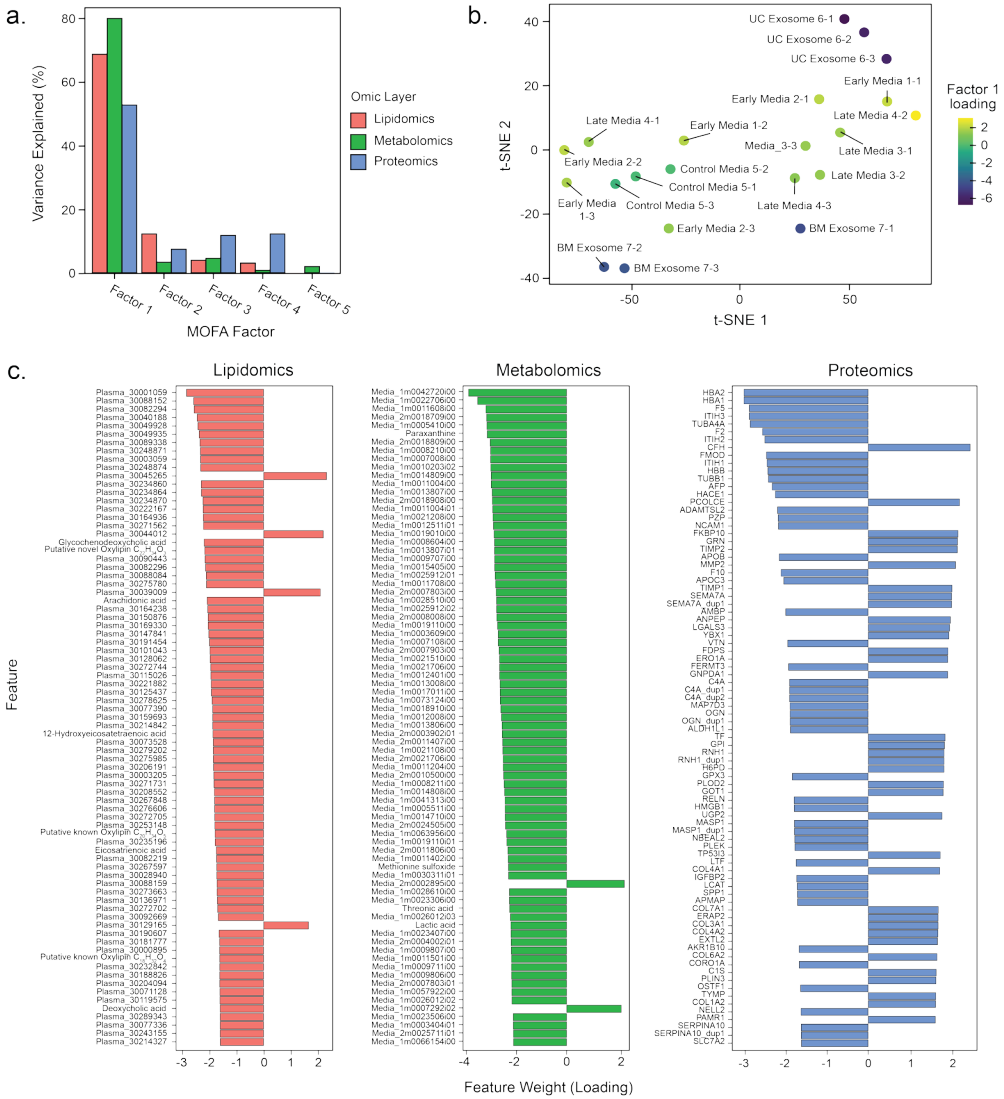


Figure 3: Multi-Omics Factor Analysis (MOFA) reveals distinct molecular signatures differentiating sample groups and identifies key contributing features.

Figure 3: (a) Variance Explained per MOFA Factor: Bar chart illustrating the percentage of total variance explained by each of the top five MOFA factors (Factor 1 to Factor 5) across the three integrated omics datasets: Lipidomics (red), Metabolomics (green), and Proteomics (blue). Factor 1 accounts for the largest proportion of variance, predominantly driven by Metabolomics and Proteomics data.

(b) t-SNE of MOFA latent space (colour = Factor 1 loading): A t-SNE (t-distributed Stochastic Neighbour Embedding) plot visualizing the samples in the latent space derived from MOFA. Each point represents an individual sample (e.g., "Exosome_6-1", "Media_1-1"). Points are coloured according to their loading on Factor 1, with the colour scale on the right indicating the loading value (yellow for high positive loadings, dark blue/purple for high negative loadings). The plot demonstrates clear separation of sample groups (e.g., "Exosome" samples clustering distinctly from "Media" samples), driven primarily by Factor 1.

(c) Feature Weights (Loadings) for Factor 1 across omics layers: Bar plots showing the top contributing features (lipids, metabolites, and proteins) to MOFA Factor 1, ranked by their feature weight (loading). Lipidomics (left, red): Displays the feature weights for individual lipid species. Lipids with positive weights (e.g., Bile AcidGlycochenodeoxycholic acid, certain Putative_Novel_Oxylipins) are positively associated with Factor 1, while those with negative weights are negatively associated. Metabolomics (middle, green): Shows feature weights for identified metabolites. Metabolites like Paraxanthine and Methionine sulfoxide show strong positive or negative contributions to Factor 1. Proteomics (right, blue): Illustrates feature weights for identified proteins. Proteins such as HBA2, ITIH3, and FMOD are among the top contributors, with their respective positive or negative weights indicating their association with Factor 1.

Protein species with the strongest positive loadings, therefore enriched in late-stage secretome, were dominated by classical SASP and inflammatory mediators. These included complement regulators and initiators (CFH, C4A, MASP1, C1S), acute-phase and coagulation factors (PZP, F10), matrix-remodeling enzymes (MMP2) and a spectrum of pro-inflammatory cytokine or DAMP-like molecules (HMGB1, LGALS3, SPP1, APOC3, SEMA7A). Additional components such as TIMP2, RELN, TYMP and multiple collagens (COL1A2, COL3A1, COL4A1/2, COL6A2, COL7A1) underscore active extracellular-matrix turnover and tissue-repair signals characteristic of chronic, unresolved inflammation. These proteins define a late-passage secretome poised to amplify complement activation, leukocyte recruitment and ECM degradation which are all hallmarks of SASP-driven tissue dysfunction.

Conversely, proteins with the most negative loadings, or enriched in early-passage exosomes, exhibited anti-inflammatory or homeostatic roles. Heavy-chain hyaluronan stabilizers (ITIH1/2/3), ECM-modulating fibromodulin (FMOD), antioxidant and redox regulators (H6PD, GPX3) and immune-tuning factors such as progranulin (GRN) and the antigen-processing enzyme ERAP2 were prominent. Their coordinated abundance suggests that youthful MSC exosomes convey a protective cargo able to dampen inflammation, reinforce ECM integrity and restore redox balance.

MOFA Factor 1 delineates not only a protein-based pro- versus anti-inflammatory divide but also a lipidomic counterpart centered on oxylipins and bile acids. All eight bioactive lipids with the strongest negative loadings-glycochenodeoxycholic acid, deoxycholic acid, a series of putative oxylipins ($C_{22}H_{34}O_3$, $C_{20}H_{34}O_5$, $C_{18}H_{32}O_4$), free fatty acids arachidonic and eicosatrienoic acids, and the eicosanoid 12-HETE-track with the early-passage, anti-inflammatory exosome signature. Oxylipins derived from poly-unsaturated fatty acids are pleiotropic: while some metabolites of arachidonic acid (e.g., leukotrienes, certain prostaglandins) propagate inflammation, others-including 12-HETE under specific enzymatic contexts-participate in resolution phases or bias macrophages toward a reparative phenotype. Their concerted enrichment alongside bile acids that engage FXR/TGR5 signaling pathways (well-known dampers of NF-κB activity) suggests that the lipid arm of Factor 1 represents a pro-resolving, metabolic-regulatory program that counterbalances the SASP. Thus, Factor 1 captures a coordinated molecular opposition in which late-passage secretome deploy classic SASP proteins, whereas early-passage exosomes co-export anti-inflammatory proteins and a specialized oxylipin-bile-acid repertoire that may actively steer recipient cells toward immune quiescence and tissue homeostasis.

The anti-inflammatory, homeostatic pole of Factor 1 is sharpened further by four small-molecule metabolites that also carry strong negative loadings. Paraxanthine, the principal caffeine catabolite, associates with neuro- and cardio-protective effects and may reflect lifestyle or xenobiotic exposures linked to favorable immune tone. Threonic acid, a downstream product of vitamin C turnover, implies adequate ascorbate availability and a robust antioxidant network that scavenges reactive species generated during immune activation. The presence of methionine sulfoxide-an oxidized sulfur amino acid normally repaired by methionine-sulfoxide reductases-suggests efficient redox-repair cycling rather than unchecked oxidative damage in this state. Finally, elevated lactic acid points to tightly coupled glycolytic flux that can act as an immunomodulatory signal, steering macrophages toward pro-resolution phenotypes. Taken together, these metabolites complement the pro-resolving oxylipin-bile-acid profile and the protein cargo of early-passage exosomes, reinforcing the view that the negative end of Factor 1 represents a coordinated metabolic and signaling program that sustains antioxidant defense, energy efficiency and immune quiescence in opposition to the pro-inflammatory SASP axis. MOFA Factor 1 can be interpreted as a pan-omic inflammatory-versus-homeostatic axis: positive scores reflect a SASP-laden, pro-inflammatory program in late MSC secretions, while negative scores denote an anti-inflammatory, reparative signature characteristic of early-passage exosomes. This

axis provides a mechanistic framework linking MSC senescence to functional shifts in exosomal bioactivity and highlights candidate protein mediators that may underlie the superior therapeutic potential of youthful MSC exosomes.

Type 1 Diabetes Metabolome Mirrors a Deficit in the MOFA-Derived Anti-inflammatory Axis To evaluate whether the protective metabolic program captured by MOFA Factor 1 is reflected in clinical samples, we compared its key metabolites with an independent dataset contrasting plasma from patients with type 1 (T1D) versus type 2 diabetes (T2D). Two metabolites that anchor the anti-inflammatory/homeostatic pole in Factor 1, L-threonic acid (a vitamin C catabolite) and L-lactic acid (a pleiotropic fuel and immunomodulatory signal), showed significant depletion in T1D ($\log_2$FC ≈ -0.25 and -0.10, respectively; $q < 0.05$), whereas T2D samples exhibited a mild increase or no change. Because higher levels of these metabolites co-loaded with early-passage, anti-inflammatory exosome signatures in the MOFA model, their reduction in T1D suggests a systemic milieu that is farther from the homeostatic end of the axis and potentially biased toward a pro-inflammatory or SASP-like state. In contrast, the relative preservation-or even elevation-of these metabolites in T2D implies partial retention of the pro-resolution metabolic circuitry identified in vitro.

This divergence is noteworthy against a backdrop of shared hyperglycemic markers (D-glucose, D-mannose were increased in both cohorts), indicating that the oxidative-stress and antioxidant balance, rather than glycemic load per se, may distinguish the inflammatory tone of T1D from T2D. Mechanistically, lower L-threonic acid could reflect diminished vitamin C turnover and antioxidant capacity, while reduced lactate might signify impaired metabolic coupling or loss of lactate-mediated anti-inflammatory signaling. Together, these findings provide an inferential bridge between the MSC exosome model and patient metabolomics: the beneficial metabolite signature that accompanies youthful MSC exosomes appears under-represented in T1D plasma, strengthening the rationale for deploying early-passage MSC-derived exosomes-or strategies that restore their associated metabolites-to counterbalance autoimmune inflammation in T1D.

In summary, our results demonstrate that early-passage MSC-derived exosomes are enriched with multi-omic factors that collectively target and mitigate diabetes-associated pathways, unlike exosomes from senescent MSCs. The multi-omics approach provides a comprehensive understanding of the exosome's therapeutic payload, from proteins and RNAs that modulate immune responses, to metabolites and lipids that influence metabolic signaling. Crucially, these exosomal contents show an inverse relationship with the molecular signatures of diabetes, supporting the idea that they could help restore balance in a diabetic milieu.

Discussion

Therapeutic Potential of Early-Passage MSC-Exosomes in Diabetes In this exploratory study, we characterized the multiomic profiles of early-passage mesenchymal stem cell-derived exosomes and related our findings to type 1 diabetes pathophysiology. A deeper understanding of MSC-derived vesicles may reveal their capacity to serve as a cell-free therapy that offers many benefits of MSC transplantation free from its associated risks. Our findings highlight that early-passage MSC-exosomes can may? mitigate β-cell injury, quell inflammation, and restore metabolic homeostasis. These effects are especially valuable in an autoimmune diabetic milieu where chronic inflammation and β-cell dysfunction drive disease progression.

Try this: Based on the findings of this study, a hypothetical outcome (A striking outcome) of early-passage MSC-exosome treatment could bethe reduction of pro-inflammatory cytokine signaling in the diabetic pancreas (Figure 5). The untreated diabetic state is characterized by elevated TNF-α and IL-6 levels (pro-inflammatory cytokines) that create a toxic inflammatory microenvironment around islets, contributing to β-cell stress and immune-mediated damage. Published studies in diabetic models support the hypothesis of MSC-derived exosome mediated suppression of pro-inflammatory cytokines (e.g., TNF-α, IL-6) and induction of anti-inflammatory cytokines like IL-10 [15]. By dampening these cytokine networks, the exosomes appear to disrupt the vicious cycle of insulitis and systemic inflammation that impairs β-cell survival in type 1 diabetes.

Additionally, the diabetic state is associated with heightened oxidative stress (ROS) and inflammatory damage that leads to β-cell dysfunction and death [ref]. Exosome treatment could interrupt this process by delivering factors that promote cell survival. Our multi-omics analysis detected anti-oxidative stress metabolites and lipids in the exosomal cargo. For example, we observed increased expression of PDX1 and other β-cell transcription factors post-exosome treatment, suggesting that exosomal signals help restore the functional identity of β-cells. These results align with prior reports that MSC-derived exosomes carry cytoprotective molecules capable of inhibiting apoptosis in injured islets [16]. Specifically, exosome-treated β-cells show reduced activation of caspase-3 and PARP (markers of apoptosis) and upregulation of survival proteins like surviving [17]. In a hypoxia-induced injury model, human MSC-exosomes dramatically improved β-cell survival, correlating with diminished ER stress markers and lower cleaved caspase-3 levels [17]. By delivering such pro-survival signals, early-passage exosomes preserve the remaining β-cell mass in an inflammatory setting and support the recovery of insulin secretory function.

Early-passage MSC-derived exosomes exert pronounced metabolic regulatory effects that can counteract diabetic dysregulation. A key observation in our study was the improvement of insulin sensitivity and glucose homeostasis following exosome treatment. In insulin-responsive tissues, exosomes appear to enhance insulin signaling pathways, contributing to better peripheral glucose uptake and utilization. Induction of GLUT-

4 translocation by MSC-derived exosomes is supported by the literature: for instance, human umbilical cord MSC-derived exosomes have been shown to augment insulin sensitivity by upregulating glucose transporters (GLUT1-4) and Akt signaling, thereby increasing glucose uptake in muscle and adipose tissue while diminishing insulin resistance in diabetic rats [18,19]. Transplantation of MSC-derived exosomes in diabetic animals has been reported to promote islet cell proliferation, increase insulin gene expression, and to reduce hyperglycemia [18]. Exosomes may also carry developmental transcription factors or induce pathways (e.g., Reg genes and Cyclin D1) that encourage β-cell proliferation [18]. Additionally, the cargo of growth factors in exosomes likely plays a role: factors such as VEGF and IGF-1, identified within the MSC secretome and possibly loaded in exosomes, could improve islet vascularization and provide direct trophic support to β-cells. Indeed, IGF-1 signaling is known to enhance β-cell survival and function, and VEGF can ameliorate islet hypoxia by improving local blood supply [ref].

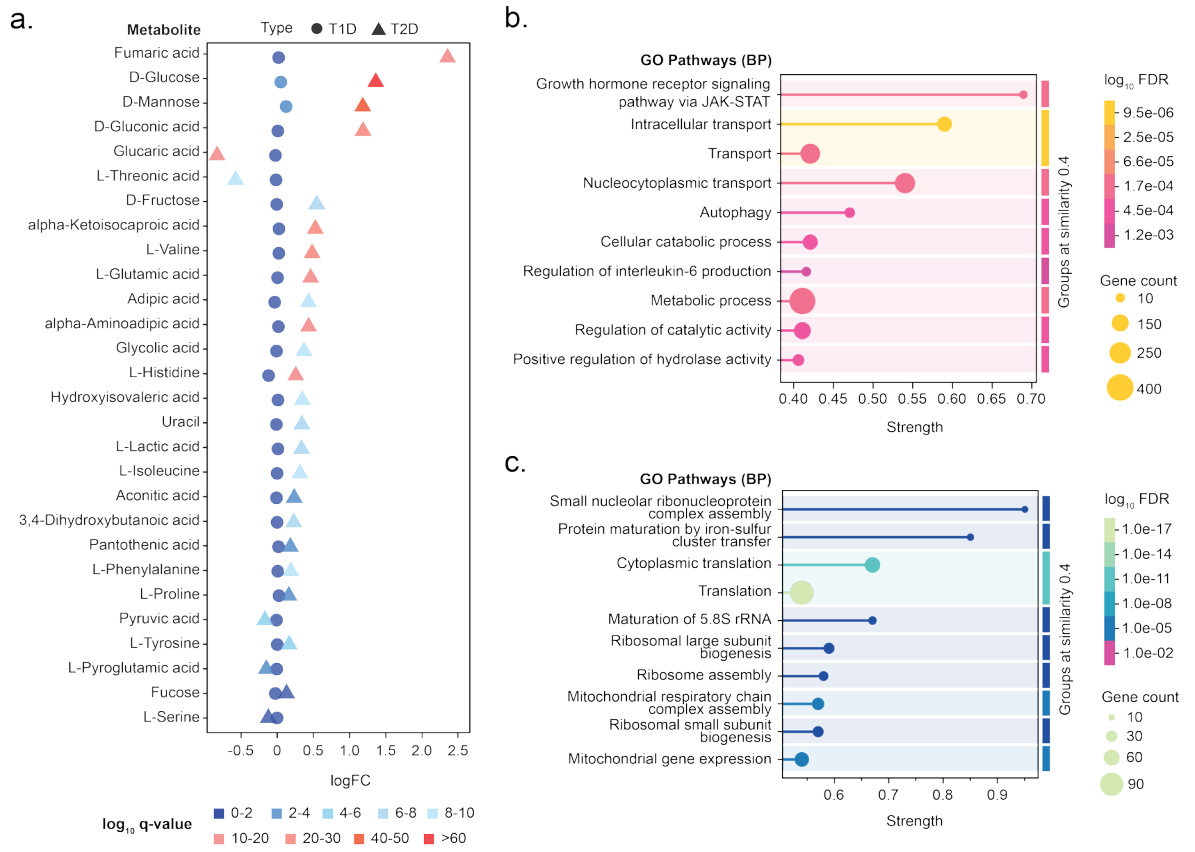


Figure 4: Comparative Metabolomic Profiles in Type 1 and Type 2 Diabetes and Functional Enrichment of Exosomal Proteins.

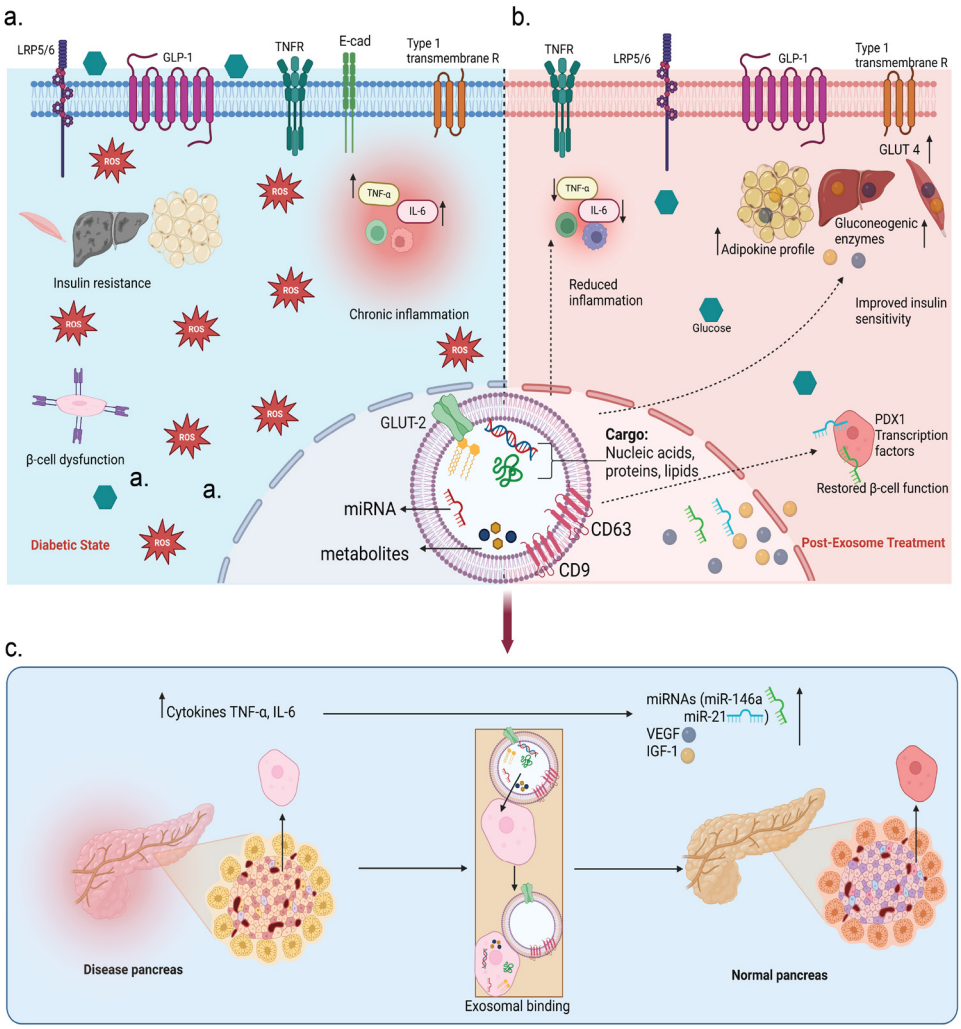


Figure 5: Proposed mechanisms of MSC exosome–mediated amelioration of diabetic pathophysiology.

Limitations of the Current Study

While the results of this preliminary study are promising, we acknowledge several important limitations in this study. First, our investigation relied heavily on multi-omics analyses on healthy human MS cultures and publicly available T2D data to infer the therapeutic functions of MSC-derived exosomes. The absence of in vivo validation (e.g., in an animal model of type 1 diabetes) means we could not confirm whether the exosomes home to the pancreas or immune organs and exert the same protective effects in a whole-body context. Future studies will need to test early-passage MSC-exosomes in diabetic animal models (such as NOD mice or STZ-induced diabetes) to verify β-cell preservation, immunomodulation, and metabolic improvements under physiological conditions.

Second, our study employed a multi-omics comparison of exosome content to elucidate mechanisms, which, while comprehensive, remains correlative by nature. There may be other active components or synergistic interactions within the complex exosome cargo that we have not functionally dissected. Isolating the contribution of individual exosomal what? would strengthen the mechanistic hypothesis proposed in this study. Additionally, exosome characterization and standardization pose technical challenges. The field lacks consensus on optimal isolation methods and potency assays for exosomes {if there is a ref to this, please cite it}.

Clinical and Translational Implications

The demonstration that early-passage MSC-derived exosomes can concurrently modulate immune responses and metabolic pathways positions them as a unique cell-free therapeutic

candidate for diabetes. Given that type 1 diabetes is driven by autoimmunity and β-cell loss, a therapy that can reduce auto-inflammatory damage while also supporting β-cell recovery and metabolic control addresses the disease on multiple fronts. Moving towards clinical application, one clear recommendation from our findings is the need to standardize the MSC culture conditions, particularly the passage number, for exosome production. We found that using early-passage MSCs is critical to obtaining exosomes with anti-inflammatory and regenerative cargo. This is supported by accumulating evidence that prolonged expansion of MSCs (late passages) induces cellular senescence and shifts the secretory profile towards a pro-inflammatory phenotype in fact, exosomes derived from senescent or late-passage MSCs have been reported to lose immunosuppressive potency and may carry senescence-associated "SASP" factors that could be counterproductive to therapy. For example, aged MSCs release exosomes enriched in miR-29b-3p, a microRNA linked to insulin resistance via Sirtuin-1 downregulation [18]. Such exosomes, when injected into young mice, were shown to impair insulin sensitivity and raise inflammatory markers, effectively mirroring aspects of the donor cell's senescent profile [18]. This underscores that exosome content reflects the physiological state of the parent MSCs. Therefore, it is imperative for clinical-grade exosome manufacturing to use early-passage, replication-competent MSCs (free of senescence markers) or to implement quality checks on exosome batches for pro-inflammatory cargo. Standardizing these parameters will help ensure consistent therapeutic efficacy and avoid inadvertent delivery of harmful signals to patients.

Beyond passage standardization, our results encourage the broader optimization of exosome-based therapies for diabetes. This could include scaling up exosome production using bioreactors or 3D culture of MSCs (which has been noted to increase exosome yield and potency and loading exosomes with enhanced levels of beneficial cargo). From a clinical trial perspective, careful dosing studies will be needed to determine the effective quantity and frequency of exosome administration required to achieve immunomodulation without immune suppression. Additionally, the route of administration (intravenous vs. pancreatic arterial infusion, for example) could influence how efficiently exosomes target the pancreatic islets or immune cells in lymph nodes.

In conclusion, our findings contribute to a growing body of evidence that early-passage MSC-derived exosomes hold significant promise as a therapy for diabetes. They act through multi-modal mechanisms: attenuating autoimmunity and inflammation, delivering protective and reparative signals to β-cells, and recalibrating metabolic homeostasis (Figure 5). While further validation is needed, this approach could pave the way for a novel class of cell-free therapeutics in endocrinology, potentially offering relief from the progressive β-cell decline and metabolic imbalance that characterize diabetes. By addressing both the immune and metabolic dimensions of the disease, MSC-exosome therapy exemplifies a comprehensive regenerative strategy - one that is speculative yet firmly supported by our current data and the mechanistic insights from the literature. With continued research to surmount the remaining challenges (standardization, mechanistic clarity, in vivo efficacy), early-passage MSC-exosomes could become a transformative addition to the therapeutic arsenal against type 1 diabetes. The translational trajectory will involve close collaboration between basic researchers and clinical investigators to ensure that this potent biologic therapy is harnessed safely and effectively for patients in the near future (Figures 4).

Figure 4: (a) Volcano plot illustrating differential expression of select metabolites in Type 1 Diabetes (T1D, circles) and Type 2 Diabetes (T2D, triangles). Data points represent the $\log_2$ Fold Change of each metabolite compared to respective control groups. The colour intensity corresponds to the $\log_{10}$ q-value, indicating statistical significance as per the legend. The T2D dataset, investigating serum metabolites in patients with T2D compared to healthy controls, was obtained from *Maet al. (2025, PMCID: PMC11880315)*. The T1D dataset was obtained from *Bramer, et al. (2024, DOI: 10.25584/2405140).* (b) & (c) Gene Ontology (GO) enrichment analysis of differentially expressed proteins identified in MSC exosomes (data derived from GSE123658). (b) Enriched biological process pathways among upregulated exosomal proteins. (c) Enriched biological process pathways among downregulated exosomal proteins. The x-axis represents the signal (b) or strength (c) of enrichment. The size of the circles corresponds to the gene count in each GO term, and the colour intensity of the bars indicates the False Discovery Rate (FDR) of enrichment.

(Figure 5)

(a) Diabetic state – Systemic insulin resistance in liver and adipose tissue, pancreatic β-cell dysfunction, chronic inflammation (↑TNF-α, ↑IL-6) and oxidative stress (↑ROS). Cell-surface receptors illustrated include LRP5/6, GLP-1 receptor (GLP-1R), TNF receptor (TNFR) and E-cadherin (E-cad). β-cells retain GLUT-2 but have impaired insulin secretion.

(b) Post-exosome treatment – Early-passage MSC-derived exosomes ($CD9^+/CD63^+$) deliver miRNAs, proteins and lipids that (i) suppress pro-inflammatory cytokines, (ii) normalize adipokine signaling, (iii) enhance GLUT4-mediated glucose uptake, (iv) reduce hepatic gluconeogenesis, (v) improve systemic insulin sensitivity and (vi) reactivate β-cell transcriptional programs (e.g. PDX1).

(c) Pancreatic focus – Left: inflamed islet with elevated local TNF-α/IL-6 and β-cell loss. Centre: MSC-exosomes bind to and are internalized by target cells. Right: exosomal cargo (e.g. miR-146a, miR-21, VEGF, IGF-1) attenuates inflammation, supports β-cell survival and restores normal islet architecture and endocrine function.

Declarations

a) Ethics approval and consent to participate

This study did not involve the use of human participants or

animal subjects. MSC-derived exosomes were obtained from in vitro cultures.

b) Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests

This study was conducted as part of industry research at GATC Health. The authors declare no other competing interests.

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Author's contributions

Dr. Jonathan RT Lakey conceived and designed the study. Samuel Kho and Lukeman Kharrat performed the multi-omics and analyses and conducted the MOFA integration. Samuel Kho wrote the manuscript. All authors reviewed and approved the final version.

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