RNA BIOLOGY
2025, VOL. 22, NO. 1, 1–7
https://doi.org/10.1080/15476286.2024.2446868

Taylor & Francis
Taylor & Francis Group

REVIEW

Relevance of RNA to the therapeutic efficacy of mesenchymal stromal/stem cells extracellular vesicles

Thong Teck Tan[a] and Sai Kiang Lim[a,b]

[a]Paracrine Therapeutics Pte. Ltd, Tai Seng Exchange, Singapore, Singapore; [b]Department of Surgery, Yong Loo Lin School of Medicine, National University of Singapore C/O NUHS Tower Block, Singapore, Republic of Singapore

ABSTRACT

Mesenchymal Stromal/Stem Cells (MSCs) are among the most frequently studied cell types in clinical trials, and their small extracellular vesicles (sEVs) are now being extensively investigated for therapeutic applications. The RNA cargo of MSC-sEVs, particularly miRNAs and mRNAs, is widely believed to be a key therapeutic component of these vesicles. In this review, we critically examine using first principles and peer-reviewed literature, whether MSC- extracellular vesicles (MSC-EVs) can deliver sufficient quantity of functional miRNA or mRNA to target compartments within recipient cells to elicit a pharmacological response. Several RNA sequencing studies reveal that miRNAs are underrepresented in the small RNA population of MSC-sEVs compared to the parent MSCs. Additionally, the majority of miRNAs are mature forms that are not associated with Argonaute (AGO) proteins, essential for their function in RNA-induced silencing complexes (RISCs). Compounding this, cellular uptake of EVs is generally inefficient, with less than 1% being internalized, and only a fraction of these reaching the cytosol. This suggests that EVs may not deliver miRNAs in sufficient quantities to meaningfully interact with AGO proteins, either through canonical or non-canonical pathways, or with other proteins like Toll-like receptors (TLRs). Further, MSC-sEV RNAs are generally small, with sizes less than 500 nucleotides indicating that any mRNA present is likely fragmented as the average mammalian mRNA is approximately 2000 nucleotides, a fact confirmed by RNA sequencing data. Together, these findings challenge the notion that RNA, particularly miRNAs and mRNAs, are primary therapeutic attributes of MSC-sEVs.

ARTICLE HISTORY
Revised 12 November 2024
Accepted 18 December 2024

KEYWORDS
Extracellular vesicles (EVs); Mesenchymal Stem/Stromal Cell (MSC); RNA; MicroRNA (miRNA); Mechanism of action

Research activity in RNA and extracellular vesicles

Extracellular Vesicles (EVs), or secreted bilipid membrane vesicles, are now recognized as major conveyers of intercellular communication through the transfer of various materials among cells. EVs are composed of lipids, proteins, nucleic acids, and metabolites. These biological materials have been postulated to mediate EV-based communication between cells, significantly enhancing their potential for therapeutic and diagnostic applications. As a reflection of this potential, of the 64,215 PubMed publications on EVs (search query: 'extracellular vesicles OR exosomes'), 21800 (34%) and 17,621 (27%) focus on therapy and diagnostics, respectively (search queries: '(extracellular vesicles OR exosomes) AND therapy" and "(extracellular vesicles OR exosomes) AND diagnosis') (Figure 1a).

RNA is a major biological material conveyed by EVs. Among the 64,215 publications on EVs 19,053 (30%) discuss RNA (search query: 'RNA AND (extracellular vesicles OR exosomes)'), indicating a substantial interest in understanding the role of RNA within these vesicles. Of the studies addressing RNA in EVs, over half (56%) focus specifically on miRNAs, 20% focus on mRNA, 4% on IncRNAs and 5% on circular RNAs (Figure 1b). This strong interest in EV miRNA reflects the critical role of cellular miRNAs as regulatory molecules that can influence gene expression. However, the functional equivalence of EV miRNA and cellular miRNA remains to be determined.

In the broader context of EV research, interest in the RNA cargo of EVs is primarily driven by its potential for clinical applications such as diagnostics (28%) and therapeutics (32%) (Figure 1c). The focus on diagnostics is not surprising, as EVs can be easily isolated from readily accessible bodily fluids such as blood, urine, and saliva, making EVs valuable non-invasive biomarkers. A deeper analysis of EV RNA in therapeutics revealed that 32% are related to stem cells (Figure 1d). Within this stem cell category, mesenchymal stem cells (MSCs) are the predominant stem cell type, accounting for 67% of the studies. The popularity of MSC-derived EVs in therapy can be attributed to their cell source, namely MSC. MSCs are the most widely clinically tested cell type and are generally found to be safe. They can be easily extracted from diverse adult tissues and expanded in vitro. They are also known to have low immunogenicity, regenerative properties, and immunomodulatory effects. Therefore, MSCs represent an easily accessible cell source of potential therapeutic EVs.

CONTACT Sai Kiang Lim saikiang.lim@paracrinetherapeutics.com Department of Surgery, Yong Loo Lin School of Medicine, National University of Singapore C/O NUHS Tower Block, Level 8, IE Kent Ridge Road, Singapore 119228, Republic of Singapore

This article was originally published with errors, which have now been corrected in the online version. Please see Correction (http://dx.doi.org/10.1080/15476286.2025.2449742)

Figure 1a. EV publications on therapy and diagnostics.

Total number of publications on EVs (PubMed search query: 'extracellular vesicles OR exosomes') is 64,215. 21800 (34%) were on therapy and diagnostics, respectively (PubMed search query: '(extracellular vesicles OR exosomes) AND therapy'. 17621 (27%) were on diagnosis (PubMed search query: (extracellular vesicles OR exosomes) AND diagnosis).

Figure 1c. Publications on EVs and RNA for therapy and diagnostics.

Of the 19,053 publications on RNA and EVs, 6,150 (32%) were on therapy (PubMed search query: 'RNA AND (extracellular vesicles OR exosomes) AND therapy') and 5,310 (28%) on diagnosis (PubMed search query: 'RNA AND (extracellular vesicles OR exosomes) AND diagnosis').

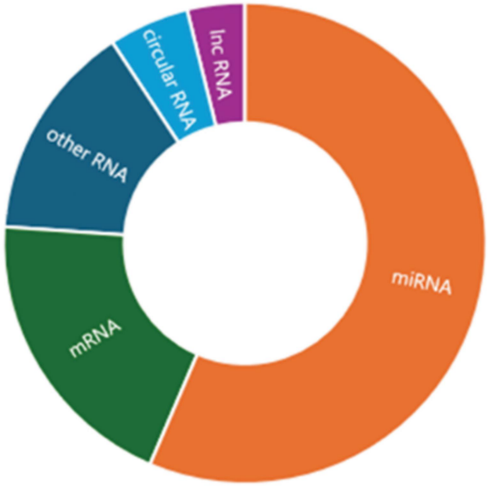

Figure 1b. Publications on EVs and types of RNA.

Of 64,215 publications on EVs 19,053 (30%) were on RNA (PubMed search query: 'RNA AND (extracellular vesicles OR exosomes)"), 10754 (56%) on microRNAs (PubMed search query: "(Extracellular vesicles OR microvesicles OR exosomes) AND microRNA'), 3,743 (20%) on mRNA (PubMed search query: 'RNA AND (extracellular vesicles OR exosomes) AND (mRNA OR messenger RNA)"), 736 (4%) were on long noncoding RNAs (PubMed search query: "(Extracellular vesicles OR microvesicles OR exosomes) AND long noncoding RNA'), and 1,030 (5%) were on circular RNAs (search query: 'RNA AND (extracellular vesicles OR exosomes) AND (circular RNA)').

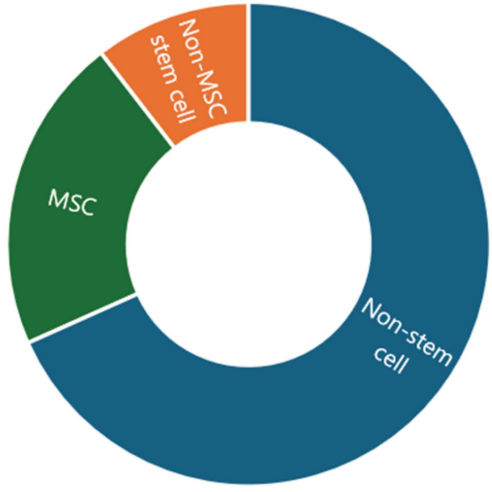

Figure 1d. Cell source of EV RNA for therapy.

The main cell source of 6,150 publications in Figure 1c on EVs or exosomes is stem cells, constituting 1949 or 32% of the publications (PubMed search query: RNA AND (extracellular vesicles OR exosomes) AND therapy AND stem cell'). Of these 1949 publications, 1,305 publications (67%) were on MSCs (PubMed search query:'RNA AND (extracellular vesicles OR exosomes) AND therapy AND stem cell AND (MSC OR mesenchymal stromal cell).

MSC EVs

MSCs have demonstrated therapeutic efficacy across a wide range of pre-clinical animal models of diseases. This success has led to their testing in 1,792 clinical trials targeting diverse diseases (Search query: Mesenchymal stromal cell OR/ Mesenchymal stem cell July 2024; www.clinicaltrials.gov). Completed clinical studies consistently find MSCs to be safe. Most MSCs are primary cells isolated from various adult and postnatal tissues such as bone marrow, adipose tissue, and umbilical cord. They are typically defined by the International Society for Cellular Therapy (ISCT) minimal criteria, which include their ability to adhere to plastic in standard culture conditions, expression of CD105, CD73, and CD90, and lack of expression of CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR surface molecules. Additionally, MSCs can differentiate into osteoblasts, adipocytes, and chondroblasts in vitro [1].

Initially, it was assumed that transplanted MSCs home to injured tissues, engraft, and differentiate to form new tissues. However, it has become increasingly evident that MSCs mediate their therapeutic efficacy through secretions. Early studies on MSC paracrine factors focused on small molecules such as cytokines or chemokines [2]. In 2008, Timmers et al. reported that the cardioprotective activity of MSC conditioned medium was in a > 1000 kD fraction [3], later shown to be enriched for 100–130 nm particles typical of exosomes [4]. Conversely, Bruno et al. in 2009 [5]. identified 80–1000 nm microvesicles as active MSC paracrine factors that alleviated glycerol-induced acute kidney injury in mice. They later found that smaller microvesicles (~160 nm) were renal protective, unlike larger ones (~215 nm) [6,7].

In direct head-to-head comparisons, EVs were shown to be as effective as their parental MSCs [5,8–10]. Consequently, small extracellular vesicles (sEVs) of 100–200 nm are now recognized as the key active factors in MSC secretions [11]. The discovery of EVs as mediators of MSC therapeutic efficacy has shifted MSC therapy from a living cell therapy to a non-living, smaller sEV therapy. This transition mitigates many challenges in the manufacturing, storage, administration, and transport of living MSCs, making sEVs a more attractive therapeutic than MSCs.

MSC RNA

Direct measurement of RNA length distribution in total RNA from MSC-sEVs using gel or capillary electrophoresis revealed that most of the RNAs are small RNAs of <300–500 nts [12–14]. Notably, 18S and 28S ribosomal RNAs (rRNAs) were not detectable. Similar characteristics – small RNAs of <300–500 nts and the absence of 18S and 28S rRNAs – were observed in total RNA from EVs derived from other cell sources, such as myeloid-derived suppressor cells (MDSCs) [15], primary liver fibroblast [16], B cell lymphoma cell line SU-DHL-6 and plasma [17].

However, other studies using EVs from other sources, including dendritic cells [18], plasma [19], B16F10 melanoma [20] and EBV infected human lymphoblastoid B-cell lines [21] showed that most RNAs fall within the < 300–500 nt range, with rRNAs present at very low concentrations. This is in sharp contrast to their high abundance of approximately 80% in cellular RNA.

Moreover, RNA presence varies among EV types. For instance, RNA was found in Shiga toxin-B-positive but not cholera toxin B chain-positive or Annexin V-binding MSC-EVs [22]. Similarly, only 6% of bronchoalveolar lavage -EVs were reported to contain RNA [23].

Overall, these findings suggest that RNA in MSC-sEVs is generally small and specific to certain EV types. Furthermore, the diverse array of these small RNA molecules suggest that RNA loading in EVs is determined by size or subcellular location rather than specific RNA sequence motifs.

miRNA in MSC-EVs

The pre-dominance of small RNAs in EVs has focused most EV RNA research on these molecules. There is a common perception that MSC-sEVs exert their RNA effects through miRNAs as evidenced by the substantial focus of research articles on MSC-EVs and miRNAs. Of the 1,028 research articles in PubMed on RNA and MSC-EVs (search query: RNA AND (extracellular vesicles OR exosomes) AND therapy AND stem cell AND (MSC OR mesenchymal stromal cell) NOT review), 873 (83%) specifically addressed miRNAs (search query: microRNA AND (extracellular vesicles OR exosomes) AND therapy AND stem cell AND (MSC OR mesenchymal stromal cell) NOT review).

However, miRNA represents only one type of RNA among the diverse RNA population in MSC-sEVs and are not a major constituent of the EV-RNA cargo. Microsequencing studies have shown that miRNAs constitutes only 2–5% [14], 3.3% [13], 1.4% [22] and 3.4% [24] of the small RNAs. The other RNA types are tRNAs, rRNA fragments, mRNA fragments and snoRNA. Many of the reads were classified as miscellaneous or unannotated. In direct comparison with the parental cells, Baglio et al. noted an under-representation of miRNA in both bone marrow- and adipose- derived MSC-EVs [14]. Similarly, Nolte-'t Hoen et al. observed that miRNAs were underrepresented in EVs, with miRNAs in dendritic cells and their EVs comprising 57.3% and 7.8% of small RNAs, respectively [18]. Notably, miRNAs make up more than 80% of small RNAs in 40 different tissues or cells [25].

Together, these findings suggest that miRNAs are generally under-represented in MSC-sEVs compared to their cellular abundance and this phenomenon is not unique to MSC-sEVs. However, there is currently no information on the copy number of specific miRNAs in an MSC-sEV. Nonetheless, it can be inferred to be very low based on estimated copy numbers of miRNAs in EVs from other cell sources. For instance, a highly abundant miRNA in C2C12 myotube EVs, miR-133a-3p, was present at approximately 1 copy per 195 EVs [26] while another abundant miRNA in EVs from Epstein-Barr Virus (EBV)-infected human lymphoblastoid B-cell lines, mature EBV-miR-BHRF1-2, was found at one copy per 300 EVs [21]. The most abundant miRNA in EVs of plasma, prostate cancer plasma, dendritic cell, mast cell, seminal fluid, ovarian cancer cells were less than one copy per EV in the range of 0.00001 to 0.1 copy per EV [27]. Conversely, the copy number of highly abundant miRNAs in human cells are often in the thousands, with miR-122 estimated to have 120 000 copies per hepatocyte [28–32].

Aside from abundance or copy number, a critical determining parameter of miRNA functionality is maturity of the miRNA. For instance, Chen et al. reported that the ratio of pre-miRNA to mature miRNA in MSC EVs was much higher than in parental MSCs [12]. However, studies by Baglio et al. and De Luca et al. indicate that most of the miRNAs in MSC-sEVs are mature [13,14]. Since mature miRNAs function only when they are an integral part of the miRNA-induced silencing complex (miRISC), they must be present in EVs as a component of miRISC to exert their functions. However, a meta-analysis of 10 publicly available MSC-sEV proteome datasets did not identify Argonaute (AGO), a critical RISC protein, as a constituent of the MSC-sEV proteome [33]. Given that RISC assembly involves the processing of pre-

miRNA by the RNase III enzyme Dicer to generate a mature miRNA duplex, which then unwinds to load the guide strand into the AGO protein, mature miRNAs in EVs cannot form functional miRISCs via this canonical pathway upon cellular internalization. On the other hand, pre-miRNAs could potentially form functional RISCs upon cellular internalization but only one report demonstrated that MSC-sEV miRNA was present predominantly in the pre-miRNA form [12]. It is however possible that the mature EV miRNA could be functional in other ways such as forming RISC with AGO protein in a non-canonical pathway [34] or binding to Toll-like receptors (TLRS) [35].

In summary, MSC-sEVs have an under-representation of miRNA in their RNA cargo and likely contain very low copy numbers of miRNA per EV, as seen in EVs from other cellular sources. Hence, the transfer of one or two copies of miRNA to a cell through the internalization of several hundred EVs is unlikely to have a biological effect on the cell, which generally contains a few hundred to thousands of copies for detectable miRNA [28]. This limitation is further compounded by the predominance of mature miRNAs and the absence of AGO proteins within MSC-EVs, which are essential for the canonical RISC-mediated gene silencing pathway. Although mature miRNAs could potentially engage in non-canonical RISC pathways or act through RISC-independent mechanisms, such as binding to TLRs, the quantity of miRNA transferred from EVs to recipient cells is exceedingly low. This is due to both the low copy number of miRNA per EV and the inherently low efficiency of EV internalization by target cells, which together reduce the likelihood of eliciting a significant biological response. Therefore, these observations challenge the notion that miRNAs mediate the activity of sEVs.

mRNAs in MSC-sEV

The first report describing the therapeutic effects of MSC EV attributed the renal protective effect against glycerol-induced acute kidney injury (AKI) in SCID mice to the transfer of POLR2E and SUMO-1 mRNA from MSC EV into tubular epithelial cells where they were translated [5]. These observations were also noted when they similarly treated a mouse-model of cisplatin-induced AKI [36]. The presence of the mRNA was determined by the presence of a 90 bp RT-PCR fragment. Since RNA in MSC-sEVs are small RNAs of < 300–500 nts (see above) and the average mammalian mRNA is about ~2314 nt [37], mRNAs in MSC-EVs are likely to be fragments and not full length mRNA. This is consistent with sequencing data of MSC-sEVs where mRNA fragments are a significant constituent of the MSC-sEV RNA cargo.

Nevertheless, there are reports of full-length mRNA in EVs. In one report, in-silico analysis of sequencing reads predicted full-length mRNA sequences [38]. Another study confirmed this by detecting the presence of overlapping 300–1400 bp RT-PCR fragments representing the complete coding sequences of several mRNAs [39]. However, both studies did not provide direct evidence for full-length mRNAs.

Recently, You et al. reported that introducing a COL1A1-GFP plasmid into neonatal human dermal fibroblasts (nHDFs) via cell nanoporation altered the RNA cargo of EVs. The EVs transitioned from having predominantly small RNAs to a population of RNA molecules greater than 4000 nucleotides, matching the size of a synthetic RNA from the same plasmid, while retaining all other key features of the wild-type EVs [40]. It remains unclear whether this remarkable transformation is specific to the cell nanoporation technique, or the particular plasmid used.

In summary, there is no conclusive evidence that MSC-sEVs contain full-length mRNA. The current evidence suggests that the mRNA present in MSC-sEVs is likely fragmented and may not be capable of being translated into proteins, even if the EVs successfully deliver the RNA to recipient cells.

Other RNA in MSC-sEVs

Apart from miRNA and mRNA, MSC-sEVs also contain other abundant RNAs, including tRNAs, snoRNAs, and rRNA fragments. Most of the literature on these RNAs in MSC-sEVs consists of descriptive characterization, and their roles in mediating the therapeutic activity of MSC-sEVs have yet to be fully understood.

Challenges to an RNA-based mechanism of action for MSC-sEVs

As discussed earlier, there are significant limitations to the role of RNA in mediating the therapeutic activity of MSC-sEVs. Despite numerous reports suggesting that miRNA and mRNA are the primary therapeutic components of MSC-sEVs, several challenges undermine this idea. The main challenges are the low abundance of miRNA and the integrity of mRNA. We highlighted these limitations in 2018 and proposed that MSC-sEVs primarily exert their effects through a protein-based mechanism rather than an RNA-based one [41].

Since then, additional challenges to the RNA-based mechanism have emerged. For miRNA or mRNA within EVs to exert biological effects, the EVs must be internalized by cells and the contents delivered into the cytosol. Two pivotal studies [42,43] have shown that the cellular internalization rate of EVs is less than 1%, and only a fraction of these internalized EVs escape the endosome into the cytosol. This, combined with the low abundance of miRNA and mRNA, presents a significant challenge to the idea that RNA in MSC-sEVs contributes to their therapeutic efficacy.

To enhance EV internalization, the fusogenic protein vesicular stomatitis virus glycoprotein (VSV-G) was proposed in a 2015 review by György et al. [44]. They suggested engineering VSV-G on the EV surface might promote EV – plasma membrane fusion and enhance cargo delivery. Indeed, several studies have shown that VSV-G can increase cellular internalization of EVs. However, given the very low baseline uptake of ~ 1% or less, even a 100% increase would lead only to 2% uptake that is not likely to be biologically significant. A proper assessment requires rigorous quantitation of cellular EV uptake with and without VSV-G, as demonstrated by

Albanese et al. [21]. They reported that in the presence of VSV-G, 1×10^4 EV particles per cell were sufficient to transduce half of the cells. Considering that a mouse liver has approximately 1.35×10^8 hepatocytes [45], an estimated 6.7×10^{11} EVs would be needed to transduce half the liver, an impractically large quantity. Furthermore, Hung et al. observed that although VSV-G increased EV uptake efficiency, the internalized EVs were rapidly degraded [46]. These findings do not inspire confidence that VSV-G could significantly improve EV-mediated RNA transfer into cells for therapeutic purposes. By extension, transferring cytoplasmic proteins from EVs into cells is unlikely to be a viable mechanism for MSC-sEV therapeutic efficacy.

Recently, we proposed that the most efficient mechanism for EV-based therapeutic efficacy may involve 'External Modulation of Cell by EV' (EMCEV) [47]. We suggest that EVs modulate cellular activity externally by altering the concentration of biologically active molecules. Unlike internalization, where one EV can engage at most one cell, EMCEV could enable a single EV to potentially influence multiple cells. EMCEV allows for a more widespread effect, though it may not be suitable for all types of cargo, particularly those like RNA that require intracellular action for their therapeutic effects. By enabling an EV to influence multiple cells through the modulation of the extracellular environment, EMCEV addresses the limitations associated with low internalization efficiency, which typically restrict the engagement to one cell per EV.

Summary and conclusions

A central focus of MSC-EV research is the investigation of their RNA cargo, particularly miRNAs and mRNAs, as these molecules are believed to play critical roles in the therapeutic potential of MSC-EVs. In this study, we rigorously assess, based on fundamental principles and peer-reviewed evidence, whether MSC-EVs can deliver sufficient quantities of functional miRNAs and mRNAs to specific target sites within recipient cells to elicit a pharmacological response (Figure 1e). Establishing this foundational understanding is essential before delving into which specific miRNAs or mRNAs within MSC-EVs might influence cellular processes.

Current research, leveraging advanced sequencing technologies and capillary electrophoresis, reveals that the RNA content of MSC-EVs is predominantly small RNAs (<500 nucleotides), with miRNAs comprising only 2–5% of the total small RNA cargo. In comparison, miRNAs constitute more than 80% of small RNAs in various tissues and cell types [25]. Notably, the most abundant miRNAs in EVs are estimated to be present at fewer than one copy per EV, a stark contrast to the approximately 11,587 copies per human cell [28]. Additionally, mRNA within MSC-EVs appears to consist mainly of non-functional fragments, as these are significantly

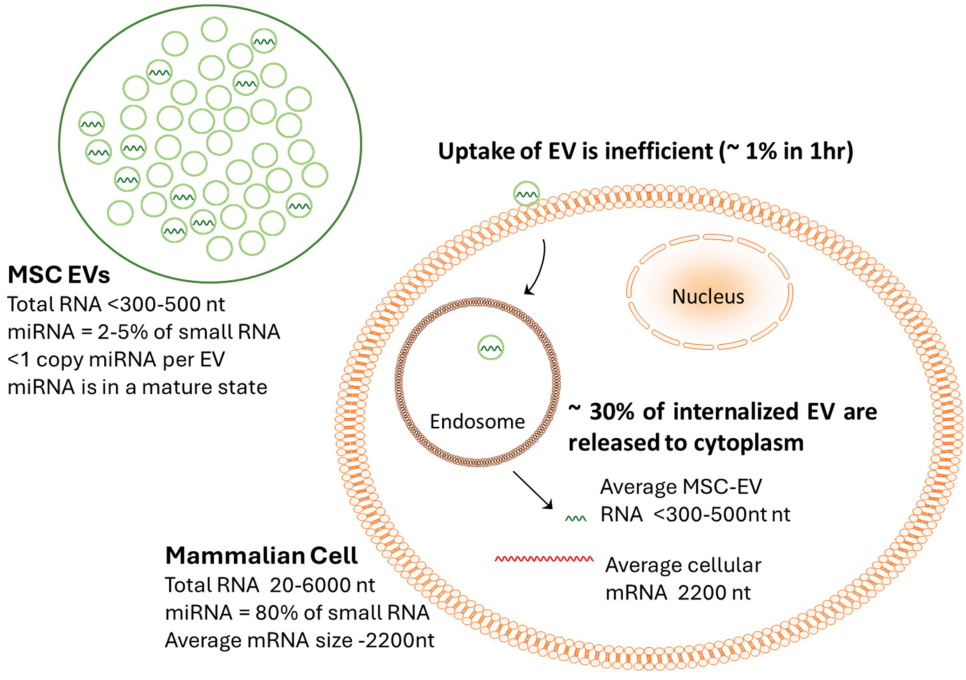

Figure 1e. The limitations of an RNA-based hypothesis for MSC-EV therapeutic activity.

RNA within MSC-derived EVs predominantly consists of fragments smaller than 300–500 nucleotides. Given that the average length of mRNA in a mammalian cell is approximately 2200 nucleotides, mRNA present in EVs is likely fragmented and thus non-functional. EV-associated miRNAs represent only 2–5% of the total small RNA population, with estimates suggesting that even the most abundant miRNA exists at fewer than one copy per EV. This is in stark contrast to mammalian cells, where miRNAs constitute around 80% of the small RNA pool with copy numbers as high as > 100 000 per cell for the most abundant miRNAs. Consequently, miRNAs are underrepresented in EVs and found at low concentrations. Additionally, the uptake of EVs by recipient cells is limited, with less than 1% being internalized within one hour and only about 30% of internalized EVs released into the cytoplasm. This implies that the amount of EV miRNA delivered into recipient cells is insufficient to elicit any significant biological or pharmacological response. Collectively, these findings suggest that mRNA and miRNA in EVs are unlikely to elicit substantial biological activity.

shorter than the average mammalian mRNA length of approximately 2,314 nucleotides [37].

Given these findings, it appears unlikely that the miRNAs and mRNAs derived from MSC-EVs can meaningfully influence the biological activity of recipient cells. This conclusion is further reinforced by reports showing the low efficiency of EV internalization and endosomal escape by cells, suggesting that even if functional RNA is present, its delivery and subsequent impact on cell biology are minimal.

Aside from the low abundance of miRNAs and the poor efficiency of EV internalization, most miRNAs within MSC-EVs are mature forms. The absence of AGO in MSC-EVs means that these miRNAs cannot engage in canonical AGO-mediated RNA interference upon entering recipient cells. Although, in theory, these mature miRNAs might still function through non-canonical AGO binding [34] or interactions with other proteins such as TLRs [35], the likelihood of these pathways compensating for the low copy number of miRNAs in MSC-EVs and the poor efficiency of EV internalization and subsequent endosomal escape is highly questionable.

Taken together, these observations suggest that miRNA and mRNA MSC-sEVs do not play a central role in their pharmacological activities.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The author(s) reported there is no funding associated with the work featured in this article.

Author contributions

TTT reviewed and edited the paper, SKL conceptualized and drafted the paper. All authors approved the final submitted version of the manuscript.

Data availability statement

The data supporting the findings of this study are available within the article.

References

[1] Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement. Cytotherapy. 2006;8(4):315–317. doi: 10.1080/14653240600855905

[2] Ghannam S, Bouffi C, Djouad F, et al. Immunosuppression by mesenchymal stem cells: mechanisms and clinical applications. STEM Cell Res Ther. 2010;1(1):2. doi: 10.1186/scrt2

[3] Timmers L, Lim SK, Arslan F, et al. Reduction of myocardial infarct size by human mesenchymal stem cell conditioned medium. Stem Cell Res. 2008;1(2):129–137.

[4] Lai RC, Arslan F, Lee MM, et al. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. Stem Cell Res. 2010;4(3):214–222. doi: 10.1016/j.scr.2009.12.003

[5] Bruno S, Grange C, Deregibus MC, et al. Mesenchymal stem cell-derived microvesicles protect against acute tubular injury. J Am Soc Nephrol. 2009;20(5):1053–1067. doi: 10.1681/ASN.2008070798

[6] Bruno S, Tapparo M, Collino F, et al. Renal regenerative potential of different extracellular vesicle populations derived from bone marrow mesenchymal stromal cells. Tissue Eng Part A. 2017;23 (21–22):1262–1273. doi: 10.1089/ten.tea.2017.0069

[7] Collino F, Pomatto M, Bruno S, et al. Exosome and microvesicle-enriched fractions isolated from mesenchymal stem cells by gradient separation showed different molecular signatures and functions on renal tubular epithelial cells. STEM Cell Rev Rep. 2017;13(2):226–243. doi: 10.1007/s12015-016-9713-1

[8] Doeppner TR, Herz J, Görgens A, et al. Extracellular vesicles improve post-stroke neuroregeneration and prevent postischemic immunosuppression. Stem Cells Transl Med. 2015;4 (10):1131–1143. doi: 10.5966/sctm.2015-0078

[9] Nawaz M, Fatima F, Vallabhaneni KC, et al. Extracellular vesicles: evolving factors in stem cell biology. Stem Cells Int. 2016;2016 (1):1073140. doi: 10.1155/2016/1073140

[10] Baek G, Choi H, Kim Y, et al. Mesenchymal stem cell-derived extracellular vesicles as therapeutics and as a drug delivery platform. Stem Cells Transl Med. 2019;8(9):880–886. doi: 10.1002/sctm.18-0226

[11] Witwer KW, Van Balkom BWM, Bruno S, et al. Defining mesenchymal stromal cell (msc)-derived small extracellular vesicles for therapeutic applications. J Extracell Vesicles. 2019;8 (1):1609206–1609206. doi: 10.1080/20013078.2019.1609206

[12] Chen TS, Lai RC, Lee MM, et al. Mesenchymal stem cell secretes microparticles enriched in pre-microRNAs. Nucleic Acids Res. 2010;38(1):215–224. doi: 10.1093/nar/gkp857

[13] De Luca L, Trino S, Laurenzana I, et al. MiRNAs and piRNAs from bone marrow mesenchymal stem cell extracellular vesicles induce cell survival and inhibit cell differentiation of cord blood hematopoietic stem cells: a new insight in transplantation. Oncotarget. 2016;7(6):6676–6692. doi: 10.18632/oncotarget.6791

[14] Baglio SR, Rooijers K, Koppers-Lalic D, et al. Human bone marrow- and adipose-mesenchymal stem cells secrete exosomes enriched in distinctive miRNA and tRNA species. Stem Cell Res Ther. 2015;6(1):127. doi: 10.1186/s13287-015-0116-z

[15] Geis-Asteggiante L, Belew AT, Clements VK, et al. Differential content of proteins, mRnas, and miRNAs suggests that MDSC and their exosomes may mediate distinct immune suppressive functions. J Proteome Res. 2018;17(1):486–498. doi: 10.1021/acs.jproteome.7b00646

[16] Fujita Y, Fujimoto S, Miyamoto A, et al. Fibroblast-derived extracellular vesicles induce lung cancer progression in the idiopathic pulmonary fibrosis microenvironment. Am J Respir Cell Mol Biol. 2023;69(1):34–44. doi: 10.1165/rcmb.2022-0253OC

[17] Gutiérrez García G, Galicia García G, Zalapa Soto J, et al. Analysis of RNA yield in extracellular vesicles isolated by membrane affinity column and differential ultracentrifugation. PLOS ONE. 2020;15(11):e0238545. doi: 10.1371/journal.pone.0238545

[18] Nolte-'t Hoen ENM, Buermans HPJ, Waasdorp M, et al. Deep sequencing of RNA from immune cell-derived vesicles uncovers the selective incorporation of small non-coding RNA biotypes with potential regulatory functions. Nucleic Acids Res. 2012;40 (18):9272–9285. doi: 10.1093/nar/gks658

[19] Enderle D, Spiel A, Coticchia CM, et al. Characterization of RNA from exosomes and other extracellular vesicles isolated by a novel spin column-based method. PLOS ONE. 2015;10(8):e0136133. doi: 10.1371/journal.pone.0136133

[20] Zhang H, Freitas D, Kim HS, et al. Identification of distinct nanoparticles and subsets of extracellular vesicles by asymmetric flow field-flow fractionation. Nat Cell Biol. 2018;20(3):332–343. doi: 10.1038/s41556-018-0040-4

[21] Albanese M, Chen Y-FA, Hüls C, et al. MicroRNAs are minor constituents of extracellular vesicles that are rarely delivered to target cells. PlOS Genet. 2021;17(12):e1009951. doi: 10.1371/journal.pgen.1009951

[22] Lai RC, Tan SS, Yeo RWY, et al. MSC secretes at least 3 EV types each with a unique permutation of membrane lipid, protein and RNA. J Extracell Vesicles. 2016;5(1):29828–29828. doi: 10.3402/jev.v5.29828

[23] Lee H, Groot M, Pinilla-Vera M, et al. Identification of miRNA-rich vesicles in bronchoalveolar lavage fluid: insights into the function and heterogeneity of extracellular vesicles. J Control Release. 2019;294:43–52. doi: 10.1016/j.jconrel.2018.12.008

[24] Kodali M, Madhu LN, Reger RL, et al. Intranasally administered human msc-derived extracellular vesicles inhibit NLRP3-p38/MAPK signaling after TBI and prevent chronic brain dysfunction. Brain Behav Immun. 2023;108:118–134. doi: 10.1016/j.bbi.2022.11.014

[25] Kuchen S, Resch W, Yamane A, et al. Regulation of MicroRNA expression and abundance during Lymphopoiesis. Immunity. 2010;32(6):828–839. doi: 10.1016/j.immuni.2010.05.009

[26] Hanson B, Vorobieva I, Zheng W, et al. Ev-mediated promotion of myogenic differentiation is dependent on dose, collection medium, and isolation method. Mol Ther Nucleic Acids. 2023;33:511–528. doi: 10.1016/j.omtn.2023.07.005

[27] Chevillet JR, Kang Q, Ruf IK, et al. Quantitative and stoichiometric analysis of the microRNA content of exosomes. Proc Natl Acad Sci. 2014;111(41):14888–14893. doi: 10.1073/pnas.1408301111

[28] Bissels U, Wild S, Tomiuk S, et al. Absolute quantification of microRNAs by using a universal reference. RNA. 2009;15(12):2375–2384. doi: 10.1261/rna.1754109

[29] Chang J, Nicolas E, Marks D, et al. miR-122, a mammalian liver-specific microRNA, is processed from hcr mRNA and may downregulate the high affinity cationic amino acid transporter CAT-1. RNA Biol. 2004;1(2):106–113. doi: 10.4161/rna.1.2.1066

[30] Denzler R, Agarwal V, Stefano J, et al. Assessing the ceRNA hypothesis with quantitative measurements of miRNA and target abundance. Mol Cell. 2014;54(5):766–776. doi: 10.1016/j.molcel.2014.03.045

[31] Ho V, Baker JR, Willison KR, et al. Single cell quantification of microRNA from small numbers of non-invasively sampled primary human cells. Commun Biol. 2023;6(1):458. doi: 10.1038/s42003-023-04845-8

[32] Liang Y, Ridzon D, Wong L, et al. Characterization of microRNA expression profiles in normal human tissues. BMC Genomics. 2007;8(1):166. doi: 10.1186/1471-2164-8-166

[33] van Balkom BWM, Gremmels H, Giebel B, et al. Proteomic signature of mesenchymal stromal cell-derived small extracellular vesicles. Proteomics. 2019;19(1–2):1800163. doi: 10.1002/pmic.201800163

[34] Ghoshal B, Bertrand E, Bhattacharyya SN. Non-canonical argonaute loading of extracellular vesicle-derived exogenous single-stranded miRNA in recipient cells. J Cell Sci. 2021;134(9). doi: 10.1242/jcs.253914

[35] Fabbri M, Paone A, Calore F, et al. A new role for microRnas, as ligands of toll-like receptors. RNA Biol. 2013;10(2):169–174. doi: 10.4161/rna.23144

[36] Bruno S, Grange C, Collino F, et al. Microvesicles derived from mesenchymal stem cells enhance survival in a lethal model of acute kidney injury. PLOS ONE. 2012;7(3):e33115. doi: 10.1371/journal.pone.0033115

[37] Ota T, Suzuki Y, Nishikawa T, et al. Complete sequencing and characterization of 21,243 full-length human cDNAs. Nat Genet. 2004;36(1):40–45. doi: 10.1038/ng1285

[38] Ransom LS, Liu CS, Dunsmore E, et al. Human brain small extracellular vesicles contain selectively packaged, full-length mRNA. Cell Rep. 2024;43(4):114061. doi: 10.1016/j.celrep.2024.114061

[39] Zeng A, Wei Z, Rabinovsky R, et al. Glioblastoma-derived extracellular vesicles facilitate transformation of astrocytes via reprogramming oncogenic metabolism. iScience. 2020;23(8):101420. doi: 10.1016/j.isci.2020.101420

[40] You Y, Tian Y, Yang Z, et al. Intradermally delivered mRNA-encapsulating extracellular vesicles for collagen-replacement therapy. Nat Biomed Eng. 2023;7(7):887–900. doi: 10.1038/s41551-022-00989-w

[41] Toh WS, Lai RC, Zhang B, et al. MSC exosome works through a protein-based mechanism of action. Biochem Soc Trans. 2018;46(4):843–853. doi: 10.1042/BST20180079

[42] Bonsergent E, Grisard E, Buchrieser J, et al. Quantitative characterization of extracellular vesicle uptake and content delivery within mammalian cells. Nat Commun. 2021;12(1):1864. doi: 10.1038/s41467-021-22126-y

[43] O'Brien K, Ughetto S, Mahjoum S, et al. Uptake, functionality, and re-release of extracellular vesicle-encapsulated cargo. Cell Rep. 2022;39(2):110651. doi: 10.1016/j.celrep.2022.110651

[44] György B, Hung ME, Breakefield XO, et al. Therapeutic applications of extracellular vesicles: clinical promise and open questions. Annu Rev Pharmacol Toxicol. 2015;55(1):439–464. doi: 10.1146/annurev-pharmtox-010814-124630

[45] Sohlenius-Sternbeck AK. Determination of the hepatocellularity number for human, dog, rabbit, rat and mouse livers from protein concentration measurements. Toxicol In Vitro. 2006;20(8):1582–1586. doi: 10.1016/j.tiv.2006.06.003

[46] Hung ME, Leonard JN. A platform for actively loading cargo RNA to elucidate limiting steps in ev-mediated delivery. J Extracell Vesicles. 2016;5(1):31027. doi: 10.3402/jev.v5.31027

[47] Tan TT, Lai RC, Sim WK, et al. Enhancing ev-cell communication through "external modulation of cell by EV" (EMCEV). Cytotherapy. 2024;27(1):1–6. doi: 10.1016/j.jcyt.2024.07.014