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Extracellular Vesicles from human umbilical cord Mesenchymal Stem Cells: a potential delivery system for RNA interference-based therapeutics in cancer

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Abstract: Stem Cell Therapy is currently being extensively investigated as a novel treatment approach in disease models and patients. Mesenchymal Stem Cells (MSCs) have garnered recognition as a promising reservoir for cancer therapy, offering potential as carriers for therapeutic compounds in cancer gene therapy initiatives. Recent research indicates that the therapeutic benefits of such approaches are driven by Extracellular Vesicles (EVs), released by cells and comprise a diverse array of biomolecules, including lipids, proteins, cytokines, messenger RNAs (mRNAs), transfer RNAs (tRNAs), micro-RNAs (miRNAs), long non-coding RNAs (lncRNAs), and mitochondrial DNA (mtDNA), which play a role in stimulating various endogenous processes in target cells or tissues. Specific types of miRNAs are commonly associated with cell adhesion, protein translation/stabilization, immune response, cell proliferation and platelet aggregation. This review delves into the intricate communication network between MSCs and cancer, with a particular focus on the involvement of MSC-derived EVs encapsulating miRNAs. Through a comprehensive survey of literature databases, we elucidate the pivotal role of miRNAs in tumor development, where they function as potent regulators of gene expression post-transcriptionally, exerting either oncogenic or tumor-suppressive effects.

Keywords: regulator; gene; expression; microRNA; cancer therapy

1. Introduction

Targeted treatments, like cancer gene therapy, are currently widely utilized as a treatment component for various types of cancer conditions [1,2]. Several strategies for cancer gene

therapy have been employed and are continuously evolving, encompassing approaches such as immunotherapy, anti-angiogenic gene therapy, pro-apoptosis therapy, and RNA interference (RNAi) therapy [3]. Anti-cancer agents are delivered to specific targets using biological carrier molecules that encapsulate specific components, primarily to malignant cells at the tumor site. As a general trend, two increasingly utilized techniques in cancer gene therapy involve viral vectors and cell-based delivery methods. Cell-based delivery is recognized as more promising for transporting anti-cancer agents to tumor cells [4,5].

Mesenchymal Stem Cells (MSCs) can serve as delivery vehicles in cancer therapy, leveraging their remarkable tumor tropism, low immunogenicity, unlimited capacity for packaging genetic material, and suitability for large-scale production. This positions MSCs as potent candidates for enhancing anti-cancer treatments [3,6,7]. EVs derived from MSCs are considered to provide superior safety profiles, along with benefits in terms of storage, transportation, and administration, in comparison to MSC transplantation therapies.

The therapeutic effects of MSCs are associated with the stimulation of endogenous tissue repair processes through secreted factors and immunomodulatory responses. Vesicles containing proteins, nucleic acids and lipids can be released into the extracellular space from eukaryotic cells [8]. These biological vesicles carry a variety of small molecules, such as lipids, proteins, cytokines, micro-RNAs (miRNAs), messenger RNAs (mRNAs), transfer RNAs (tRNAs), long non-coding RNAs (lncRNAs), and mitochondrial DNA (mtDNA) which can be transferred between cells [9]. MSC-derived EVs demonstrate advantages, specifically regarding safety, stability, and ease of storage and transportation, which are more favorable compared to MSCs [3].

EVs originating from MSCs play a pivotal role as central mediators in the therapeutic mechanisms of MSCs and are increasingly recognized as a promising alternative to cell-based therapy. The unmodified counterparts of MSC-derived EVs exhibit a dual capacity to either promote or inhibit tumors. Conversely, modifications applied to MSC-derived EVs can enable precise manipulation, potentially enhancing their therapeutic efficacy and safety profiles in cancer treatment contexts can be tailored to act as cancer growth suppressors by enhancing specific therapeutic molecules, such as chemotherapy drugs, miRNA, anti-miRNA, and specific siRNA (small interfering RNA) [3].

2. Mesenchymal Stem Cells

Mesenchymal Stem Cells (MSCs) have been identified as significant entities in cancer therapy, serving as a versatile resource with multiple potential applications. Beyond their primary role in cellular therapy, MSCs can be harnessed as effective carriers for a diverse array of therapeutic compounds, facilitating targeted delivery in cancer gene therapy applications. This dual functionality underscores the considerable potential of MSCs in advancing the development of novel treatment modalities for cancer. MSCs can directly influence cancer development through interactions with tumors, mediated by cell-to-cell contact or the secretion of soluble molecules [10]. Numerous studies have shown that MSCs

possess pro-tumor functions [11], yet it's also known that MSCs can inhibit tumor growth through various mechanisms such as suppressing the tumor cell cycle and inducing apoptosis.

MSCs exhibit a remarkable versatility as multipotent stem cells, showcasing their capacity to differentiate into a spectrum of cell types including adipocytes, chondrocytes, osteocytes, and various other lineages [12]. This inherent plasticity is prominently observed when MSCs are cultured in specific induction media, where they undergo differentiation processes tailored to the environmental cues provided [13]. To date, MSCs have been extracted from diverse tissues, with the most common sources being adipose tissue, umbilical cord, and bone marrow. MSCs possess regenerative and immunomodulatory properties that contribute to tissue repair and wound healing [14].

MSCs have demonstrated significant therapeutic potential and play a pivotal role in the tumor microenvironment. They can express chemokines, cytokines, growth factors and regulatory non-coding RNAs that contribute to antitumor, immunosuppressive and migratory properties, as well as modulating the host's innate cellular immune response [9]. MSCs possess the ability to migrate and settle at primary inflammatory sites [15]. However, several studies indicate that MSCs exhibit dual effects in tumor inhibition or progression. In various *in vitro* studies and rodent models of colorectal, prostate, gastric, glioma, and ovarian cancers, these cells have demonstrated the capability to promote tumor growth and facilitate tumor development [3].

3. Extracellular Vesicles

EVs are characterized as vesicles comprising nucleic acids, proteins, and lipids, which have the capacity to be discharged into the extracellular space from eukaryotic cells [8]. According to the International Society for Extracellular Vesicles (ISEV), EVs are defined as naturally secreted particles produced by cells, encased within a lipid bilayer membrane, and lacking the ability to replicate [16]. EVs discharged from cells are nano-sized particles bound by a lipid bilayer found in diverse biological fluids like serum, saliva, urine, cerebrospinal fluid, nasal secretions, and breast milk. In MSC cultures, EVs are present in the cell culture media. Based on size and biogenesis, EVs are categorized into three primary subclasses: Apoptotic Bodies (500–2000 nm), Exosomes (40–120 nm) and Microvesicles (50–1000 nm) [17].

The structure of EVs enables protection during the delivery of their cargo compounds and directed transfer between cells. Initial studies discovered that stable mRNA and non-coding RNAs (ncRNAs), such as miRNA, are contained within EVs. These molecules, along with other EVs cargo, can be transferred to recipient cells in culture, with functional consequences. Different cell types can generate distinct repertoires of vesicles containing different components [18].

MSCs are known to be one of the most efficient human cell types in producing EVs [19]. MSC-derived EVs have the potential to serve as therapeutic agents in various pathological conditions, including cancer, due to their anti-inflammatory and pro-resolving properties. Several studies have indicated that MSC-derived EVs can influence the growth and invasion of cancer cells and enhance the effectiveness of conventional therapies like chemotherapy.

Furthermore, MSC-derived EVs can also act as potential biomarkers for the diagnosis and monitoring of specific clinical conditions.

3.1. Components of Extracellular Vesicles

These biological vesicles contain a multitude of small molecules that can be transferred between cells. The complex cargo composition of EVs can consist of hundreds to thousands of different proteins, lipids, some DNA, and numerous small non-coding RNAs (sncRNAs), such as small nucleolar RNAs, microRNAs (miRNAs), mitochondrial RNAs, Y RNAs, and Vault RNAs. Additionally, EVs also contain long non-coding RNAs (lncRNAs) and messenger RNAs (mRNAs), which are typically already fragmented [9,18]. Proteins can be found both on the outer and inner surfaces of EVs, serving as indicators of biogenesis mechanisms and potential communicative functions.

3.2. Isolation and Identification of Extracellular Vesicles

The most common techniques used for EVs isolation are ultracentrifugation and polymer precipitation. However, polymer precipitation techniques can result in EVs and culture media component co-precipitation, leading to low recovery rates [20]. Isolating EVs from MSCs holds potential for continuous and reproducible production, making it easier to produce than MSCs themselves. EVs can express a variety of biomarkers depending on their MSC source. Some common markers of MSC-derived EVs include CD81, CD82, CD63, CD9, TSG101, and Hsp70, which are regularly used for characterizing EVs from MSCs [21].

3.3. Extracellular Vesicles as mediators of intercellular communication

EVs were initially considered as cellular waste [22], as the release of EVs was thought to be a cellular disposal mechanism to rid the cell of unwanted proteins and molecules. EVs were first clearly defined in 1983 by Pan and Johnstone [23]. Following years of research subsequent to 1983, the release of EVs came to be recognized as a significant mediator in cell-to-cell communication. EVs were acknowledged to be involved not only in normal physiological processes but also to play a role in disease development [24]. EVs play a vital biological role in maintaining cellular homeostasis and function by engaging in autocrine, paracrine, and endocrine signaling. They achieve this by delivering various compounds to neighboring and distant cells [25].

EVs facilitate intercellular communication by transferring functionally relevant biomolecules, and as such, they can be harnessed for therapeutic purposes in a manner similar to their parent stem cells [25]. EVs secreted by MSCs readily communicate with their target cells and initiate biological processes through specific receptor-ligand interactions [26,27]. Following endocytosis in the target cell, EVs release mRNA, miRNA, and proteins into the target cell, mediating biological processes such as mRNA translation, translation inhibition, and modulation of other target signaling pathways [28–31].

Cross-talk between MSCs and cancer cells can occur directly or indirectly. The mechanisms of these interactions contributing to MSC-mediated cancer cell growth stimulation include nanotube formation, intercellular gap junctional communication, Notch signaling, cytokine exchange, Extracellular Vesicles and exosomes [10]. MSC-derived EVs contain functional proteins, mRNA, and micro-RNAs that contribute to cellular interactions between MSCs and cancer cells within the tumor microenvironment, thereby altering the functionality of the target cells, as illustrated in Figure 1 [32].

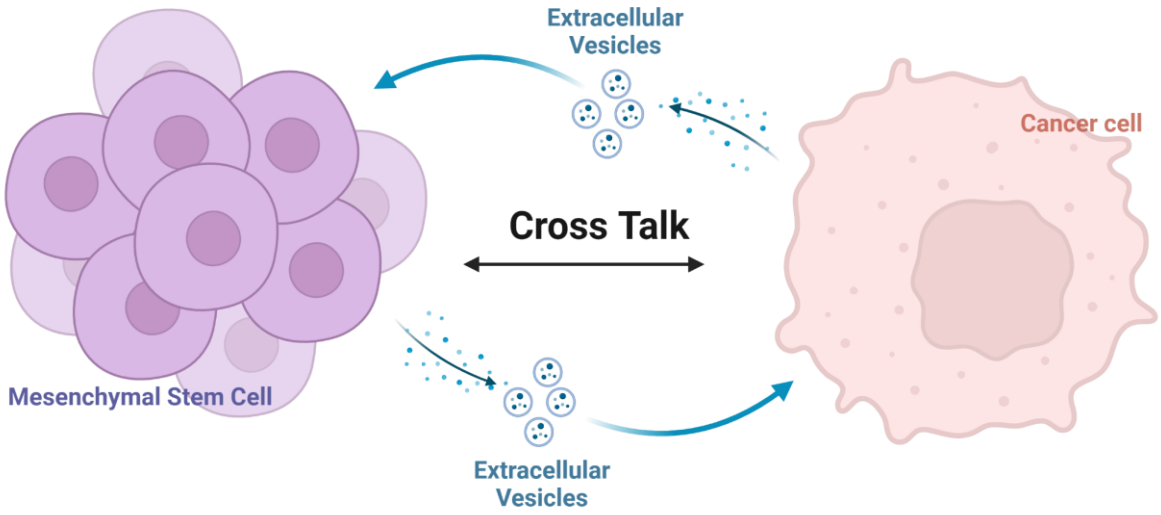

Figure 1. Cross talk between MSC and cancer cells.

3.4. Potential applications of Extracellular Vesicles

Unmodified EVs derived from MSCs may demonstrate either tumor-promoting or tumor-inhibiting properties, depending on various factors. Conversely, modified EVs derived from MSCs are implicated in inhibiting tumor development and supporting cancer progression through the targeted delivery of diverse therapeutic molecules. These include chemotherapy drugs, miRNAs, anti-miRNAs, and small interfering RNAs (siRNAs) directed towards specific genes. Additionally, EVs originating from bone marrow-derived MSCs have been shown to influence the Hedgehog signaling pathway and its involvement in tumor development in osteosarcoma and gastric cancer cells [33]. A study conducted on MCF7 cells (a breast cancer cell line) treated with MSC-derived EVs revealed an augmentation in migratory capacity, attributed to the activation of the Wnt signaling pathway [34].

Unmodified MSC-derived EVs are also involved in suppressing tumor growth. EVs enriched with miR-16 have been found to exert anti-angiogenic effects *in vitro* and *in vivo* by downregulating VEGF regulation in breast cancer cells [35]. Comparable studies have indicated that non-genetically modified EVs derived from MSCs possess the ability to convey miR-100 to breast cancer cells, resulting in the suppression of angiogenesis through the inhibition of the mTOR/HIF-1α/VEGF signaling axis [36]. MSC-derived EVs are recognized for their enhanced safety profiles owing to their low immunogenicity relative to MSCs. Moreover, they lack the potential to differentiate into mature cells or tumor cells. These

attributes render them a promising therapeutic tool for cell-based cancer treatments [3]. EVs derived from MSCs have also exhibited therapeutic effects in diverse animal models.

4. MicroRNA

One crucial component of EVs is RNA, which plays a role in their ability to mediate intercellular communication and be a part of various signaling pathways [19]. MiRNAs are a class of small non-coding RNA molecules, typically consisting of around 20 nucleotides in length. They are initially transcribed as primary miRNA transcripts (pri-miRNAs) by RNA polymerase II, occasionally by RNA polymerase III, in conjunction with associated transcription factors. These pri-miRNAs undergo processing mediated by the ribonuclease III enzyme Drosha, leading to the formation of precursor miRNAs (pre-miRNAs). Following this, pre-miRNAs are transported from the nucleus to the cytoplasm, where they undergo further processing facilitated by Dicer, another member of the RNase III endonuclease family. This processing culminates in the production of miRNA duplexes comprising 22 nucleotides [37].

Following processing, the mature miRNA dissociates from the miRNA duplex and becomes incorporated into the RNA-induced silencing complex (RISC). In concert, the RISC and mature miRNA collaborate to modulate gene expression by binding to mRNA transcripts of target genes, predominantly in the untranslated region at the 3' end (3'UTR). This intricate molecular complex possesses the capability to attenuate the translation of the target mRNA into protein and/or induce the degradation of the target mRNA transcript [37].

The RNA content within EVs can differ from that in the parent MSCs. In a study conducted by Goldie [38], it was found that the proportion of miRNAs is higher in EVs compared to their parent cells. MiRNAs in EVs can serve as biomarkers in disease diagnosis, provide insights into target gene information, analyze regulatory pathways, and molecular regulation of drugs. Several studies have demonstrated that miRNAs in EVs can detect cancers, heart diseases, and neurological disorders with high sensitivity and specificity. EVs can also be utilized as vehicles for delivering drugs to target cells.

4.1. MicroRNA regulation

Numerous investigations have scrutinized the regulation of miRNAs at the transcriptional level, which entails a comprehensive exploration of mechanisms such as direct interactions with transcription factors, modifications in the methylation status of miRNA promoters leading to hypermethylation or hypomethylation, and alterations in the organization of histones and chromatin structure. MiRNAs are acknowledged for their pivotal roles in regulating post-transcriptional gene expression through a diverse array of mechanisms. These mechanisms include the binding of miRNAs to various regions of the target messenger RNA (mRNA), such as the 3' untranslated region (3'UTR), coding sequence, or 5' untranslated region (5'UTR). This interaction ultimately culminates in the suppression or degradation of the mRNA, thereby modulating gene expression [39]. MiRNAs exert their regulatory influence by suppressing the expression of thousands of genes via a mechanism known as

RNA interference, wherein they bind to messenger RNA (mRNA) and hinder translation. However, a study by Jame-Chenarboo *et al*. [40] suggested that miRNAs may also have the capability to enhance gene expression.

mRNA regulation by miRNA can also involve mRNA degradation, with mRNA destabilization being a prominent aspect of repression in mammalian species. MiRNAs exhibiting a high level of complementarity to the target mRNA sequence can instigate mRNA degradation. Although numerous intricate components contribute to mRNA degradation regulation, processes such as deadenylation, decapping, and exonucleolytic mRNA digestion mechanisms are also implicated [39].

Based on the interactions between miRNAs and target mRNAs in the target cells, miRNAs are categorized as oncogenic (oncomiRs) and tumor suppressor miRNAs (miR-TS). Oncogenic miRNAs exert a crucial influence in the intricate process of tumor development, orchestrating significant alterations within the tumor microenvironment to facilitate and augment cancer metastasis. Complex interactions within the tumor microenvironment can drive cancer development and metastasis through various mechanisms, including oxidative stress, hypoxia, angiogenesis, lymphangiogenesis, and regulation of cancer stem cells [41].

MiRNA profiles can vary among different cell types and control different gene expressions. Changes in miRNA profiles within different cell types can influence their activities and functions, affecting processes such as development and responses to the environment. This underscores the significance of miRNAs in controlling cellular activities and how alterations in miRNA profiles can impact their functionality. For example, miR-124 plays a crucial role in neural cell differentiation, ensuring that cells develop into appropriate neural cell types. However, miR-124 also plays a significant role in cancer and helps suppress oncogenic activities [42].

The miR-181 family has been described to play roles in the malignancy of liver, breast, and colorectal cancers. However, in Acute Myeloid Leukemia (AML), the miR-181 family acts as a tumor suppressor. On the other hand, miR-126 was previously recognized as a tumor suppressor in breast, lung, and colorectal cancers. Recently, it has been discovered that miR-126 functions as an oncogene in leukemia development, particularly in core-binding factor (CBF) leukemia [37].

There is a potential scenario where distinct target genes are expressed in conjunction with miRNA regulators, leading to their regulation by miRNAs in varying cell types or tissues. Another potential aspect is the recruitment of regulatory miRNAs to diverse target transcripts within different cell types or tissues. Recent research has highlighted the presence of numerous RNA-binding proteins that aid in the processing of miRNAs [43].

4.2. MicroRNAs from Extracellular Vesicles of Mesenchymal Stem Cells

Recent investigations have unveiled the microRNA composition within EVs derived from MSCs [43], revealing the presence of over 150 microRNAs [44]. These microRNAs have been extensively studied for their roles in transferring bioactive molecules involved in various physiological and pathological processes. These processes encompass a wide range

of biological phenomena, spanning from organismal development and epigenetic regulation to immune modulation, which involves miRNAs such as miR-146 and miR-155. Additionally, these processes extend to tumorigenesis, where miRNAs such as miR-23b, miR-24, miR-31, miR-122, miR-125b, miR-214, miR-223, and miR-451 are implicated [45–48].

MSC-derived EVs have been found to harbor a diverse repertoire of miRNAs, showcasing their heterogeneity and functional diversity. Among the miRNAs identified within MSC-derived EVs are miR-10b, miR-19a/b, miR-21, miR-29a/b, miR-143, miR-145, miR-146a, miR-148a, and miR-221/222. Notably, certain miRNAs have been recognized for their role as tumor growth suppressors, including miR-15a/16-1, miR-34a, miR-107, miR-143, miR-145, miR-148a, miR-203, and miR-375. Conversely, a subset of miRNAs, referred to as oncogenic miRNAs (oncomiRs), exhibit tumorigenic properties. This group includes miR-17-92, miR-21, miR-155, miR-221/222, miR-10b, miR-27a, miR-106b-25, and miR-18a [3,9,49].

Functional analysis of miRNAs has uncovered that 18 distinct miRNAs within MSC-derived EVs actively participate in the regulation of cell death pathways. This analysis highlights a subset of miRNAs that are particularly influential in this process, including miR-16-5p, miR-21-5p, miR-23b-5p, miR-25-3p, miR-34a-5p, miR-134-5p, miR-143-3p, miR-146a-5p, miR-181b-5p, and miR-222-3p. These miRNAs have emerged as key regulators, orchestrating the intricate balance between cell survival and programmed cell death, thereby contributing to the overall understanding of how MSC-derived EVs influence cellular dynamics and disease progression [9].

5. MicroRNAs in Extracellular Vesicles of Mesenchymal Stem Cells as a novel cancer therapy strategy

EVs from umbilical cord-derived MSCs (UC-MSCs) play a significant role in tumor development regulation. The expression profile of UC-MSC-derived EVs and miRNAs within them indicates tumor-suppressive properties that could serve as a novel therapeutic approach in cancer research [9]. MiRNAs found in EVs derived from UC-MSCs are implicated in a range of cellular functions, including apoptosis, cell proliferation, cell cycle control, and necrosis. Those originating from bone marrow-derived MSCs regulate processes such as blood cell proliferation, immune cell functions, cell migration, leukocyte infiltration, cellular maintenance and function, and apoptosis. Meanwhile, miRNAs sourced from adipose tissue-derived MSCs play a role in modulating cell proliferation, differentiation, and migration [50].

Transfected MSCs can deliver growth-inhibitory miRNAs to tumors, either through direct cell-cell communication or via the release of Extracellular Vesicles (EVs). Recent studies demonstrate that EVs released from MSCs containing high levels of miR-381-3p, miR-34a, miR-193a, and miR-146a effectively combat triple-negative breast cancer, non-small cell lung carcinoma, and ovarian cancer [51]. The therapeutic delivery of miRNAs (anti-miR-9) to chemotherapy-resistant glioblastoma multiforme via MSC-derived EVs has been found to be more efficient than direct cell-cell communication between MSCs and

cancer cells [19]. Utilizing EVs derived from MSCs as delivery vehicles for miRNA presents a promising avenue for developing an effective therapeutic strategy for various cancer conditions [3].

5.1. In vitro test results

EVs derived from dental pulp-derived MSCs that overexpress miR-34a, a tumor-suppressive miRNA, have been shown to inhibit tumor growth *in vitro* [19]. Downregulation of miR-148a has been discovered to facilitate metastasis in skin cancer, highlighting its role as a critical regulator in cancer progression. Conversely, miR-148a functions as an inhibitor of metastasis in gastric cancer, exerting its effects through the Dock6/Rac1/Cdc42 protein within the Rho GTPase signaling pathway. The decreased expression of miR-148a has been shown to suppress cell proliferation, reduce colony formation, and inhibit cell migration *in vitro*. This dual role of miR-148a underscores its significance in modulating metastatic behavior across different cancer types, offering potential therapeutic targets for managing cancer metastasis [52–54].

A study conducted by Yu *et al.* demonstrated that EVs derived from MSCs, enriched with high levels of miR-199a, significantly inhibit the proliferation, invasion, and migration of glioma cells. Furthermore, these EVs were shown to enhance the chemosensitivity of glioma cells to temozolomide *in vitro* by targeting AGAP2, a protein integral to endosomal trafficking [55]. In a separate investigation, Lou employed a lentivector carrying miR-199a, revealing its efficacy in increasing the sensitivity of hepatocellular carcinoma (HCC) cells to doxorubicin. This sensitization was achieved through the negative regulation of mTOR expression, effectively disrupting the mTOR signaling pathway. These findings underscore the potential of miR-199a-enriched MSC-derived EVs in enhancing the therapeutic response of cancer cells to chemotherapy [56].

EVs derived from human bone marrow-derived MSCs, enriched with miR-1231, have demonstrated significant *in vitro* inhibition of cancer cell growth and cellular adhesion in pancreatic cancer cell lines BxPC-3 and PANC-156 [57]. Additionally, EVs from MSCs that are enriched with miR-139 and miR-9 have been shown to specifically target the PRC1 and ESM1 genes. This targeting results in the suppression of tumorigenic characteristics in bladder cancer cell lines, also observed *in vitro*. These findings illustrate the potent anti-cancer effects of MSC-derived EVs enriched with specific miRNAs, highlighting their potential role in disrupting critical pathways involved in cancer cell growth, adhesion, and overall tumor progression [58].

5.2. In vivo test results

In a study utilizing EVs derived from human adult liver stem cells, researchers observed a significant induction of apoptosis in both HepG2 hepatoma cells and primary hepatocellular carcinoma cells [59]. This pro-apoptotic effect was further supported by *in vivo* experiments, where a marked reduction in tumor growth was recorded in SCID mice that had been inoculated with primary hepatocellular carcinoma cells. The pronounced antitumor effect

observed in these models was primarily attributed to the selective delivery of miRNA by the EVs. This selective miRNA delivery mechanism underscores the therapeutic potential of EVs derived from human adult liver stem cells in targeting and suppressing hepatocellular carcinoma.

EVs derived from bone marrow MSCs, enriched with miRNA-584, were found to significantly reduce tumor growth when administered to a glioma animal model. The mechanism underlying this inhibitory effect was identified as the direct targeting of the CYP2J2 gene by miRNA-584. This targeting disrupts the expression of CYP2J2, a gene implicated in promoting tumor growth and progression, thereby highlighting the therapeutic potential of miRNA-584-enriched EVs in glioma treatment. These findings suggest that bone marrow MSC-derived EVs could serve as an effective vehicle for delivering miRNA-based therapies to combat glioma and potentially other types of cancer [60]. Similarly, EVs from MSC overexpressing miRNA-3940 effectively hindered the tumor microenvironment *in vitro* and the invasion of colorectal cancer cells. This therapeutic strategy was also demonstrated to effectively impede both tumor growth and metastasis in an animal model of colorectal cancer by specifically targeting the ITGA6 gene. ITGA6 is recognized as a noninvasive biomarker for the early detection of colorectal cancer due to its overexpression in primary tumors and colorectal cancer cell lines. Furthermore, ITGA6 plays a crucial role in the progression of colorectal cancer by upregulating the TGF-β pathway. By targeting ITGA6, the therapeutic approach not only suppresses tumor development but also mitigates the mechanisms underlying metastasis, offering a promising avenue for the treatment and early detection of colorectal cancer [61,62].

In a study conducted by Naseri *et al.*, the therapeutic potential of EVs derived from bone marrow MSCs was evaluated for their ability to deliver miR-142 inhibitors to breast cancer cells. The investigation revealed that the downregulation of miR-142 and miR-150 facilitated apoptosis in breast cancer cells. This apoptotic effect was achieved by enhancing the regulation of the APC and P2X7R genes, which are crucial for cell cycle control and apoptosis. Additionally, *in vivo* experiments demonstrated that intravenous administration of MSC-derived EVs loaded with LNA-anti-miR-142 into BALB/c mice bearing tumors resulted in a significant reduction in tumor size. These findings underscore the efficacy of using bone marrow MSC-derived EVs as a delivery system for miRNA inhibitors, offering a promising approach for targeting and treating breast cancer [63].

6. Conclusion

MSCs have shown considerable promise as cellular delivery vehicles for a range of anticancer agents. Recent studies have highlighted the pivotal role of EVs derived from MSCs in mediating the therapeutic effectiveness of these stem cells. These EVs are increasingly recognized for their potential in cancer therapy due to their capability to deliver genetic material and bioactive molecules directly to tumor cells, thereby modulating their function. This targeted delivery system enables the alteration of tumor cell behavior, which can lead to inhibited tumor growth and progression. Consequently, MSC-derived EVs represent a significant advancement in the development of innovative and effective cancer

treatment strategies. EVs can encapsulate a diverse array of molecules, including proteins, lipids, and nucleic acids such as microRNAs (miRNAs). These miRNAs are small RNA molecules that act as genetic regulators, capable of influencing various cellular signaling pathways, including those implicated in cancer. In the context of cancer therapy, miRNAs delivered via EVs can be harnessed to modify gene expression within tumor cells, thereby inhibiting tumor growth and progression, as illustrated in Figure 2.

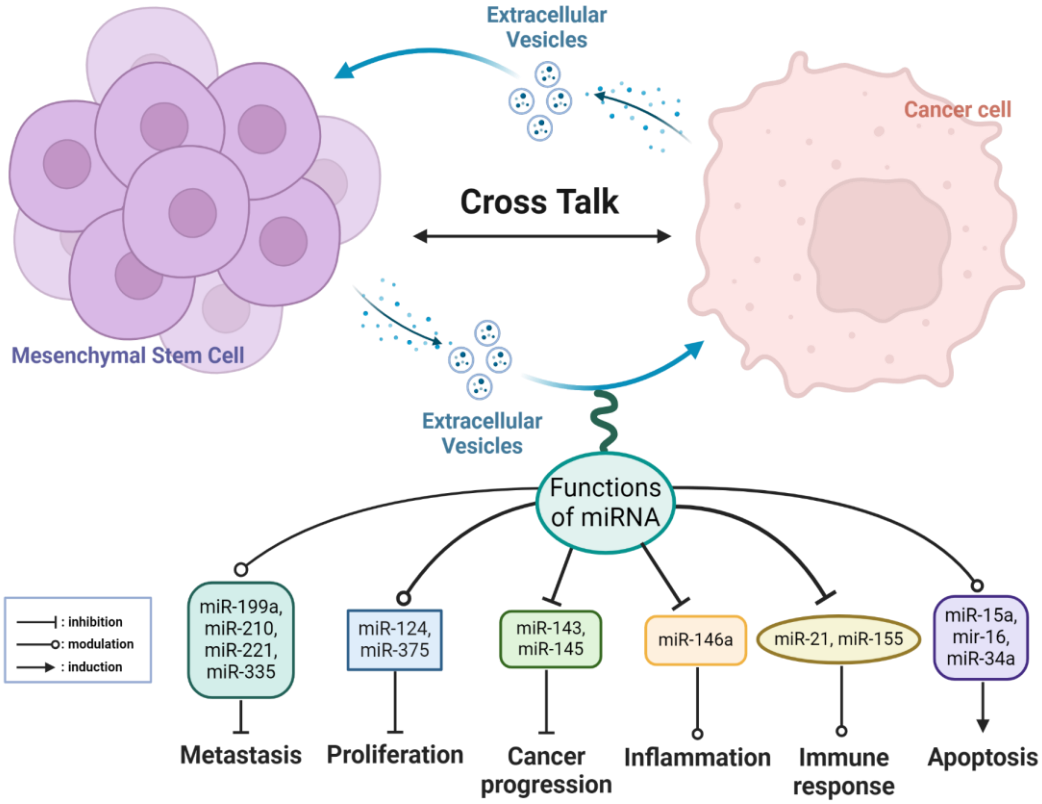

Figure 2. MSC-EVs cancer communication RNAi.

Unmodified MSC-derived EVs have shown the potential to either promote or inhibit tumor development, depending on the specific molecular cargo they carry and the context of their release. On the other hand, modified MSC-derived EVs are engineered to deliver therapeutic molecules, including chemotherapy drugs, miRNAs, anti-miRNAs, and small interfering RNAs (siRNAs), directly to cancer cells. These modifications enhance the EVs' ability to suppress tumor growth effectively. The therapeutic application of these tailored EVs involves altering key signaling pathways within the tumor microenvironment, ultimately contributing to the inhibition of tumor development and metastasis. This sophisticated delivery system underscores the significant potential of MSC-derived EVs in advancing cancer treatment methodologies. The use of EVs derived from MSC as carriers for miRNA in cancer therapy is still in the early stages of development, and further research is needed to understand the mechanisms and effectiveness of delivering miRNA through EVs. However, preliminary study results indicate significant potential in utilizing EVs from MSC as mediators for miRNA delivery in cancer therapy.

One significant limitation is the heterogeneity of EVs, which can lead to inconsistent therapeutic outcomes. The isolation and purification methods for EVs also vary, impacting their quality and efficacy. There are controversies regarding the precise mechanisms through which EVs exert their effects, as well as their stability and biodistribution *in vivo*. Additionally, the potential for unintended immune responses and off-target effects poses challenges to their clinical application. Furthermore, large-scale production and standardization of EVs-based therapeutics remain problematic, making it difficult to ensure reproducibility and safety across different batches. These limitations and controversies underscore the need for further research to fully understand and harness the therapeutic potential of hUC-MSC-derived EVs in cancer treatment.

This review brings new insights and perspectives to the field by highlighting the potential of Extracellular Vesicles (EVs) from human Umbilical Cord Mesenchymal Stem Cells (hUC-MSCs) as a novel delivery system for RNA interference (RNAi)-based therapeutics in cancer. It emphasizes the unique properties of hUC-MSC-derived EVs, such as their low immunogenicity, ability to cross biological barriers, and natural cargo of therapeutic molecules, which make them superior carriers compared to other delivery systems. The review also explores recent advancements in engineering EVs to enhance their targeting capabilities and therapeutic efficacy. Additionally, it discusses the dual role of EVs in both promoting and inhibiting tumor growth, providing a comprehensive overview of their complex biological functions. By addressing the limitations and controversies in current research, this review offers a balanced perspective, paving the way for future studies to optimize EVs-based therapies and bring them closer to clinical application.

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Conflicts of interests

The authors declare no conflicts of interest.

Authors' contribution

Conceptualization and design: M.U.A., S.I.W.; methodology: M.U.A., I.K.L., S.I.W.; software and validation: M.U.A.; formal analysis: M.U.A., I.K.L., S.I.W.; investigation and data curation: M.U.A., I.K.L., S.I.W.; resources: M.U.A., I.K.L., S.I.W.; writing and original draft preparation: M.U.A., I.K.L., S.I.W.; writing, review and editing: M.U.A., I.K.L., S.I.W.; supervision: I.K.L., S.I.W.; project administration: M.U.A., S.I.W.; funding acquisition: M.U.A., I.K.L. All authors have read and agreed to the published version of the manuscript.

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