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Review

Current understanding of the mesenchymal stem cell-derived exosomes in cancer and aging

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ABSTRACT

Mesenchymal stem cells (MSCs) are being widely researched upon for several years with translational application in regenerative medicine. Many studies acknowledged trophic factors from MSCs, attenuating dreadful ailments. The beneficial properties of MSCs are attributed to their secretion of paracrine factors as extracellular vesicles/exosomes in the tissue microenvironment. Exosomes are nano-sized vesicles involved in genetic material transportation and intercellular communication. Exosomes have been recently reported to play a role in cell-free therapy in treating many diseases like cancer and aging and are reported in regulating tumor cell fate. This review highlights the recent advances and current understanding in assessing mesenchymal stem cell-derived exosomes for possible cell-free therapy. The sources and composition of exosomes, drug delivery effectiveness, immunomodulatory property, therapeutic advances in cancer, and aging targeting exosomes as cargo or its effect to moderate the tissue microenvironment are also discussed. We summarize the regenerative mechanisms induced by MSCs derived exosomes.

1. Introduction

Cell-cell communication plays a vital role in determining the fate of a cell and its capability to comb external threats, hence maintaining cellular homeostasis or leading to disease. The understanding of Cell-cell communication was limited to autocrine, paracrine, and endocrine interactions [54] until the role of extracellular vesicles in antigen-presenting cells of the immune system function as miRNA and mRNA carriers was discovered [49]. These observations established extracellular vesicles as modulators of cell communication. Though the molecular mechanisms are not understood, their pathophysiology and function have aided in deducing these organelles' overall role in the cell.

Extracellular vesicles are membrane-adhered cellular components with the chief role of hauling molecular cargos such as nucleic acid and proteins between cells [68]. They are either classified based on the biogenesis as microvesicles, exosomes, and apoptotic bodies [68] or based on the molecular components and origin as ectosomes and exosomes [12]. Microvesicles derived directly from the budding of the plasma membrane, have a size of 1micrometer, unlike exosomes, which are developed by fusion of plasma membrane and multi-vesicular bodies through the endo-lysosomal pathway. These exosomes range from 40 to 120 nm and carry an extensive range of cellular cargo while compared to microvesicles [68].

Though these different extracellular vesicles conduct similar cargo through their bilipid layers, such as the cytoplasmic and lipid-raft interacting proteins and microRNAs, the exosomes carry some further established cell components. This includes other non-coding RNAs, protein receptors, components of the major histocompatibility complex, Tetraspanins, PDCD6IP, TSG101, flotillin, and tumor susceptibility gene 101 proteins, to name a few [3]. Exosomes possess this feature of transporting a more comprehensive range of molecular cargo with the aid of the endosomal sorting complex required for transport (ESCRT) [3]. This peculiarity of the exosomes makes it a widely studied extracellular vesicle compared to the other types. This characteristic has further helped understand cancer prognosis, drug resistance, and novel therapies [42]. Hence, the cargo-sorting mechanism of the extracellular

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vesicles, especially exosomes, is widely studied.

The usage of exosomes from MSCs has been critically studied regarding the clinical setting as transferors of biomarkers for diagnostic examinations. MSCs have been widely used as encouraging therapeutic options in clinical or pre-clinical researches. The reported way of action attributed to MSCs is their prolific proliferative ability, their fundamental property to differentiate, and even exhibit trans-differentiation into various cell types. It has recently been proved that the trophic effects of MSC play a significant role in tissue regeneration. The paracrine effects are mostly because of the MSC-derived exosomes. MSC-derived exosomes can be defined as the dynamic biological constituents of MSCs, with functional cargo that has raised extensive interests in multiple tissue repair and regeneration. Here, we critically summarise the advances in exosomes derived from different cell sources, especially the mesenchymal stem cell-derived exosomes, and we mainly highlight the exosomes derived from adult bone marrow, umbilical cord, adipose tissue, and fibroblasts which communicate intensively to facilitate proper immune function through the paracrine interactions or direct cell-cell contact. The purview of this review article is not only to introduce to the different types of extracellular vesicles that can be derived from MSCs but also to summarize their similarities and differences and then discuss other analysis methods currently used to detail the various potential uses in cancer and aging-related disorders.

2. Exosome biogenesis

The 1990s contributed to the advancement of modern biology, one such progression was the discovery of exosomes secreted by dendritic cells and B lymphocytes and were speculated to operate in immune regulation. Following which, exosomes were discovered in reticulocytes and diverse cultured cells, it was further identified to control a common 5'-nucleotidase activity. Exosomes' origin through internal budding during maturation of multi-vesicular endosomes and cell surface membrane confirmed its involvement in the endosomal systems [57]. Extensive research in this organelle has proven it to have various cellular origins, including stem cells. Its function as an intercellular communicator in diseased and normal cells has been widely accepted [57].

Exosomes habitually differentiated based on the tissue or cell of origin could also be classified based on the cellular components, typically their protein content. The common cell-specific exosomal proteins include adhesion molecules, integrins, MHC I and II, transferrin receptors, and other surface binding exosomes. While non-specific protein exosomes such as fusion and transporting proteins, heat shock proteins, and cytoskeleton proteins help in multi-vesicular body formation and other cellular processes [42]. These exosomal proteins are restricted to the plasma membrane, cytosol, and endosomal elements but are absent in other cell components, including the nucleus, mitochondria, or endoplasmic reticulum [42].

Exosomes are similar to their cell of origin is not just their cellular composition but also their sorting mechanism. The diverse range of exosomes originated by sorting relies on the multivesicular endosomes, post-translational modification, and maturation stage of the cell. Hence the sorting mechanism plays a significant role in determining cell fate and function and serves as a critical aspect in the differentiation of the exosomes [57].

3. Composition of exosomes

Exosomes, derived from Mesenchymal stem cells, have a wide array of therapeutic applications explored in the recent past. The major step in understanding this cellular organelle is the comprehension of its cellular components and molecular mechanism. The bilipid layer of MSC-derived exosomes preserves its endurance and stability, hence maintaining the cell's biological potency. It further protects cellular cargo from external degradation. The bilipid layer is also involved in the conduction of proteins such as fatty acid-binding proteins, arachidonic acid, phosphatidic acid, to name a few. There are about 2000 proteins recognized in MSC- derived exosomes, including a range of heat shock proteins, sorting proteins, MHC-I and MHC-II antigen-presenting proteins, proteins involved in maintaining cell structure and composition such as integrin, actin, tuberin, and growth factors. MSC- derived exosomes further contain enzymes, signaling molecules, and cytokines. But its major cellular component is the miRNAs. It includes both pre-miRNAs and mature miRNAs such as miR-21, miR-34, miR-16 that are involved in maintaining cellular homeostasis [42].

4. Sources of exosomes

Exosomes are procured from vesicle originated from the endosomes, which has been well documented to have a critical role in cell-to-cell communication. Exosomes are secreted in all cell types and are found in several biological fluids, including serum, saliva, urine, breast milk, serum, synovial fluid, amniotic fluid, lymph, bile, tears gastric acid and cerebrospinal fluid (CSF). In animals, exosomes are secreted primarily by the immune cells such as lymphocytes, platelets, dendritic cells, red blood cells, and tumor cells. Previously the exact function of the exosomes was not defined, and they were viewed as tiny trash sacs tossed from cells. However, recent reports stress that exosomes have been seen to enhance immune responses by performing as antigen-presenting vesicles. They play a crucial role in cell-cell communication, cell maintenance, specific myelin formation in the nervous system, and in some cases in tumor progression.

5. Characterization and isolation of exosomes

The basic challenge in developing the translational tools involving the exosomes lies in the purification of exosomes. Exosomes must be distinguished from other distinct populations of EVs, such as microvesicles or ectosomes that are shed from the plasma membrane and apoptotic bodies at the first step. The major sources of MSC in most adult tissues are the bone marrow, umbilical cord, and adipose tissue. The major techniques involved in the isolation and purification process are differential centrifugation, including ultra-centrifugation, charge neutralization-based precipitation, size-exclusion chromatography and lab-on-a-chip devices that include acoustic nanofiltration, immune-affinity, trapping on nanowires like techniques. However, due to overlapping size range, composition and the lack of specific markers, current isolation of extracellular vesicles (EVs) including exosomes do not allow purification of specific EVs. Consequently, current EV/exosome preparations are highly heterogenous [56]. The below section lists the characterisation and isolation technique of MSC derived exosomes from the major sources.

6. Exosomes from adult bone marrow-derived MSCs

Mesenchymal stem cells (MSCs) are adult stem cells that are mostly isolated from bone marrow, extra-embryonic tissues like the umbilical cord, cord blood, amniotic fluid, and adipose tissues. MSCs offer a prodigious therapeutic potential, and several remedies based on these cells have been established to treat a wide range of ailments. It is recognized today that, besides releasing paracrine factors like some cytokines and growth factors, MSC also secrete extracellular vesicles (EVs), which play an essential role in tissue regeneration and immunomodulation [2]. Recently the EVs derived from MSC form a core part of the cell-free therapeutic aspect. The significant development proposed in this new therapeutic strategy is that since no cells are introduced, transformed, or injured, the genetic material that could adversely affect the recipient cell or target tissue can be avoided. One of the most potent sources of MSCs is adult bone marrow (BM). Previous reports state that BM exosomes are being rigorously worked upon, and the potential for treating various pathologies seems evident. BM-derived MSCs and exosomes have been successfully used to treat degenerative diseases, such as

intervertebral disc degeneration (IDD) [58], cardiovascular disorders [59], and liver fibrosis [60]. They have also advocated by a group of researchers against glioblastoma in a mouse model [61]. BM-MSC has been employed in the treatment of graft versus host disease (GvHD) in clinical practice since [31] published their encouraging results for the treatment of refractory GvHD [31].

7. Exosomes from umbilical cord-derived MSCs

The chief application listed for the umbilical cord (UC) derived exosomes are in cutaneous wound healing. The possible mechanism for such positive modulation of these exosomes is attributed to enhanced angiogenesis through the Wnt/β-catenin pathway [4,23]. Many studies have reported the benefits of such cell free therapy in combatting colitis in a mouse model, inflammatory bowel diseases, in fracture healing, in immunosuppression, and in a mouse model of acute liver failure, myocardial infarction, and autoimmune uveitis [2]. Most of the studies on UC-MSC derived exosomes and their clinical application is mostly in the initial stage. Still, recent reports emphasize their translational potentials against various ailments [53].

8. Exosomes from adipose tissue-derived MSCs

The application of adipose tissue (AT) derived MSCs and exosomes are mainly reported on skin-related diseases. AT derived exosomes have been applied against atopic dermatitis, for cutaneous wound healing, in heart and neural conditions. The mechanism behind the positive effect of AT-derived exosomes is the release of certain paracrine factors like growth hormones or cytokines that enhance cell proliferation in the affected area, which, as a result, accelerates the wound healing process. Some reports also state that ATMSC derived exosomes can inhibit ovarian cancer cell proliferation and effective in reversing the apoptosis induced by oxidative stress in the cardiomyocytes [37,50].

9. Immunomodulatory function of exosomes

Other than cellular communication and transport of molecular cargo across the cell, the exosome is also involved in a series of functions for maintaining cellular homeostasis. This includes regulating tumorigenesis by influencing the tumor microenvironment (TME), angiogenesis, metastasis, and immune system regulation [25].

The fact that a cancer patient has a compromised immune system is broadly admitted, and the cause was speculated as to the tumor metastasis. Though the facts remain unchanged, the underlying or contributing factor for the metastasis could be the effect of exosomes. While the cell advances in tumor development, exosomes trigger the discharge of cytokines or chemokines of the immune cells. Molecular stressors commonly found in TMEs are leading components of exosome modification and immune alteration [1]. These tumor-derived exosomes (TDEs) or tumor-related exosomes (TEXs) can modulate various immune functions, including the NK cell activation, DC cell growth, trigger myeloid precursor cells, and evolve macrophages into pro-tumor cells. The entire phenomenon of TEX-influenced immunosuppression has led to the alteration of TMEs, and eventual drug resistance. With the surfacing evidence of TEX-host interaction, exosome analysis in the tumor has opened a more comprehensive range of possibilities in cancer therapy [10].

Similarly, the analysis of exosomes originated from umbilical cord mesenchymal stem cells, by Liu et al. proved the other immunomodulatory effect of exosomes [36]. The team analysed the generation of CD4 and CD8 T cells and reported that these immune cells' inhibition by mesenchymal stem cells obtained exosomes. At the same time, there was a reduction of IFN-γ, IL-6, and TNF-α levels, but an increase in TGF-β1 was reported [36]. This sudden escalation of exosome modulated cytokine/chemokine release could result in arousal of anti-tumor immune responses or an immunosuppressive effect. Hence, exosomes could be both a boon and a bane when left untreated. Therefore, exosomes could be both a boon and bane when left untreated. This could be a possible effect of long-term intercommunication of the immune cells with raised levels of tumor-specific exosomes [1].

10. Exosomes: a double-edged sword

10.1. Exosomes in the tumor microenvironment

The prevailing tumor environment in a cell is known as tumor microenvironment (TME); other than tumor cells, the TME comprises signaling molecules, blood vessels, non-malignant cells, and extracellular matrix (ECM). ECM also houses essential growth factors and gives a physical scaffold in the TME for all cells. They play a crucial part in tumor development, which deregulates, and later in the tumor progression stage, it becomes inefficient.

One of the important components of various cancers TME is cancer-associated fibroblasts (CAF) [4]. Exosomes obtained from cancer-associated fibroblasts are critical factors that regulate oncogenic transformation. Exosomes secreted by tumor cells are called "oncosomes" which actively involves delivering oncogenic signals to cellular targets. Their major function includes regulation of autocrine pathways of tumor promotion in tumor cells and modulates stromal cell function in the TME. The exosomal exchange at the distant sites and within the TME might affect tumor metastasis, therapeutic effect, and progression. Unraveling the regulatory mechanisms of exosomal release and cellular activities in recipient cells can recognize novel means to inhibit the tumor progression by targeting cell-cell communication in cancer [5].

Mesenchymal stem cells exhibit pertinent receptors for growth factors, chemokines, and cytokines generated in the tumor microenvironment (TME). This interplay of receptor-ligand binding favors tumor homing of MSCs, which around the bend allows researchers to make it possible to apply them as a unique cargo for targeted delivery in anticancer therapy.

Meanwhile, studies done by Kidd et al. [29] suggested that MSCs exhibit both anti-tumor and pro-tumor activities among the tumor microenvironment in cancer-oriented studies. MSCs might induce immunosuppression by remodeling tissue activity at inflammatory positions that eventually cause the formation of a tumor or tumor progression. Here the MSC-secreted exosomes enable the transformation of non-cancer stem cell (non-CSC) to CSC in the microenvironment. The study further proved that M2 macrophage-derived exosomes provided over-expression of miR-155-5p and miR-21-5p in colorectal cancer, which controls invasion and migration of colorectal cancer cells [32].

Exosomes also influence CSC by targeting specific cancer stem cells signaling pathways, like nuclear factor kappa-light-chain-enhancer of activated B (NF-κB), Hippo, transforming growth factor-β (TGF-β), Notch, and Hedgehog pathways. These pathways are remarkable in sustaining events like differentiation, tumorigenesis, and self-renewal of cancer stem cells. However, direct targeting of CSC by employing exosome-loaded inhibitors, siRNA, or miRNA, through the mentioned pathway is attainable. Meanwhile, exosome signaling initiates the CSC production in the tumor microenvironment via epithelial-mesenchymal transition (EMT) and provokes positive resistance to treatment. In the tumor-microenvironment, fibroblasts and the stromal cells can release factors like CXCL12 chemokine, hepatocyte growth factors (HGFs), and fibroblasts growth factors (FGFs) which not only facilitate growth and malignant cell survivance but serve as a chemo-attractant that triggers the migration of various cells, into the TME.

Exosomes isolated from hypoxic bone marrow-derived MSCs (BM-MSCs) arbitrated delivery of miRNA, such as miR-193-3a, miR-5100, miR-210-3p, from BM-MSCs to cells of lung cancer, accelerated total expression and phosphorylated STAT3, thereby further the progress of invasion via STAT3-mediated EMT activation in cancerous lung cells. However, hypoxic CRC-secreted exosomes comprise miR-23a, which led to the suppression of PDH1/2 when acted upon CRC cells, and

subsequently increased HIF-1α, an autonomous activator of EMT.

Making changes to the microenvironment can have a significant impact on the properties of drug resistance. Lugini et al. [39] highlighted that exosome released from the tumor cells provoke a disorder in colon-derived mesenchymal stem cells (cMSCs). The colon cancer cells might modify the cMSC niche to maintain their components of stem cells that accordingly turn into resistant to chemotherapy [39].

Shuai Wu et al. in their study, indicated that hWJMSC-MVs firmly exert pro-apoptotic and anti-proliferative effects/properties in bladder tumor T24 cells *in vivo* and *in vitro*. It is clearly stated that hWJMSC-EVs provoked/prompted apoptosis and arrest of the cell cycle are mainly initiated by inhibition of Akt phosphorylation pathways and upregulation of caspase-3 cleavage in T24 cells study [62]. Additionally, the hypoxic tumor-microenvironment is crucial for efficacious angiogenesis-targeted therapies in metastatic CRC. It is also indicated that hypoxic colorectal cancer-derived exosomes could trigger β-catenin in endothelial cells by providing Wnt4 mRNA and, further, results in migration and proliferation of endothelial cells. These findings might provide a new and useful target for cancer therapies and have summarized that TME plays a crucial part during the development of a tumor, drug resistance, and tumorigenesis [62]. The critical dual role of MSC derived exosomes is depicted in Fig. 1.

10.2. Exosomes as a tumor promoter

MSC-derived exosomes could impact tumor progression in vivo and in vitro by relocating their contents to surrounding neoplastic cells or giving rise to in recipient cell with a phenotypic alteration. MSC-derived exosomes were seen to be associated with the induction of angiogenesis in tumorous cells via promoting vascular endothelial growth factor (VEGF) production and elevating ERK1/2 and p38 mitogen-activated protein kinase pathways. Exosomes released from MSCs were noticed in transferring miR-221 to HGC27 cells, thereby facilitating migration and tumor cell growth. These studies are known to acknowledge the significant role of MSC-Exo on tumor progression *in vivo*. Zhu et al. [72], demonstrated a co-implantation of human colon cancer and gastric cancer cell lines subcutaneous with MSC-derived exosomes or MSCs into BALB/c-nu/nu mice. This showed a greater degree of proliferative capacity in the co-implantation group. Additionally, their results showed that MSC-Exos were involved in an indirect tumor growth promotion by intensifying pro-angiogenic function; MSC exosomes with greater strength activate VEGF and CXCR4 expression triggering p38 MAPK and ERK1/2 pathways [72].

However, multiple myeloma (MM) BM-MSC released exosomes in MM cells are assimilated by multiple myeloma cells, which have increased the number of cytokines, adhesion molecules, and oncogenic proteins tumor growth transition comparative with normal BM-MSC exosomes. Eventually, normal BM-MSC exosomes suppress the MM cell growth, whereas MM BMSC secreted exosomes further progress MM tumor growth. Indeed, transference of exosomal miRNAs from the bone marrow (BM) might advance breast cancerous cell dormancy in a metastatic niche [51].

A study reported that tumor-derived (TD) exosomes mediate myofibroblast function and phenotype through SMAD-induced signaling pathways in adipose-derived MSCs (ADSCs). TD-exosomes provide support to malignancy and progression of tumor cells by converting MSCs into tumor-associated myofibroblasts, within tumor stroma, in the TME. The results of this research demonstrate the critical role of TD-exosomes in the tumor stromal generation. This study further highlights in providing a foundation for new target break-through focusing on suppression or blockade of molecules associated with TD-exosomes, as an unconventional approach in cancer therapeutics [[11,12].

Nevertheless, the clinical treatment of gastric cancer (SGC7901) and osteosarcoma (MG63) cells, respectively, with hBMSCs-released exosomes in the absence or presence of small-molecule inhibitors (SMIs) Hedgehog pathway. This study concluded that through the Hedgehog signaling pathway's stimulation, the hBMSCs secreted exosomes enhanced SGC7901 and MG63 cell proliferation and has the capability/potential associated with tumor progression. Suppression of the Hedgehog signaling pathway substantially inhibits the approach of hBMSCs-secreted exosomes on tumor growth. Therefore, this evidence might be considered as important therapeutic intermediation in reducing gastric cancer.

The inclusion of MSC-Exos was also closely associated with obtained ecto-5′-nucleotidase activity by SCCOHT-1 tumor cells; this whole interactive involvement contains mRNA and protein assimilation. Literature findings support the idea of acquisition of 5′-nucleotidase activity by NK cells and SCCOHT-1 tumorous cells as a result of co-culture with mesenchymal stem cells, which are transferred by the exosomes or/and by the cells. Along with the metabolizing ability of 5′ AMP into adenosine, the pro-inflammatory activity can be repressed and regulated by SCCOHT-1 cells through the actuation of adenosine receptor signaling present on the immune cell surface. Another study indicated that conversion of 5′ AMP into adenosine by exosome could suppress the activation of T-cell in TME. Moreover, the existing evidence favors the concept that conditioned medium (CM) of MSC released

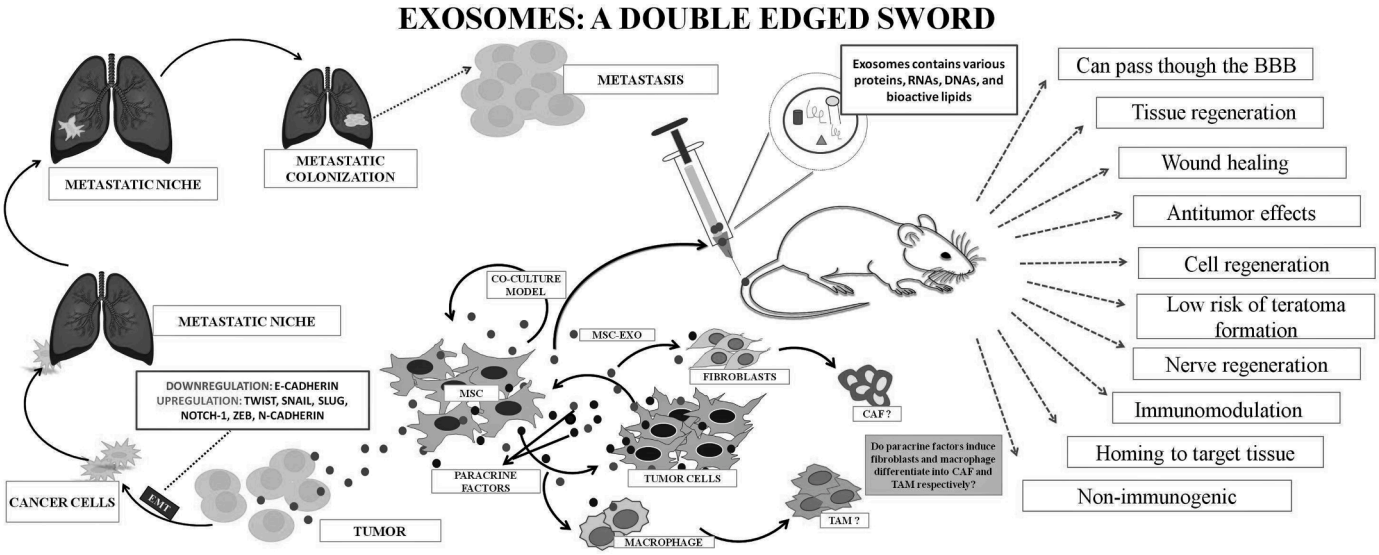

Fig. 1. *Exosomes as a double-edged sword:* The figure depicts the dual function of exosomes in tumor promotion and tumor suppression.

exosomes enhances migration activity of MCF-7 cells along with upregulatory functions, like Wnt signaling, of many pathways related to cancer.

HUC-MSC-secreted exosomes can initiate lung cancer cell growth and inhibit apoptosis; meanwhile, hUCMSC-EV transferred miR-410 suppresses the expression level of PTEN. The cellular communication between the cancer cells and mesenchymal stem cells was observed through MSC-EV-miRNA and further indicated that hUCMSC-EVs might be a novel therapeutic approach to decrease the side-effects. Besides, the existing evidence put forward that MSC secreted exosomes could modify tumor cells' functional capability by way of initiating ecto-5′ nucleotidase and MMP-2 activity and, consequently, contribute to elevated tumor heterogeneity and transformed tumor microenvironment (TME).

MSC-derived exosomes illustrate a beneficial / effective transporter in delivering anti-tumor cargo. As a result, functional alterations by MSC secreted exosomes could endorse prevention of tumorous cells against chemotherapeutic purpose and enhance tumor cell resistance. Collectively, these studies concluded that MSC-derived exosomes influence tumor growth in promoting and inhibiting ways, dependent on the MSC paracrine functions. The paracrine interaction between MSCs and cancer cells in the TME is displayed in Fig. 2.

10.3. Exosomes as tumor suppression

Exosomes obtained from MSC exhibit a vital role in delivering miRNAs, proteins, and lipids to the neighboring cells. They are also involved in suppressing inflammation, injured tissue repair, modulating the immune system, and function as key regulators of cell-to-cell activities. Roccaro et al. [51], however, observed that extracellular vesicles obtained from BM MSCs of multiple myeloma (MM) patients could promote multiple myeloma (MM) cell progression whereas EVs of normal healthy individuals, by the transference of lower miR-15a content, possibly prevents the development of multiple myeloma cells/tumor. Likewise, extracellular vesicles derived from normal human BM MSCs were shown to avoid proliferation and stimulate apoptosis in Kaposi sarcoma, ovarian tumor, and liver carcinoma cell lines. In summary, this literature findings suggested that micro-vesicles (MVs) released from human BM-MSCs promote apoptosis in various cell lines and provoke cell cycle arrest in vitro, and also suppresses in vivo tumor growth [51].

As well as exosomes released from adipose MSC demonstrated that prostate cancer is restrained through the delivery of miR-145 by suppressing the Bcl-xL activity and encouraging the apoptotic process via the caspase-3/7 pathway. In their study, Lee et al. [33] revealed that exosomes obtained from MSCs inhibited tumor progression and angiogenesis by suppressing the VEGF expression in tumor cells in vivo and in vitro. Moreover, their study also supported the concept of MSC-derived exosomes delivers miR-16, which is partially accountable for the VEGF down-regulation. Likewise, Huang et al. [19] showed that the MSCs angiogenic function was arbitrated via MVs while paracrine factors and exosomes were seen in inhibiting HIMF and Smad2, further results in anti-vascular remodeling [19].

Colorectal cancer is outlined as one of the aggressive cancer types due to its worldwide high incidence and mortality rate. It has been noticed that a particular microRNA, miR-16, exhibited tumor-suppressive characteristics in invasion as well as proliferation; it also mediated the apoptotic process of CRC cells. Overexpression of exosomal miR-16-5p (exo-miR-16-5p) repressed tumor growth, simultaneously enhanced apoptosis of colorectal cancer cells (CRC) via the integrin α2 (ITGA2) inhibition. As a result, BMSC-secreted exosomes with an increased expression of miR-16-5p suppressed the CRC progression and has shown the potentiality in the lack of biomarkers for effective CRC therapeutic strategies. Furthermore, a study also explored the other exosome-associated specific protein markers, like TSG101 and CD63, present in mesenchymal stem cells. In BMSCs, noticed that miR-223 expression was increased, this functions necessarily to maintain the liver against autoimmune hepatitis.

Primarily, retrieved analytical data demonstrated the upregulation of ITGA2 while miR-16-5p was suppressed in colorectal cancer and miR-16-5p targeted ITGA2. A particular antibody blocks the expression of ITGA2, which in turn suppresses the expression of PAK, LIMK, and N-WASP to inhibit actin organization and cellular migration of GC cells. Previous studies also supported that ITGA2 is over-expressed in gastric cancer (GC), and over-expression of hypo methylated ITGA2 in pancreatic ductal adenocarcinoma (PDAC) provided critical survival rate. In recent years Kamerkar et al. [26] conducted a study which

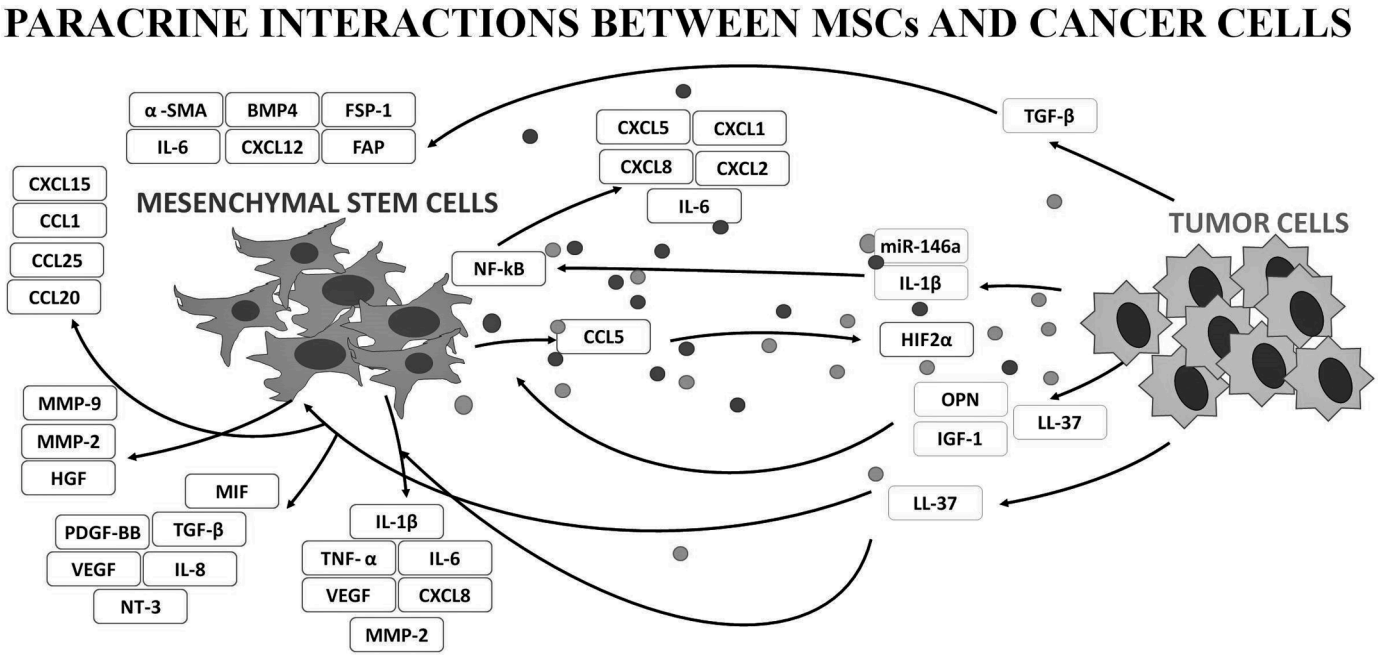

Fig. 2. *The paracrine interactions between MSCs and cancer cells:* In the tumor microenvironment, the MSCs influences the cancer cell growth/regression via releasing various trophic factors and the paracrine factors released by tumor cells aid in MSCs homing and proliferation in the niche.

reported that electroporated MSC obtained exosomes with siRNA, against oncogenic Kras, inhibited cancer in the pancreatic tumor of mouse models and exceptionally enhanced survival rate overall. Exosomes secreted by WJ-MSC reduces the cellular migration of U87 glioblastoma multiform (GBM) and delivers miR-124. MSC-secreted exosomes suppressed high expression of miR-14b and decreased the growth of glioma in rat models. Certain studies have revealed that exosomes obtained from MSCs have transported anti-tumorigenic miRNAs. Furthermore, one of them suggested that the expression of miR-124 was suppressed in glioma cells compared to normal tissues [26].

As seen in publications, a crucial role of MSC secreted exosomal cargos on cancerous cells are seen to affect angiogenesis to stimulate cellular proliferation. MiR-140 displays an effective tumor-suppressive function in the SOX9, SOX2, and WNT regulating pathways of stem cells. As the tumor rate elevates, a gradual decrease in miR-140 is noticed. The inhibitions of these regulating pathways are eliminated by the miR-140 down-regulation, resulting in a higher population of cancer stem cells and progress breast cancer. Additionally, Wu et al. [62], in a study, showed that EVs obtained from human umbilical cord Wharton's jelly MSCs (hWJSCs) by suppressing the response of phosphorylation of Akt protein kinase and up-regulation of cleaved caspase-3 led to reversal action of the advancement of bladder carcinoma cells. Conclusively, these studies propose that exosomes obtained from MSC might provide a prominent cell-cell communication mediator within the TME and inhibit the angiogenesis process by transferring anti-angiogenic molecules [62]. The paracrine interaction between MSCs and cancer cells in the TME is displayed in Fig. 2.

10.4. Emerging anti-tumor therapies from MSC-derived exosomes

Exosomes are biologically active delivery modules, exists as a supporting platform for advanced carriers of cargo *in vivo*. Understanding the exosomal organization, packaging of internal components, and stability could be engineered for effective usage as a cargo. Exosome-based therapeutic delivery or vaccination will become progressively significant standard-of-care in several cancer therapies corresponding to existing immunotherapy. A study demonstrated in the mouse models was observed that exosomes released from fibroblasts and increased expression of CD47 were designed to carry short hairpin RNA, particularly targeting oncogenic KRAS effectively resulted in tumor growth reduction in pancreatic ductal adenocarcinoma. Currently, human MSC is the only cell type that has a remarkable potentiality involved in the mass production of exosomes. MSCs have numerous cellular properties that are considered to be the desired features of drug delivery vehicles.

A study found that taxol-loaded MVs derived from MSC544 had no observable effect of viability in the tested cell lines of tumor. On the other hand, taxol-treated MSC544-derived exosomes manifested cytotoxicity in cancer cells relatively similar to the effect of taxol in various tumor cell populations of lungs, breast, and ovarian cancer cells. However, these exosomes obtained from MSC544 could be modified or loaded with many other chemotherapeutic agents, which could be essential in tumor therapeutic approaches [43].

Providing further emphasis on the chemotherapeutic potential, hUCMSC-Exo-delivered miR-375 could inhibit cell migratory events, tumor growth *in vivo*, cell proliferation, and invasion and also promote apoptotic activity. Thus, delivery of miR-375 by hUCMSC-Exo could repress the expression level of its putative target enabled homolog (ENAH) and eventually impede the esophageal squamous cell carcinoma (ESCC) initiation and proliferation event [40]. Chemotherapeutic drug-loaded exosomes are the finest exosomes used in cancer therapy, particularly cancer stem cells (CSCs) *in vivo*. Existing evidence supports that, in animal models, exosome-induced chemotherapeutic delivery has enhanced anti-tumor effects better than free drugs. For instance, a chemotherapeutic drug called doxorubicin, used in treating hematological malignancies and other tumors. It is tracked easily because of fluorescence; thus, it is possibly a well-studied drug in cancer therapy mediated by exosomes. *In vivo* experiments demonstrated on mice models showed that exosome-encased doxorubicin reduced the size of tumor effectively compared to liposome-delivered or free doxorubicin in colon adenocarcinoma. Moreover, increased concentrations of doxorubicin could be possibly used in treating ovarian tumors and breast tumors by decreasing off-target effects.

Another broadly used chemotherapeutic drug is paclitaxel, in its exosome-encapsulated form could target drug-resistant cancer stem cells (CSCs) *in vivo*. Additionally, paclitaxel loaded into prostate cancer cell-secreted exosomes has enhanced cytotoxic effect to autologous prostate cancer cells [52]. MVs-mediated-co-delivery of paclitaxel is known to retain anti-tumor effect, it also reveals that during the physiological biogenesis of microvesicles pharmacological function of paclitaxel was not influenced or affected. Ultimately, this study demonstrated that MSCs are an effective cell type in delivering active drugs via their microvesicles along with greater specificity of cell-target.

Withaferin A is an effective inhibitor of cancer growth and angiogenesis. In the xenograft mice model, the administration of exosomes-loaded withaferin A compared with free drugs showed a better enhancement of anti-tumor effect in human lung cancer [46]. Similarly, an anti-apoptotic protein, Survivin, plays a vital part in cancer cells by inhibiting apoptosis activation. In several cell lines of pancreatic adenocarcinoma, survivin-T34A-loaded exosomes could enhance apoptotic activity and induce their sensitivity to gemcitabine.

Consistent with the results of a few studies reported the functional characteristics/properties of tumor derived (TD)-exosomes, which involve various biological events like pharmacological and immune modulation, and their association with the host-environment. Nevertheless, TD-exosomes could be a practical approach for the anti-cancer vaccine to enhance their anti-tumor effects via non-genetic or genetic modification; certainly, TD-exosomes acquire and exhibit the properties of tumor cells in vivo since they accumulate in body fluids of cancerous subjects. Thus, MSCs are a well-known cellular candidate for producing many exosomes for effective therapeutic delivery of drugs.

10.5. Role of exosomes in aging

Skin is a vital and self-repairing organ, functions as a protective barrier against external environmental factors, and maintains homeostasis. Also, it plays a chief role in the process, metabolism, and synthesis of structural biomolecules. Skin aging is a biologically complex process that can be visible through a person's appearance, triggered by extrinsic and intrinsic factors. The skin regeneration characteristics are critical in maintaining the skin barrier during the process of average growth and wound healing. The skin regeneration property is generally regulated by stem cells (SCs) to contribute to cell turnover at the time of skin repair and homeostasis upon injury. Several studies have shown the importance of adult mesenchymal stem cells expressed wound healing and potential regenerative properties. In a recent study, Kato et al. reported that tissue repair/regeneration's better progress / regeneration is noticed when the cutaneous wound was treated with adult's BM-MSC or their ADSCs [27].

Various studies have indicated advantageous effects of adult stem cell-released-exosomal paracrine factors on the aging of the skin. Aging induces modifications in dermal fibroblast proliferation, increases degradation of existent skin by MMPs, decreases the repair and collagen synthesis, and further reduces skin regeneration ability. On the other hand, published reports indicated that aging-associated reduction in collagen synthesis and cellular proliferation of fibroblasts is associated with the down-regulation of the growth-related factors, TGF-β, and platelet-derived growth factor (PDGF). Additionally, in the aged fibroblasts, up-regulation of matrix-degrading metalloproteinase (MMP) expression is observed.

A particular study investigated the role of iPSC-derived exosome in the treatment of skin aging. As a result, Oh et al. [47] found that iPSC-Exo promoted migration and cellular proliferation of human

dermal fibroblasts (HDFs) under regular conditions. The preliminary treatment with iPSC-secreted exosomes prevents the damage of HDFs and reduces the high expression of MMP enzymes triggered by UVB radiation. Additionally, in photo-aged HDFs, iPSC-Exo potentially suppressed the MMP-1/3 and SA-β-Gal expression, and restoration of collagen type-1 expression level was also observed. These findings collectively intimate the therapeutic potentiality of iPSC-derived exosomes in the treatment of skin aging [47].

The aging process is mostly linked with the accumulation of pro-inflammatory senescent cells and chronic low-grade inflammation, which remodels the body's immune system. Mainly, senescent cells are supplemented by the release of immunosuppressive exosomes; also, exosomes from senescent cells are concerned in the spreading of senescence into surrounding neighbor cells, it also induced myelopoiesis in the bone marrow by activating myeloid lineage.

In addition, aging is also linked with immune remodeling, which includes both adaptive and innate immunity. A common sign in the process of aging is chronic-low grade inflammation, such a condition termed as inflammaging. Helenius et al. [17] revealed that the NF-κB signaling pathway was activated in the tissues of old mice, which are critically involved in the actuation of inflammatory responses. Benayoun et al. [7], in their study discussed age-associated changes in transcription and epigenetic regulatory events, which consequently determines the up-regulation of inflammatory and innate immune responses along with aging [7].

On the other hand, a study reported that administration of exosomes derived from young mice involved in the reversal action of age-associated changes in the aged mice, meanwhile the expression of IGFR, p16, and mTOR was also reduced upon administration. Zhang et al. [70] revealed that exosomes obtained from hypothalamic stem cell of younger mice shown decreased inflammation in the aged mice hypothalamus and delayed the process of aging. A study provided convincing data that EVs or exosomes obtained from the serum of aged mice and humans exhibit cellular senescence of MSCs. Ultimately, Davis et al. [14] indicated that the elevated expression of miR-183-5p, which increased during the aging of BM-derived exosomes, decreased BM stem

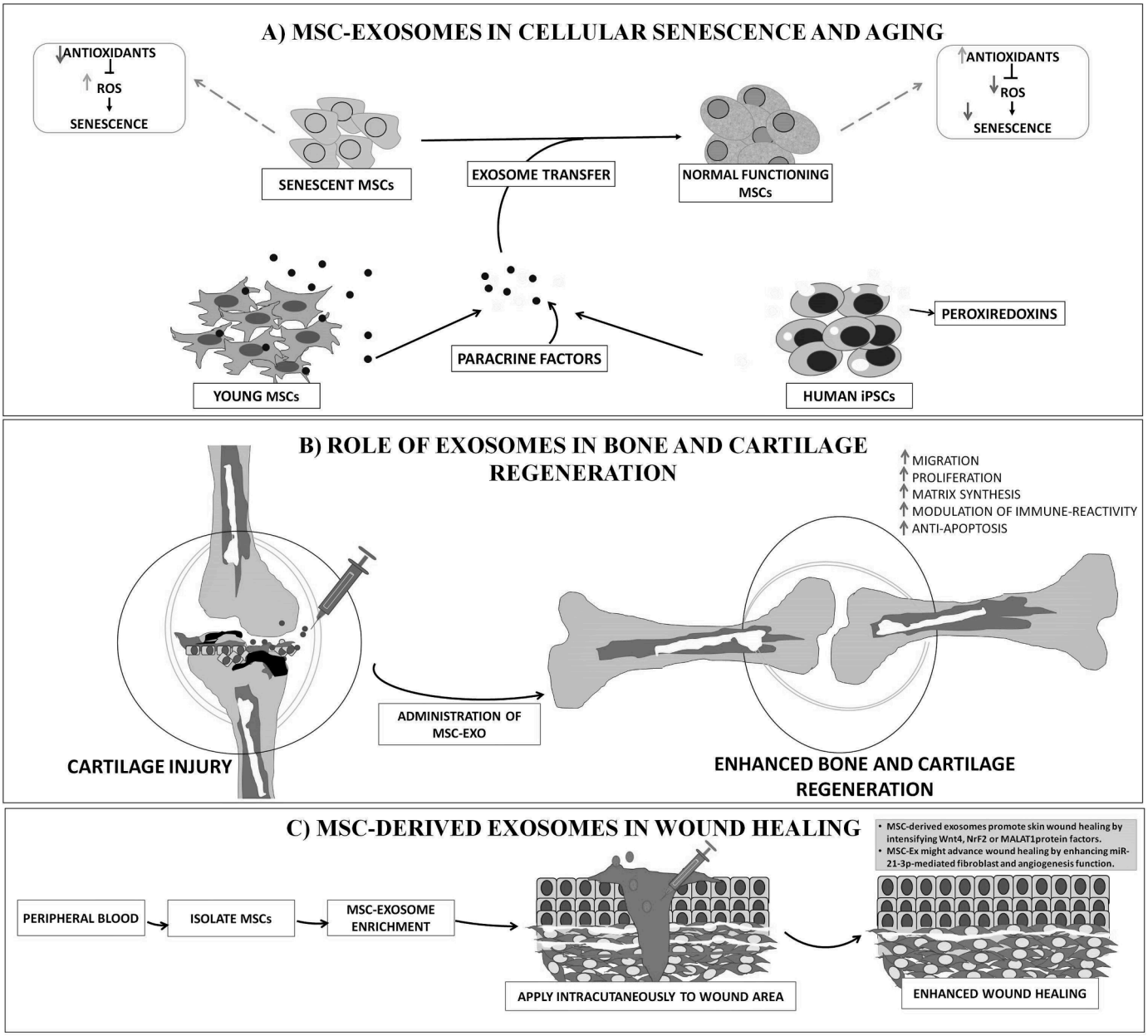

Fig. 3. *Precise role of MSC derived exosomes in:* (a) cellular senescence and aging; (b) in bone and cartilage regeneration; (c) in wound healing.

cells' proliferation, inhibited the osteogenic differentiation, and provoked cellular senescence. Similarly, a study reported that in exosomes secreted by muscle, miR-34a level has augmented along with aging in mice model. This indicated so that the exosomes derived from old mice enhanced the senescence of bone-marrow stem cells. Thus, this existing evident point out the critical role of exosomes in the process of aging [14,70]

Biancone et al. [8] found that MSCs can alter gene expression by secreting EVs to regulate physiological processes [8]. Also, it reported that old MSCs treated with young MSC-secreted EV indicated increased expression of Oct4 and Nanog. Meanwhile, the culture of young MSCs with EV obtained from old MSC has shown reduced expression of transcription factors. This denotes that self-renewal capacitance of mesenchymal stem cells is affected by EV at the genetic level, consistent with Jo et al. [22], who reported that nanovesicles promote the cellular proliferation of mesenchymal stem cells of a murine, enhances signal pathway associated with proliferation, but do not regulate/influence the murine MSCs properties. Finally, these vesicles could provide stable MSCs for other therapeutic applications and regenerative medicine [22].

This evidence discusses that EVs obtained from old MSC holds "age-promoting" factors, which might be accountable for an age-related reduction in stem cells, pluripotency, and self-renewal, such are affected by aging. Additionally, understanding the concept of the aging process helps in the progress of EV-based therapeutics. The prominent role and application of MSC-exosomes in aging research are highlighted in Fig. 3 a–c.

10.6. Anti-aging therapies targeting the exosomes

Li et al. [35] reported high expression of miR188 in BMSCs of old mice models and humans. Further, it is identified that BMSCs modulated by miR-188 bifurcate into adipocytes and osteoblasts during the process of aging. In mice models, knocking out of miR188 reduced age-related cortical bone loss, trabecular, and deposition of marrow fat. Thus, these evidences suggest therapeutic objectives for age-associated bone loss [35].

Another study indicated that EVs are a promising tool in altering the expression of target families, mTOR, engaged in aging via their miR cargo. By suppressing miR's expression level, miR-188-3p, in EVs obtained from bone marrows of young mice incubated with aged MSCs induced a high level of Rictor, also led to decreased phosphor-AKT which paves the way for developing new therapies applying MSC-secreted EVs combating age-related diseases.

Another study demonstrated by Zhang et al. [69] revealed that combined application of sponge Haliclona sp. spicules (SHSs) and hUCMSC-Exos in mice models exhibited anti-photoaging effects that reduced micro-wrinkles, induced expression of ECM, and also diminished histopathological changes. In contrast, hucMSC-Exos solely generated significantly weaker effects. Conclusively, this study showed an effective approach to treating photo-aging. SHSs considerably enhance the skin delivery of MSC-secreted exosomes in a safer method [69].

Other literature findings indicated that by delivering novel IncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), UMSC-secreted exosomes inhibit aging-mediated cardiac dysfunction around the bend prevents the NF-Kb/TNF-alpha signaling pathway. These findings will lead to therapeutic ideas that delay aging and in the treatment of age-associated diseases.

10.7. Exosomes in wound healing and in aiding cellular senescence

Certain studies reported that MSC-Ex might advance wound healing by enhancing miR-21-3p-mediated fibroblast and angiogenesis function. Also, MSC-derived exosomes promote skin wound healing by intensifying Wnt4, NrF2, or MALAT1 protein factors that arbitrate angiogenesis, migration, and cellular proliferation. A study found that, in the wound microenvironment, exosome-derived from stem cells actuates dormant stem cells by controlling immune and inflammatory responses. Zhao et al. [71], in their study indicated that direct injection of MSC-Ex might treat skin wounding as hUCMSC-Ex promotes wound healing by stimulating the activity of HaCaT cells. [71].

By the provoked expression level of IGF1, SDF1, HGF, and NGF led to the activation of Stat3, Akt, Erk signaling, MSC-derived EVs from BMMSC, ADMSC, iPSCs and UCMSC tissues up-regulated cellular proliferation, collagen generation, migration of cell, and the formation and maturation of newly developed vessels along with the reduction of scar width and re-epithelialization. Most exosomes contain factors such as HGF, VEGF-A, PDGF-BB, and FGF-2 under xeno- and serum-free conditions, unlike exosome released from UCMSC merely containing TGF-β. MSC-derived exosomes from the primary sources enhanced migration, dermal fibroblast, and keratinocyte proliferation. A slight potential belongs to BMMSC released exosomes to fibroblasts UCMSC-secreted exosomes to keratinocytes. Further, this could be an advantageous therapeutic tool in the treatment of wound healing.

Senescent cells secrete a more significant number of EVs that might be a non-canonical element of the senescence-associated secretory phenotype (SASP). Senescence-related EVs emerge as SASP factors associated with age-related lung diseases. For instance, in the serum, high levels of exosomal miR-21 have been related to idiopathic pulmonary fibrosis; thereby play a more significant role as a SASP factor. The role of small extracellular vesicles (sEVs) is well-known in causing paracrine senescence and speculates that sEVs might be engaged in rendering senescent bystander effect during the process of aging or therapy-induced senescence. Various studies suggest that exosomal secretion is enhanced in senescent cells. Meanwhile, Mensa et al. [44] analyzed that secretion of exosomal miR-217 and miR-21 from senescent human umbilical vein endothelial cells (HUVECs) triggered a significant reduction in the SIRT1 (a member of Sirtuin family) and DNMT expression levels in the non-senescent cells (control). Concurrently, it is shown that DNMT1 regulates the status of DNA methylation; it seems that exosomes obtained from senescent cells spreads senescence through epigenetic regulation [44].

Terlecki-Zaniewicz et al. [55] investigated the exosomal miRNA cargos released from senescent human dermal fibroblasts. Also, it analyzed that these released miRNAs from exosomes decrease the apoptotic activity by targeting mRNAs of pro-apoptotic proteins, therefore a typical senescence phenotype of a cell. Similar to human studies, mucinous analysis indicated the downregulation of exosomal microRNAs such as miR-294 and miR-133b-3p in aged rats might be an influential factor in causing renal aging and renal EMT. These findings indicated that extracellular vesicles obtained from human SC provide lower-risk and an effective alternate method for targeted-cellular therapies and regenerative medicine [55].

10.8. Exosomes in tissue regeneration

Regeneration of tissues by stem cells, especially mesenchymal stem cells, is widely practiced, but a better alternative is the utilization of MSC-derived exosomes. Its paracrine effect on the damaged tissue and aid in regeneration could be utilized in various cases, including liver, kidney, cardiovascular, and skin regeneration. The ability to cross biological barriers between cells is a contributing factor for MSC-derived exosomes' regenerative capacity. The application of MSC-Exos is favourable to the rigid MSC as MSC-Exos are more comfortable to collect as they could be secreted by most cells, they are stable during long term storage. They are ideal molecular cargo carriers that are specific to the target site or the microenvironment. The effect of MSC Exos in acute kidney and liver injuries have also been reported to be beneficial.

The regenerative capacity of MSC-Exos is further explored in skin cell regeneration by various cutaneous wound healing models. Healing of skin cells requires the united response of multiple cells surrounding the damaged cells and cytokines. MSC-Exos can hasten skin regeneration

and diminish redundant scar generation. They are involved in overall re-epithelialization and scar width reduction and the delivery of proactive miRNAs and support the discharge of collagen and elastin that aids in overall skin healing. This increasing evidence states that MSC-Exos are reliable than MSC in the regeneration of tissues [20].

10.9. MSC-derived exosomal therapy in various cancers

Exosome-based cancer treatment strategies are actively being tested in virtue of their various advantages, leading to several clinical trials for cancer therapy. Reports highlight that exosome are considered natural nano-carriers with absolute prevalence in biocompatibility, which can be applied in clinical applications, such as transfer some proteins, lipids, and regulatory mi, and specific mRNAs. Since exogenous cargo can be loaded into them to transport therapeutics to tumor sites, the exosomes are thought to be crucial with an essential role in cell-to-cell communication. In the current period, many researchers have found that MSC-derived exosomes are intricately involved with a significant role in cancer cell-to-cell interaction both in vivo and in vitro. Hence, these might provide a possible therapeutic option for cancer in future clinical medicine [28].

10.10. Gastric cancer

Gastric cancer (GC) is understood to be the fifth most often diagnosed cancer and the third leading reason for cancer death globally. Despite recent advances in therapeutic methods the prospects for advanced GC patients remains very poor. EVs acts as natural carriers of anti-cancer agents, which suggested that exosomal based therapy of gastric cancer is also a valuable approach. The past decade has witnessed a renewed interest in exosomes. These nano-scale vesicles of the endocytic source are secreted by nearly every type of cell.

Exosomes aid the anti-mir-214 transfer by acting as nanoparticles to overturn chemo resistance to Cisplatin in GC are identified. Hepatocyte protein (HGF) siRNA packed in exosomes is delivered into GC cells, suppressing proliferation, thereby continuing migration of both vascular and cancer cells. Besides, in vivo exosomes, by inhibiting the tumor growth rates and blood vessels, moved HGF-siRNA. These outcomes suggested that exosomes by delivering HGF siRNA could function as nanoparticles to suppress tumor growth and angiogenesis in gastric cancer [21]. Another study demonstrated that the exosomal delivery of tripartite motif containing-3 (trim-3) protein caused the suppression of GC growth and metastasis both in vitro and in vivo. These studies contribute to the effective use of exosomes within the therapy of gastric cancer [16].

10.11. Colorectal cancer

Colorectal cancer (CRC) is one of the foremost familiar cancers diagnosed in humans and maybe an explanation of mortality worldwide. It arises from the epithelial cell established within the lining of the colon/rectum of the gastrointestinal tract and is characterized by lymphocyte infiltration.

One of the prime unmet concerns in CRC targets mutated kind of RAS kinases since these are considered mainly unresponsive to existing drugs. Attractive anti-tumor activity was displayed by small interfering-RNAs (siRNA) for specific KRAS point mutations in non-small cell lung cancer (NSCLC). It possibly will be directly translated into CRC models [6,24,41]. Yan et al. examined the role of bone marrow-derived mesenchymal stem cells (BM-MCSs) and exosomal mir-16-5p were investigated by regulating integrin α2 (ITGA 2) in CRC. Consequently, in vivo experiments established that the BM-MCSs-derived exosomes overexpressing mir-16-5p blocked the tumor growth. Simultaneously, BM-MCSs-derived exosomes up-regulating mir-16-5p restricted CRC development by downregulating ITGA2 [63]. There is still an extended way ahead to guide the diagnosis and treatment of CRC.

10.12. Pancreatic ductal adenocarcinoma (PDAC)

The pancreas' most familiar cancerous disease with the prevalence of 90 percent of all pancreatic malignancy is pancreatic ductal adenocarcinoma (PDAC). To date, PDAC is the fourth most recurring cause of cancer deaths globally, with an overall endurance of less than 8%.

Study shows that the human umbilical cord MSC-exos aid in the delivery of Mir145-5p which inhibits PDAC progression by direct effect in arresting cell proliferation, and reduction in SMAD-3 expression. Additionally, the vivo study showed a reduced xenograft tumor growth following mir-145-5p overexpression in mice [64]. Inhibitors of apoptosis (IAPs) facilitate cell death, but their expression pattern is continuously decreased in various cancers. In pancreatic cancer, the IAPs are constitutively enhanced by Nf-κb in tissues and cell lines. Cancer progression and chemotherapy resistance are associated with ectopic upregulation of IAPs. It was found that exosomes carry mRNA IAPs and protein. The quantity of IAP protein or mRNA in the cytoplasm continued to remain unchanged when exposed to chemotherapy or was moderately up-regulated. Likewise, the expression pattern of IAPs in exosomes reveals no significant alteration [66]. Therefore, exosomes are an evolving means and an essential candidate for pancreatic cancer treatment.

10.13. Prostate cancer

The most common cancer with solid malignancy and a very high mortality rate in men is prostate cancer (PC). Due to the incomplete depletion of tumor cells and tumor relapse, cancer therapies fail in most cases. Traditionally, anti-PC treatments for patients affected with advanced/ metastatic disease is not beneficial. Exosomes can transport Natural products, Chemotherapeutics, and RNA to treat PC to different types of specific cells. Exosomal mir-145 from adipose-derived stromal cells (ASCs) could lessen Bcl-xl activity and promote prostate cancer cell apoptosis via the Caspase-3/7 pathway. Hence, these exosomes are used in prostate cancer therapy [48]. MSC-derived exosomes could act as suppressors of the prostate cancer-induced angiogenesis on PC-3 neoplasm cells, and, thus, they might be appropriate for anti-cancer therapies.

These results highlight the significant role of MSC-derived exosomal mir-143 in the advancement of prostate cancer. Prostate cancer was suppressed by downregulating TFF3 through the overexpression of mir-143. These findings ascertain the Mir-143 and TFF3 genes as potential therapeutic targets for managing prostate cancer [48]. Additionally, preclinical studies are needed to validate the potential of exosomes in prostate cancer therapy.

10.14. Hepatocellular carcinoma

Primary liver cancer is widespread in hepatocellular carcinoma (HCC) in adults. MSC-excreted paracrine factors have shown mediated effects on tumor progression, which are transported by Extracellular Vesicles. Only a very few studies have expressed the potential results of MSC exosomes on HCC. Cell cycle progression is hindered by BM-MCSs derived exosomes from MVs and also induces apoptosis in hepg2 cells. HepG2 cells loaded subcutaneous injection was administrated in SCID mice, which extensively inhibits tumor growth by the intra-tumor management. The anti-cancer immunity is shown to promote by the anti-cancer effects of AD-MSC-derived exosomes [38]. The role of tumor growth remains indistinct, and therefore caution should be taken before the use of MSC-derived exosomes in cancer therapy [30].

10.15. Exosomes as cancer biomarkers

A separate study reported that miRNAs and proteins' exosomal composition emerges as a promising biomarker for the diagnostic process. Each tumor is distinguished by a particular microRNA expression

level or protein profiling. Growing data evidence has revealed that proteins and microRNAs are positively associated with various tumor cells progression stages. Tetraspanin is a group of scaffold protein, which is greatly enhanced in exosomes. Also, CD63 is included in both the member of tetraspanin family and an exosomal marker. In 2013, Yoshika et al. demonstrated a balanced assessment of protein markers present in various other cancer cells' exosomes. They also revealed that protein-marker, CD63, is present considerably at a greater level in cancer cell-derived exosomes than exosomes released from non-CSC, which further proves that CD63 is a potential biomarker for cancer diagnosis.

Additionally, this study discussed the exosomal-miRNAs, these miRNAs released from exosomes were guarded against RNAse-dependent degradation and most often subjugated as a clinical biomarker. Therefore, these exosomal-miRNAs are a supreme biomarker as it can be persistently detectable in both serum and plasma, which could be applied in clinical diagnostics. Mitchell et al. [45] have pointed out, in prostate cancer, that robust expression of circulating mir-141 is employed as a diagnostic biomarker [45,67]. Moreover, researchers have found that exosomal mRNAs might be a novel biomarker used in clinical diagnostic applications along with microRNAs. Further, this investigation is considered appropriate and effective in diagnosis by employing exosomal biomarkers. The multifaceted role of exosomes and their hallmarks are depicted in Fig. 4.

10.16. Anti-tumor vaccine

Clinical significance moves along the treatment of cancer using the immune system. The utilization of exosomes as delivery molecules contributes to the insight of gene and biological therapies in oncology. It's very well-known that booming tumor treatment lies within the capacity to preferentially target tumor cells while minimizing damage to normal healthy tissue. Exosomes provide major advantages for improving the curative index of cancer treatment because they contain the potential to target cells for intracellular delivery of their contents.

Dendritic cells (DC) derived-exosomes could also be functional as anti-cancer vaccines because of the nature of DC as antigen-presenting cells (APCs). Major histocompatibility complex (MHC)-I & 2, and co-stimulating component like CD86 are articulated on the surface of DC-derived exosomes. Besides the APC, the natural killer cells are activated by surface protein like nkg2d ligand and il-15rα of the dc-derived exosomes [45].

Cancer cells contain Exosomes, which may also be practically used as anti-cancer vaccines since they contain antigens. The effective antigen presentation of APC can be triggered by the tumor antigen carried by the Tumor cell-derived exosomes (TEXs). Diverse investigations have revealed that TEXs can interfere with the maturation of DC, transform macrophages into the tumor-promoting phenotype, also, induce suppressor cells of myeloid origin, and weaken the activation of NK cells. One of many immune inhibitory mechanisms of TEXs is activated by the CD8+ effector T- cells in the circulation system of cancer patients where TEXs induced apoptosis. Therefore, it suppressed the patient's general immune system [65]. Alternatively, exosomes derived from cancer cells reported that they also play different roles like cancer progression, drug resistance, and metastasis. Hence, safety assessment is fundamental for utilizing the exosomes derived from cancer cells.

10.17. MSC derived exosome as a nano-drug delivery tool

Particular befits are present in an exosome-based delivery system like safety, stability, and specificity. Emerging curiosity within the application of exosomes as bio-nanostructures in the delivery system is attributed to the enormous benefits compared to other synthetic nano-carriers like liposomes. Exosomes are natural nano-sized lipid bilayer compositions that make them safe by exhibiting less immunogenicity and added biocompatibility [13]. Unlike most contemporary drugs, exosomes possess the stupendous ability to penetrate the blood-brain barrier, they serve as reliable transporters of cellular cargo. Further, exosomes are acquiescent to membranous changes that improve cell-type-specific targeting.

MSC is the ideal candidate for the mass manufacture of exosomes and they can also be encapsulated with miscellaneous cargoes because of their capability to maintain minute molecules like DNA, miRNA, siRNA, drugs, and proteins. MSC-derived exosomes have been used as drug delivery vehicles in tumor therapy and regenerative medicine in some studies [15]. Exosomes uniquely possess a curative potentiality to treat a diverse range of disorders such as neurodegenerative disorders, cell injuries, regenerating tissues, and cancer. However, it's essential to do more research to modify the description, procurement procedures, and standardize the production of MSC- derived exosomes for drug delivery systems [9].

10.18. Challenges in bringing exosome-based therapeutic options

With so much encouraging *in vitro*, animal, and preclinical findings in numerous disease models of various types of cancer and aging-related ailments, researchers are now tasked with developing a reproducible, achievable, and risk-free MSC-exosome therapy [34]. Arguably, the

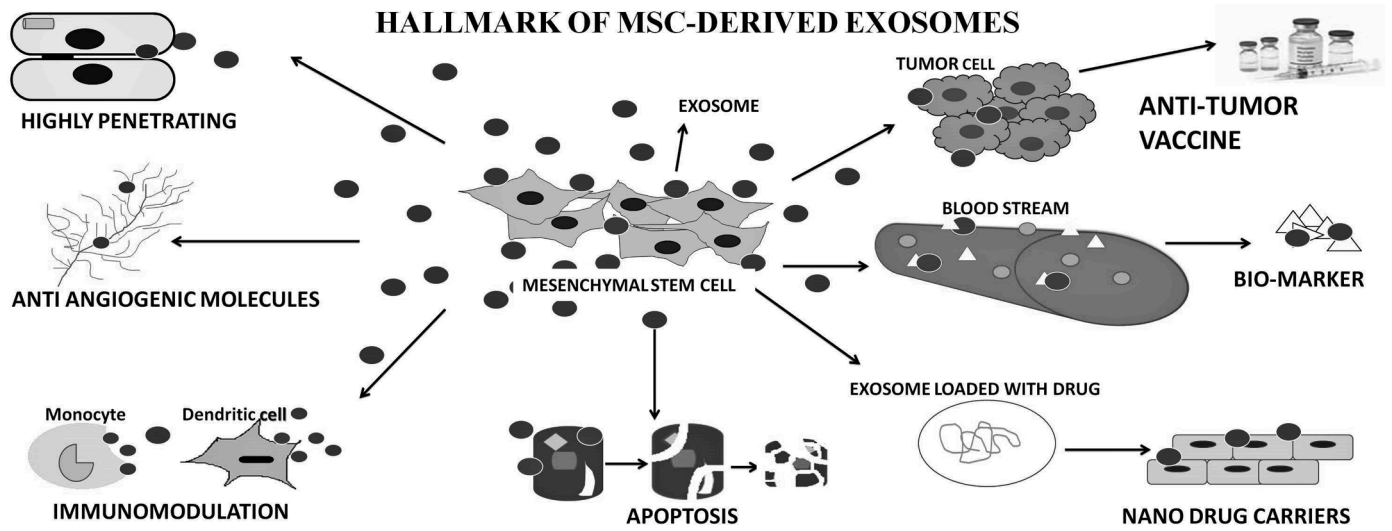

Fig. 4. *Hallmarks of MSC derived exosomes:* The figure depicts the major attributes of exosomes derived from mesenchymal stem cells.

significant steeplechases are the indifferent procedures for exosome isolation and characterization and the deficiency of enrichment in exosome purification with the highest yield and stability. Ways to harvest and enrich exosomes should be unified. More precise isolation and storage are important to be listed in guidelines to make this work more professional since many variables associated with the broad variety of MSC sources, culture systems and sEV enrichment processes should be taken in consideration. In addition to the course of action, MSC-therapeutic small EV's potential will also be impacted by micro-environment of an agonistic or antagonistic disease, path of delivery and timespan for therapeutic treatment in specific diseases [18]. It should be noted that different diseases should be handled in another way when it comes to therapeutic strategies involving exosomes. Exosome-based therapeutics' regulatory background is still developing, and representative toxicology testing methodologies may not be considered appropriate for such biologic medicines because of complex bioactive properties that may include tumorigenicity and unknown off-target activities.

11. Concluding remarks and future perspectives of MSC derived exosome therapies

Mesenchymal stromal cell-derived exosomes are an evolving stream of treatment that had displayed a beneficial role in various inflammatory and degenerative conditions and are on the verge of translation from preclinical models into early phase clinical trials. One of the major points to be noted in the isolation, purification, and its clinical translation thereafter, for various diseases ranging from cancer to aging-related disorders not only will therapy like MSC-exosomes, MSCs, and MSC exosome-educated macrophages need to be verified for safety and efficiency, but they will also be required to retain their function after cryopreservation/thawing in the context of clinical applications. MSC exosomes are perceived to be less immunogenic than their parental cells, as considered by the less expression of significant histocompatibility complex-II. But still, a potent question that remains unanswered is that about the susceptibility of these MSC-derived exosomes towards the cytotoxicity by the natural killer cells or the endogenous T cells of the host. Although, exosome-based therapeutics may represent the next-generation as a targeted drug delivery tool, providing an unparalleled usefulness for the treatment of various diseases deficient of effective pharmaco-therapeutic strategies. But it should be noted that careful consideration of the key issues raised, including the mechanistic and safety issues of procuring and transplantation of exosomes to be kept in mind that would aid in giving exosome-based therapeutics a more logistic approach for direct clinical applications.

Declarations

Ethics approval: Not-Applicable. This research does not involve human/animal participants, human/ animal material, or human

Consent to participate: Not applicable

Consent for publishing: All authors have approved the final version of the manuscript and gave consent for publication.

Availability of data and material: This is a review article, so no data are involved. However, all data from the published article analysed during this study are included as references.

Code availability: Not applicable.

Author's contributions: AB was involved in the conception of the study and designed the study protocol, and wrote the manuscript. MMK, SJM, and HG wrote parts of the manuscript. AB and MMK and SJM were involved in designing the images. SP and AKDR critically reviewed the manuscript. All authors read, reviewed and approved the final manuscript.

Declaration of Competing Interest

The authors report no conflict of interest.

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