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Research article

The role of the hematopoietic stem/progenitor cells-derived extracellular vesicles in hematopoiesis

Ezzatollah Fathi [a], Behnaz Valipour [b], Sevda Jafari [c], Abdolhassan Kazemi [d,e], Soheila Montazersaheb [f,*,1], Raheleh Farahzadi [g,1,**]

[a] Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
[b] Department of Basic Sciences and Health, Sarab Faculty of Medical Sciences, Sarab, Iran
[c] Nutrition Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[d] Medical Philosophy and History Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[e] Department of Parasitology and Mycology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[f] Molecular Medicine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[g] Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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ABSTRACT

Hematopoietic stem cells (HSCs) are tightly regulated by specific microenvironments called niches to produce an appropriate number of mature blood cell types. Self-renewal and differentiation are two hallmarks of hematopoietic stem and progenitor cells, and their balance is critical for proper functioning of blood and immune cells throughout life. In addition to cell-intrinsic regulation, extrinsic cues within the bone marrow niche and systemic factors also affect the fate of HSCs. Despite this, many paracrine and endocrine factors that influence the function of hematopoietic cells remain unknown. In hematological malignancies, malignant cells remodel their niche into a permissive environment to enhance the survival of leukemic cells. These events are accompanied by loss of normal hematopoiesis. It is well known that extracellular vehicles (EVs) mediate intracellular interactions under physiological and pathological conditions. In other words, EVs transfer biological information to surrounding cells and contribute not only to physiological functions but also to the pathogenesis of some diseases, such as cancers. Therefore, a better understanding of cell-to-cell interactions may lead to identification of potential therapeutic targets. Recent reports have suggested that EVs are evolutionarily conserved constitutive mediators that regulate hematopoiesis. Here, we focus on the emerging roles of EVs in normal and pathological conditions, particularly in hematological malignancies. Owing to the high abundance of EVs in biological fluids, their potential use as biomarkers and therapeutic tools is discussed.

1. Introduction

Hematopoiesis is a regulated biological process that occurs in complex cellular ecosystems. In this complex environment, cellular

* Corresponding author. Molecular Medicine Research Center, Tabriz University of Medical Sciences, Tabriz, 5166614731, Iran.
** Corresponding author. Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
E-mail addresses: montazersahebs@tbzmed.ac.ir (S. Montazersaheb), farahzadir@tbzmed.ac.ir (R. Farahzadi).
[1] These authors contributed equally to this work.

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and noncellular factors communicate to produce all types of blood and immune cells under homeostasis or stressful conditions [1,2]. It is well established that hematopoietic stem cells (HSCs) exhibit multilineage differentiation capabilities [3,4]. HSCs can differentiate into hematopoietic progenitor cell lineages and produce mature blood cells, thereby creating a hematopoietic hierarchy. In light of this, the preservation of hematopoietic homeostasis is related to the normal function of HSCs. A specific microenvironment called the hematopoietic niche is involved in the protection and support of HSCs [5]. Malignant cells can remodel this niche to create a self-sustaining environment to support their growth and inhibit the normal production of hematopoietic cells [6,7]. Understanding the components and dynamics of the niche and its contribution to hematopoietic disorders has expanded considerably in recent decades [8]. It is now evident that HSCs function is influenced by active intercellular interaction between niche cells and HSCs. Although human and murine HSCs have different immunophenotypic surface markers, they exert similar activities under strict control of their microenvironment [9].

A growing body of evidence indicates that extracellular vesicles (EVs) play key roles in intercellular signaling and cell-to-cell communication (Fig. 1A–C) [10,11]. Indeed, by releasing these mediators from parental cells, EVs carry biological cargo such as RNA, proteins, lipids, and some metabolites into body fluids [10,12]. The behavior of recipient cells can be altered by the recognition and internalization of EVs, there is a continuous and organized process in the biogenesis, regulation, and heterogeneity of EVs. Due to technical limitations, it has been challenging to identify the precise subcellular origin of EVs; thereby, the term “exosome” is used [13].

2. Extracellular vesicles

2.1. Structure and classification

EVs are membrane-enclosed structures of varying sizes between 30 and 10000 nm. These lipid bilayers of cell-derived microparticles mediate local and distant communication between cells [11]. EVs can be divided into four subtypes based on size and release procedure. Microvesicles (MVs) and exosomes are two essential types of EVs. The size of MVs (intermediate-sized EVs) is more than 100 nm they are released from the plasma membrane, while the size of exosomes (the smallest type of EVs) varies between 30 and 100 nm and is generated via the fusion of endosomes with the plasma membrane [14]. These molecules can transmit multiple biomolecules between cells, thus affecting many physiological and pathological processes in both the recipient and donor cells. The other two classifications of EVs are large vesicles and apoptotic bodies with a size of >1000 nm [15]. Although platelet-derived vesicles were first discovered by electron microscopy more than 50 years ago [16], the full spectrum of EVs types and functions of EVs have become a

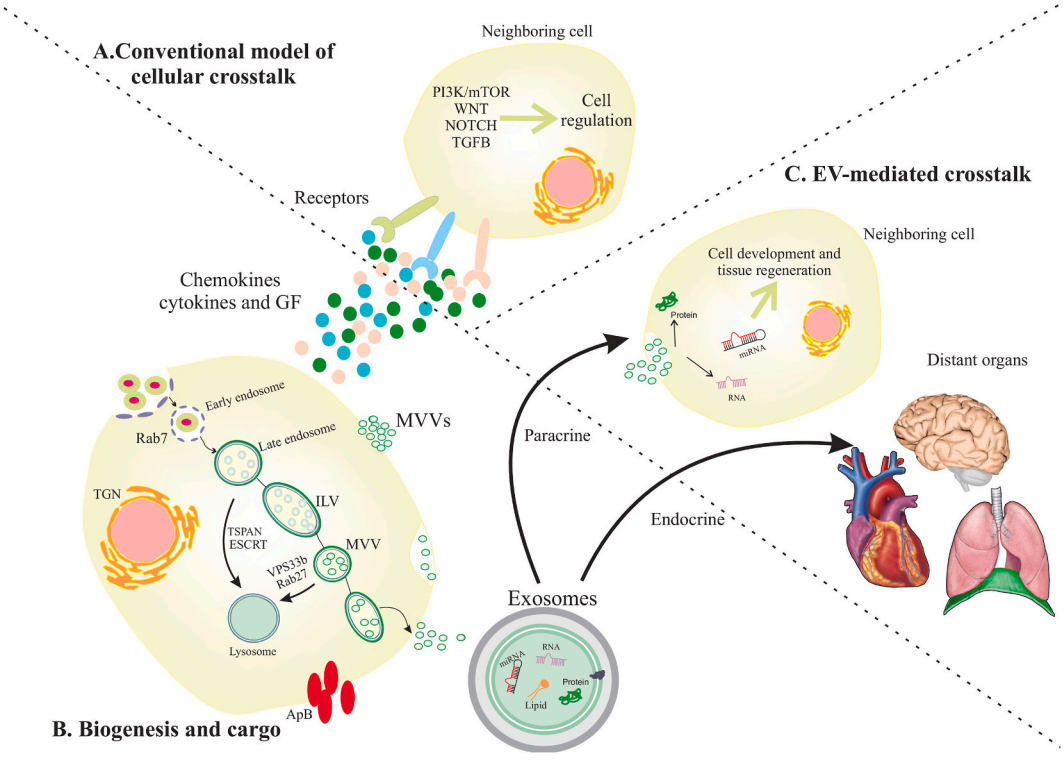

Fig. 1. The process of extracellular vesicle (EV) biogenesis and distinctive features of their transport. (A) Traditional cellular communication involves interactions between receptors and ligands. (B) Exosomes are produced as early endosomes mature into late endosomes containing Rab7, resulting in the formation of intraluminal vesicles. (C) EV-mediated communication involves the transfer of vesicle-associated proteins, lipids, and RNA components to neighboring cells or distant organs. This figure adapted from Fig. 1 of reference number 11.

major research topic. In the early 1980s, it was revealed that sheep reticulocytes released transferrin receptors in their EVs during red cell enucleation and maturation, which reflects cellular waste [17].

2.2. Concise overview of the role of EVs

Recent studies have found that EVs are substantially involved in the regulation of hematopoiesis, and the activation of immune cells and act as crucial mediators of homeostasis [18]. In the case of cancer, EVs can alter the tumor stroma and angiogenic factors by influencing gene expression, resulting in cancer development and metastasis. It has been found that the transmission of biomolecules by EVs is involved in pathogenic events during the progression of hematological malignancies [19].

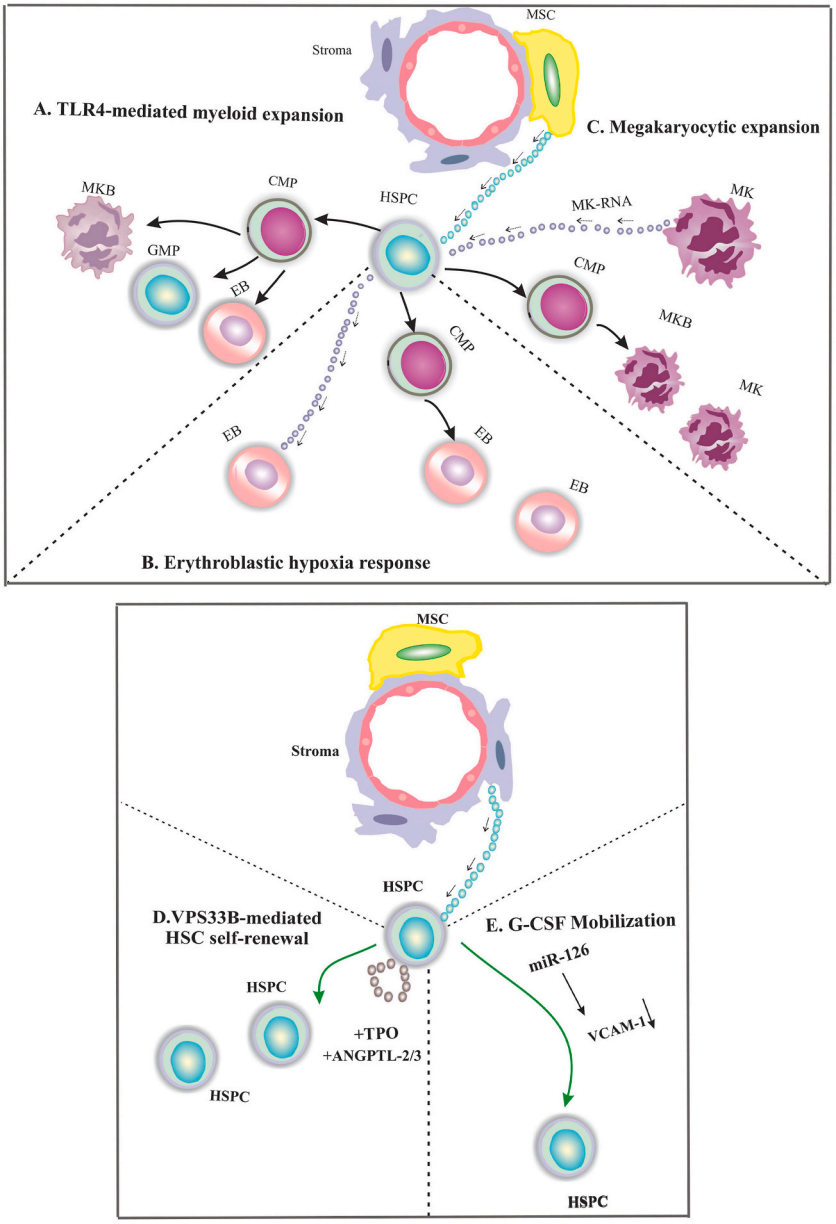

Fig. 2. The existing evidence of extracellular vesicle (EV) communication within the balanced bone marrow microenvironment. (A) Extracellular vesicles derived from mesenchymal stem cells (MSCs) transmit signals to hematopoietic stem/progenitor cells (HSPCs) via the TLR-4 pathway. (B) Erythroblastic hypoxia response. (C) Macrovesicles (MVs) from megakaryocytes are taken up by HSPCs. (D) HSPCs self-regulate their stem cell potential by packaging and releasing essential secretory proteins through the exosomal pathway. (E) Infusion of granulocyte colony-stimulating factor (G-CSF) triggers the release of EVs with miR-126, which down-regulates vascular cell adhesion molecule-1 (VCAM-1) in HSPCs. This figure adapted from Fig. 2 of reference number 11.

In the context of leukemic bone marrow (BM), EVs can affect niche cells to promote cancer progression and metastasis. While existing literature indicates that acute myeloid leukemia (AML) cells alter the BM environment, diminishing its capacity to support hematopoietic stem/progenitor cells (HSPCs) and normal hematopoietic function, the precise mechanism through which leukemic cells modify the normal functions of HSPCs remains unclear [20,21].

Essentially, extracellular vesicles (EVs) have the ability to impact the function and behavior of recipient cells by transporting bioactive contents [22]. Specifically, EVs derived from neoplastic cells can enhance cancer growth and disturb the balance of healthy tissues [23]. In hematologic cancers, neoplastic EVs play a role in advancing tumor development by fostering self-sufficiency through an autocrine loop, thereby heightening aggressiveness [22]. Furthermore, studies have shown that EVs released by AML cells may play a role in regulating BM function [24]. In the context of leukemia, EVs from AML have the ability to reprogram mesenchymal stem cells (MSCs) and stromal cells, leading to the suppression of the niche retention factor stromal cell-derived factor 1 (SDF1). This process results in the release of HSPCs from the BM. Both AML and myelodysplastic syndrome cells have been observed to diminish the supportive capacity of MSCs for hematopoiesis by transmitting miR-7977 through EVs [25]. Furthermore, studies have indicated that exosomes, small EVs in the nanometer range, can transfer cargo from AML cells to BM stem cells, contributing to the establishment of a leukemia-supportive microenvironment in the BM [26].

EVs are also involved in drug resistance and hypercoagulable state in hematological malignancies, which may be partly attributed to the enhanced number of EVs. Collectively, it can be declared that hematological malignancies are associated with EVs-mediated mechanisms, such as EVs trafficking, disruption of homeostasis, chemotherapeutic resistance, invasion, and metastatic behaviors [27,28].

3. Hematopoiesis

Hematopoiesis is tightly regulated by specialized microenvironments in the BM, which are composed of hematopoietic and non-hematopoietic cells. This microenvironment provides a dynamic regulation of hematopoiesis to ensure that mature blood cells are formed from HSCs and function properly [29]. Most HSCs are maintained in a quiescent or dormant state. The emergence of HSCs from a quiescent state to an activated state can be triggered by alterations in oxygen levels, hemorrhage, and chemotherapy and radiotherapy. Much evidence suggests that EVs contribute to the regulation of BM function during homeostasis and injury (Fig. 2 A, B, D, E) [11,30]. The involvement of EVs-derived from several cells has been confirmed in the regulation of HSPCs and HSCs. Additionally, recent evidence reported the potential role of EVs as physiological mediators for signaling and communication across the immunological synapse [31].

4. Bone marrow niche

The BM niche refers to the structural environment found within the trabecular spaces of spongy bones and the cavities of long bones. Here, HSCs preserve their undifferentiated state and ability for self-renewal through the involvement of vascular and nervous networks, metabolic pathways, transcriptional and epigenetic regulators, as well as hormonal signals. Within this niche, HSCs engage with various cell types like osteoblasts, endothelial cells, macrophages, and MSCs. These interactions either maintain HSCs in a dormant state or support their proliferation, differentiation, and movement based on the body's requirements. Under normal circumstances, the BM niche facilitates the daily generation of all blood and immune cells, allowing their entry into the bloodstream. Nevertheless, disturbances to this intricate microenvironment can trigger the development and advancement of malignancies like those found in myeloid neoplasms. Additionally, such disruptions can enhance resistance to conventional pharmacological treatments. Changes in the MSC population and their communication with HSCs due to factors originating from tumors play a role in creating a cancerous environment. Growing evidence indicates that modifications in the composition of the niche are linked to various malignancies such as myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN). These alterations also impact the osteohematopoietic niche, which can be influenced by medical interventions [32,33].

Subsequent investigations that specifically target the various cell types within the niche have concentrated on myeloproliferative disorders, specifically MPN [34], AML [35], and chronic myeloid leukemia (CML) [36].

Inflammation triggers a "neuropathy" within the BM in several malignancies like AML and other MPNs. The persistently active inflammatory state disrupts the interaction between glial cells and MSCs, potentially leading to the development of leukemia, MPNs, or BM fibrosis [36]. Therefore, targeting the dysregulation of the neural niche appears to be a promising therapeutic approach that could impact inflammation. Ongoing research is investigating the use of a Beta3-adrenergic agonist, neural-glial protection, and an IL-1R antagonist [36]. The presence of fibrosis in the BM microenvironment signifies significant alterations in the surroundings and is associated with metabolic disorders, hematological and solid conditions, infections, and immune-related issues, the aging process [37]. To elaborate further, a more substantial side population of Gli1-$^{+}$ cells, which play a role in the restructuring of bone under normal circumstances, is also situated close to the vascular regions [38]. When fibrosis is induced in animal models, these cells are triggered from their typical niche and undergo rapid expansion in BM fibrosis. Remarkably, the removal of Gli1^{+} cells alleviates BM fibrosis.

5. The role of extracellular vesicles in regulating hematopoiesis

However, little is known about how vesicles are involved in hematopoiesis during steady state or BM regeneration. The release of EVs is strongly influenced by a variety of cellular stimuli such as cytokines, irradiation, and tissue oxygen tension. An example of a stimulus that enhances the release of vesicles from hematopoietic progenitors is granulocyte colony-stimulating factor-induced

mobilization. Granulocyte colony stimulating factor (G-CSF) is commonly used for the mobilization of BM progenitor cells of HSCs for transplantation in the treatment of hematological malignancies. Indeed, G-CSF mobilizes HSCs into the blood by influencing niche dynamics and bone formation by specific macrophages within the BM [39]. As a common mobilizer, G-CSF can increase the harvest of HSPCs within BM and peripheral blood, promoting myeloid hematopoiesis and granulopoiesis [40,41]. However, the mechanism by which G-CSF regulates hematopoiesis remains controversial.

EVs are released following injury and may assist in miRNA and other cargo delivery [42]. The majority of studies on EVs function have focused on miRNAs, proteins, exosomal lipids, and non-coding RNAs, which exert important functions. Circulating miRNAs are associated with EVs, proteins, and lipids [43]. This explains the enhanced regenerative and angiogenic capacities of distant tissues after hypoperfusion injury. Angiogenesis is a process that mediates tissue regeneration and functional recovery [44]. Several studies have examined the release and function of EVs by the BM stroma, including MSCs and endothelial cells. Endothelial cells can generate EVs under the pro-angiogenic actions of miR-126 [45]. The proangiogenic effect of miR-126 has recently been established [46]. Besides, it has been revealed that miR-126 can restrain osteogenic differentiation within the BM [47].

Cellular quiescence refers to a halt in the cell cycle that can be reversed by a mix of internal cellular factors and external signals. Within HSCs, a harmonious equilibrium between quiescence and active proliferation is crucial for their long-term survival [48,49]. Earlier research has demonstrated that the transforming growth factor beta (TGF-β) signaling pathway consistently plays a role in regulating the subtype and quiescence of HSCs, primarily by impeding their re-entry into the cell cycle [50]. The TGF-β ligands attach to TGF-β receptors (TGF-β-RII and TGF-β-RI) on the cell membrane, leading to their clustering and triggering the activation of the protein kinase function of the type I receptor. Co-receptors aid in attracting ligands to the signaling receptor. Among these, the most extensively researched co-receptor for TGF-β is TGFβRII. TGFβRIII binds strongly to all three TGF-β ligands and delivers them to the TGFβRI and TGFβRII signaling complex. Once activated, TGFβRI and TGFβRII recruit and activate numerous signaling proteins. A widely recognized target of TGFβRI is the SMAD protein family, which, following phosphorylation, combine with SMAD4, amass in the nucleus, and control gene expression by binding to regulatory regions in the genome alongside other transcription factors and chromatin proteins during HSC quiescence [51]. TGF-β is secreted in an inactive form by various cells, and non-myelinating Schwann glial cells within the BM niche maintain HSC dormancy by regulating the activation of latent TGF-β. Research has indicated that when regulatory molecules for TGF-β were identified in EVs, they triggered downstream Smad2/3 signaling in target cells [52]. EVs obtained from MSCs extracted from human BM enhanced the ex vivo proliferation of HSCs. Among the contents found in abundance within these EVs, researchers identified numerous proteins associated with the TGF-β pathway, including Smad.

5.1. MSC-derived EVs

There are many studies on the release and function of MSC-EVs [53,54]. EVs trafficking from BM-derived MSCs to HSCs affects progenitor cell commitment. Goloviznina et al. (2016) reported that MSC-released EVs contribute to paracrine crosstalk, which can affect hematopoietic function [55]. They exposed c-Kit-enriched murine HSPCs to concentrated EVs *in vitro*. A significant expansion of the HSPCs with markers of KSL c-Kit$^+$, Sca-1$^+$, and lineage$^-$ compartments was observed following EVs exposure. MSC-released EVs induce HSPC activation and differentiation through a MyD88 (myeloid differentiation factor). Indeed, EVs trafficking to HSPCs elicited myeloid-biased progenitor expansion via Toll-like receptor (TLR) and MyD88 mechanisms. This process may be abrogated in mice lacking TLR4 or MyD88 [56].

MSC-derived EVs (MSC-EVs) can also regulate the angiogenic capacity of endothelial cells, implying that MSC-EVs can regulate specific cells within and/or outside the hematopoietic niche [57]. The impact of EVs on the hematopoietic system is not restricted to the supportive role of the stromal cells. Megakaryocytes also play a regulatory role in HSPCs via microvesicle release. Megakaryocytic microparticles are the most abundant microparticles in circulation. The surface molecules that mediate HSPC uptake include CD54 (ICAM-1), CD18, CD43, and CD11b. Upon cell-surface interaction, the microvesicles are internalized by endocytosis. Direct fusion is another mechanism involved in the cargo delivery of microparticles to HSPCs. In addition, lipid raft-mediated macropinocytosis contributes to the uptake of megakaryocytic microparticles by HSPCs. The internalization of these megakaryocyte microparticles could redirect HSPCs differentiation toward functional megakaryocytes with high specificity and limited effects on the phenotype of other related cells (e.g. stromal cells and endothelial cells) (Fig. 2C) [58].

5.2. EVs-derived miRNAs

It has been found that microRNAs (miRNAs) are important regulators of hematopoietic cell differentiation. In better words, each stage of hematopoietic differentiation can be identified by a specific miRNA signature [59]. Several reports have shown that EVs-derived miRNAs (EVs-miRNAs) play a significant role in regulating the hematopoietic fate in terms of HSPC differentiation. In other words, EVs can regulate stem cell fate by transferring bioactive components including miRNAs [25]. Another study reported that miR-486, a known regulator of erythroid differentiation, responds to hypoxic conditions in erythroleukemia cells. Sirt1 as a target gene of miR-486 can regulate hypoxia-induced erythroid differentiation in erythroleukemia cells by targeting Sirt1 [60]. Previous studies have demonstrated that mir-486 regulates normal erythropoiesis in CML progenitor cells. miR-486 was considerably upregulated in CML CD34$^+$ cells compared with normal CD34$^+$ cells. In contrast, suppression of miR-486-5p inhibited the growth of CD34$^+$ cells *in vitro* and *in vivo* and reduced the differentiation and survival of erythroid cells. There might be a similar mechanism modulating hypoxia-responsive erythropoiesis for increasing oxygen delivery to hypoxia tissues [61]. *Ex vivo* expansion of HSPCs without affecting their self-renewal potential would significantly improve the efficacy of hematopoietic stem cell transplantation (HSCT) in clinical settings. HSCT has been applied for the treatment of hematopoietic diseases and immune disorders, and various reports have

confirmed the value of EVs in achieving this goal [62]. Morhayim et al. (2016) demonstrated that human osteoblast-derived EVs comprise a large number of miRNAs that may have regulatory functions in hematopoietic development [63]. Treatment of HSPCs with osteoblast-EVs alters the expression levels of some mRNAs targeted by osteoblast-derived EVs miRNAs. As reported, mir-29a plays a crucial regulatory role in the hematopoietic system and is highly enriched in osteoblast-EVs. miR-29a targets genes responsible for apoptosis (BCL2), proliferation (PTEN), and cell cycle regulation (HBP1). EVs treatment of $CD34^{+}$ HSPCs altered the expression of miRNA targets such as BCL2, PTEN, and HBP1. Altogether, osteoblasts-EVs can secrete miRNA that is necessary for the expansion of human umbilical cord blood cells [64].

A growing body of evidence shows that EVs within the BM niche can modulate HSCs behavior in different ways. Treatment with G-CSF, a mobilizing agent of HSPCs for transplantation purposes, increased EVs containing miR-126 within the BM. By internalizing these EVs into the stroma, HSPCs, and endothelial cells, miR-126 is delivered into the cells, where it suppresses the expression of vascular cell adhesion molecule-1(VCAM-I). As a result of the reduction of VCAM-I, HSPC adhesion is decreased and blood is moved to the peripheral circulation for leukapheresis [65]. Human $CD34^{+}$ cell-derived EVs are enriched with miR-126. In experimental models, the release of miR-126 from EVs of mobilized human CD34 cells could promote ischemia repair by proangiogenic activity [66].

Another study by Davis et al. (2017) showed that during aging, the miRNA content of EVs might be altered within the BM niche and contribute to age-induced stem cell dysfunction. Based on their investigation, EVs from both young and aged mice are highly expressed in miRNAs; however, the miRNA profile of BM-derived EVs (BM-EVs) differs between these two groups. For instance, the miR-183 cluster is highly enriched in aged EVs. *In vitro* studies have revealed that aged EVs can be endocytosed by primary BM stromal cells, reducing cell proliferation, inhibiting osteogenic differentiation, increasing senescence, and decreasing the expression of the target protein of miR-183-5p, heme oxygenase-1 (Hmox1), an essential enzyme in the heme catabolic pathway. Together, microRNA-183 elevates with age in BM-EVs and suppresses the proliferation of BM stromal cells, inducing stem cell aging [67].

In addition, secretory signals and autocrine loops play critical roles in the maintenance of HSCs stemness. Gu et al. (2016) provided evidence that several secretory proteins produced by HSCs undergo a process of maturation by exosomes that is regulated by vacuolar protein sorting protein 33b (VPS33B). When human or mouse HSCs were depleted of VPS33B, exosome maturation and secretion were impaired and stemness was lost. Notably, VPS33B deficiency causes a remarkable delay in leukemogenesis in cancer models. According to their findings, VPS33B has a crucial function in exosome pathways in HSCs and leukemia-initiating cells [68]. Collectively, it can be concluded that HSPCs release and internalization of EVs are regulated by vesicle trafficking in the physiological BM microenvironment for the maintenance of hematopoiesis.

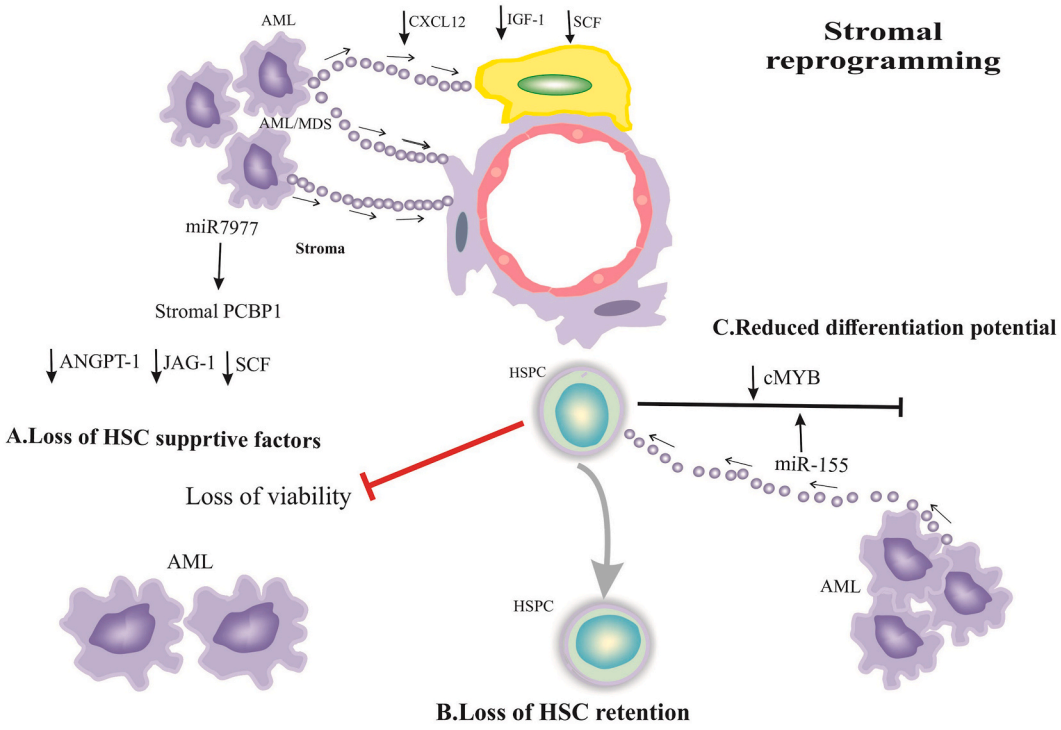

Fig. 3. Current evidence for extracellular vesicles (EVs) crosstalk in the leukemic microenvironment. (A) AML and myelodysplastic syndromes (MDS)-EVs promote the loss of HSPCs supportive factors, C-X-C motif chemokine ligand 12 (CXCL12), stem cell factor (SCF), Insulin-like growth factor 1 (IGF-1). (B) AML EVs reprogram mesenchymal stem cells (MSCs) and stromal cells, and downregulate niche retention factor CXCL12. (C) EVs from acute myeloid leukemia (AML) blasts traffic miR-155 to hematopoietic stem/progenitor cells (HSPCs). This figure adapted from Fig. 3 of reference number 11.

6. Potential implication of EVs in the pathophysiology of hematopoiesis

In addition to EVs' important role in cellular interaction within BM under physiological states, EVs trafficking exert a significant role in the deregulation of hematopoiesis in some disease such as hematological malignancies and extramedullary cancers [19]. For instance, it has been found that MSC-EVs were capable of repairing irradiation-induced marrow injury. Intravenous administration of MSC-EVs to mice exposed to 500 cGy resulted in significant restoration of BM stem cell engraftment, as well as a partial recovery in the peripheral blood count. These findings implied the potential effect of MSC-EVs in reversing radiation-induced damage to BM stem cells [69].

EVs also contribute to the pathology and progression of hematological malignancies such as acute leukemia. It is well known that patients suffering from AML experience the impaired generation of normal blood cells from HSCs in the BM, thereby there is a need for BM transplants [70]. It has been reported that EVs released from AML cells can inhibit the function of HSPCs indirectly by reprogramming the stem cell niche. Indeed, the impaired function of hematopoiesis is partly attributed to the consequence of AML-derived exosomes containing miRNAs, such as miR-150 and miR-155, targeting c-MYB. c-MYB is a known transcription factor involved in HSPC proliferation and differentiation. Indeed, there is a direct impact of AML EVs on HSPC function mediated by a stroma-independent mechanism through exosome trafficking of regulatory agents within the tumor microenvironment (Fig. 3A–C) [11,71]. Other studies have also provided evidence that AML EVs downregulate the expression of CXCL12 (as an HSC-supporting molecule) and suppress hematopoiesis, osteolineage development, and bone formation through overexpression of Dkk1 (a suppressor of normal osteogenesis and hematopoiesis) in BM stromal cells. AML blasts can remodel the BM niche to leukemia growth-permissive by EVs secretion [72].

Another similar report by Trino et al. (2022) revealed that by releasing EVs from AML cells, normal hematopoiesis is suppressed, creating a favorable niche for neoplastic development [73]. EVs released from CML cells are also involved in modifying the BM microenvironment by stimulating epithelial growth factor receptor (EGFR) signaling in stromal cells. There is an altered interaction between CML and MSCs that affects leukemia survival. EVs released by tumor and non-tumor cells into the BM environment provide an appropriate niche for cancer progression. EVs originating from patients with CML contain a high amount of amphiregulin (AREG), an agent that induces the EGFR pathway in stromal cells. Upon activation of EGFR signaling cascades, the expression of the transcription factor Snail and its target genes, interleukin-8 (IL-8) and matrix metalloproteinase-9 (MMP9), were upregulated (two known genes regulated by Snail). In this context, EVs promote the proliferation, survival, and invasiveness of leukemic cells [74].

Multiple myeloma (MM) is one of the most incurable hematological malignancies, and is characterized by clonal plasma cells in the BM. It has been shown that MM patients had more EVs enriched with CD38 and CD9 markers. EVs isolated from MM patients exerted procoagulant function visualized by an enhanced level of thrombin and increased procoagulant phospholipids (PPL) and tissue factor (TF) that have a role in thrombogenesis. In contrast, another study by Razmkhah et al. (2017) revealed that AML EVs increased HSCs numbers while preserving their stemness and clonogenicity. In this regard, they evaluated leukemic EVs in healthy umbilical cord blood HSCs. EVs isolated from patients with AML were co-cultured with healthy HSCs. According to their results, there was a large number of HSCs after incubation with leukemia EVs compared to the control group. In addition, microRNA-21 and microRNA-29a were upregulated in the co-culture group, while preserving colony-forming ability and stemness signs (for example, $CD34^{+}$, $CD34^{+}CD38^{-}$, $CD90^{+}$, and $CD117^{+}$ phenotypes, as HSC-specific CD markers). Together, leukemia EVs promoted cells in healthy HSCs [75].

The release of cytokines by steady-state or reprogrammed malignant cells has been previously demonstrated. As mentioned, EVs contain critical mediators involved in immune-related pathologies [76]. EVs in the lungs carry cytokines that have critical functions in crosstalk between immune cells. For instance, in mouse models of lung disease, EVs released into the airway enriched with pro-inflammatory cytokines such as IL-1β and IL-18 [77]. In the case of hematological malignancies, EVs also contribute to the cross-talk between the immune system and. malignant cells. AML-derived EVs contain high amounts of the immune suppressor agent TGF-β1 [78]. Overall, malignant cell-derived EVs can facilitate escape from immune surveillance in the tumor niche of most hematological malignancies [27]. In light of the fact that most cytokine analyses do not distinguish vesicle-bound from vesicle-free cytokine activity, hence cytokine activity that regulates HSPC function can reflect EVs-mediated trafficking [11].

Other hematologic disorders have been found to affect the hematopoietic process by altering the activity of non-hematopoietic stromal cells. Analysis of EVs-miRNAs revealed that the amount of miR-7977 in AML cells was higher than that in normal $CD34^{+}$ cells. Notably, miR-7977 copy number in BM was increased in AML and MDS. The transfection of the miR-7977 mimics could reduce the expression level of poly(rC) binding protein 1 (PCBP1) in MSCs. Moreover, the miR-7977 mimic elicited an aberrant decline in hematopoietic growth factors in MSCs, leading to a reduced hematopoietic supporting impact of BM $CD34^{+}$ cells. In addition, the aberrant reduction of hematopoietic growth factors, such as Jagged-1, and angiopoietin-1 was ameliorated by target protection of (PCBP1), indicating the potential role of PCBP1 in stabilizing growth factors. Together, miR-7977 in EVs can induce failure of normal hematopoiesis through PCBP1-mediated suppression [79]. MSCs isolated from MDS patients can release EVs that mediate the trafficking of miR-10a and miR-15a to $CD34^{+}$ progenitor cells. This can regulate the transcription of P53 and MDM2 genes, affecting the viability and clonogenicity of HSPCs. Collectively, EVs play a pathophysiological role in hematological malignancies.

7. Using EVs in the treatment of hematologic disorders

As discussed earlier, HSCs transplantation is an effective therapeutic modality for some chronic diseases such as hematopoietic diseases [80]. However, the lack of an efficient *in vitro* approach for expanding HSCs and the difficulty in collecting sufficient HSCs from the BM have impeded the clinical use of HSC-based interventions. In other words, clinical transplantation of HSCs is limited by

several factors, such as the low number of isolated HSCs in patients [81]. EVs are considered an attractive and possible approach for the application of novel therapeutics in immunotherapy. Therapeutic application can be exogenous or autologous administration of EVs to the desired site in conjunction with chemotherapeutics or immunotherapy. Moreover, EVs can be applied as therapeutic targets [82]. Given that EVs levels are often enhanced in parallel with cancer severity, decreasing circulating EVs to normal values would be a therapeutic approach. With this view, many investigations are designed to inhibit EVs-mediated communications: reducing the biogenesis and release of EVs; blocking the uptake of EVs by target cells; disturbing their path in target cells; and eliminating EVs from circulation [83].

Some reports have indicated that EVs released by tumor cells contain specific antigens that may display immunotherapeutic activities. In this regard, EVs isolated from lymphoma cells express some proteins involved in antigen presentation, indicating the potential role of lymphoma cell-derived EVs in immune system regulation along with communication between lymphoma cells and their microenvironment [84].

When using EVs as an immunotherapeutic modality, it is crucial to underline the function of MSC-EVs. MSC-EVs can improve the engraftment of stem cells during allograft transplantation. In this setting, BM-MSC-EVs can modify the phenotype of $CD34^{+}$ HSCs and enhance their migration from circulation to BM in an animal model [85].

Autologous EVs from expandable cells *in vitro*, such as MSCs, NK cells, and T cells, can be used as promising therapeutic agents for EVs. *In vitro* studies have demonstrated that human MSC-EVs shuttle miR-155 and miR-146, which suppresses NK-, B-, and T-cell activity [86]. In a murine model, MSC EVs harboring CD73 could efficiently restore graft-versus-host disease by promoting the metabolism of adenosine. As a result, Th1-induced inflammation was suppressed. In fact, pathogenic Th1 cells exposed to adenosine show a remarkable decline in the expression of CD39 (linked to pathogenic Th1 cells), undergoing apoptosis [87]. Another study used MSC-EVs for the treatment of graft-versus-host disease (GVHD) (a complication of allogeneic HSCT) since prior work reported therapeutic application of pooled MSCs in GVHD [88]. This can be attributed to anti-inflammatory cargo of EVs such as interleukin-10 (IL-10), TGF-β, HLA-G, and, IDO, thereby EVs administration led to a significant decline in an inflammatory response in patients [89].

It has been found that Rab27a and Rab27b double-knockout mice with deficiency in exosome secretion, exhibited chronic inflammation characterized by an elevated level of inflammatory cytokines. This inflammatory phenotype was ameliorated in these mice by grafting wild-type HSCs or generating EVs from GM-CSF–expanded wild-type HSCs. In addition, double-knockout mice showed blunted responsiveness to bacterial lipopolysaccharide (LPS), displaying endotoxin tolerance. This defect could be restored by exposure to wild-type HSCs-EVs but not EVs isolated from miR-155−/− cells. This finding indicates that the uptake of miR-

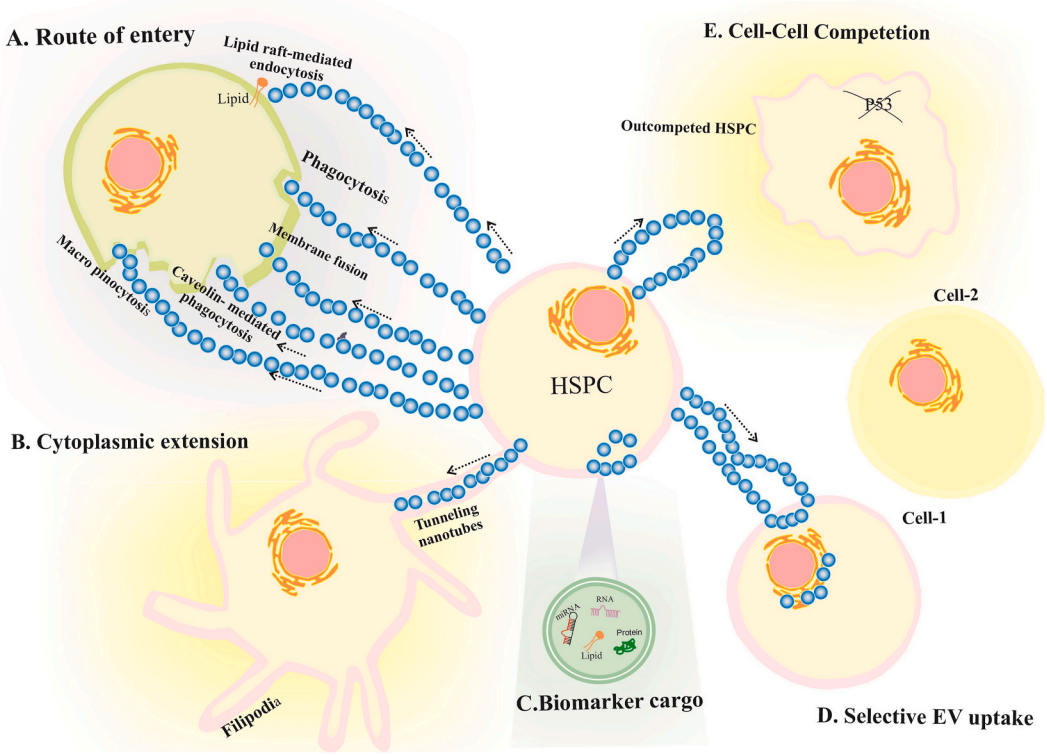

Fig. 4. Unresolved aspects of extracellular vesicle (EV) biology in the regulation of hematopoiesis. (A) EVs enter recipient cells. (B) Cytonemes as cytoplasmic extensions are considered exosomal transfer modes to adjacent bystander cells. (C) Vesicles consist of cargo including uniquely packaged miRNAs proteins, and RNAs regarding as promising biomarkers for disease diagnosis. (D) Vesicles from hematopoietic stem/progenitor cells (HSPCs) as well as other cells of the bone marrow niche show preferential targeting to certain recipient cells for entry. (E) Exosome-associated crosstalk can explain the intercellular competition of neighboring cells. This figure adapted from Fig. 4 of reference number 11.

155–containing EVs is important for producing a proper response to LPS. Together, Rab27-dependent EVs production contributes to maintaining homeostasis within the hematopoietic system as well as providing an appropriate response to inflammatory stimuli [90].

8. Using EVs in the diagnosis of hematologic disorders

In diagnostic applications, EVs are regarded as strong diagnostic biomarkers for several malignancies due to their potential to carry biologically active cargo that represents the status and nature of the cells' origin [91]. As EVs are small and rapidly equilibrate between tissues and the bloodstream, there is great interest in EVs as potentially minimally invasive biomarkers. EVs-based detection can be employed to identify disease-specific markers, particularly in hematological neoplasms (Fig. 4A–E) [11,92]. In hematological malignancies, EVs can be used as a liquid biopsy method because of their high abundance in biological body fluids. In the case of hematological disorders, there is a lack of appropriate monitoring tools that make diagnosis and treatment difficult. Monitoring hematological malignancies is a crucial step in choosing the most appropriate intervention. Because of a lack of adequate monitoring tools concerning other malignancies, there is a need to develop novel non-invasive tools applicable in the clinical setting [93,94]. For this reason, non-invasive clinical modalities such as liquid biopsy are being developed that enable proper diagnosis and monitoring of disease progression and the effectiveness of treatment [95]. Indeed, liquid biopsies are considered noninvasive diagnostic tests that provide clinical molecular signatures. In other words, EVs provide accurate diagnostic tools to distinguish biomarkers for monitoring treatment response and disease progression [27].

As discussed in previous sections, EVs are present in a wide range of biological fluids; therefore, they may serve as accessible diagnostic biomarkers for diagnosing many pathological conditions, including cancers. Noteworthy, the level of EVs in circulating blood is correlated with prognosis, thereby, the potential use of EVs as biomarkers in cancer has been addressed in recent years [96]. Additionally, exosome-loaded miRNAs have been suggested as prognostic and diagnostic markers of cancer [97], including colorectal cancer [98], breast cancer [99], lung cancer [100], ovarian cancer [101], bladder cancer [102], etc. In addition to miRNAs, exosomal long noncoding RNAs (lnc RNAs) have been developed as new tumor biomarkers for cancer patients [103]. Several lines of evidence have revealed that circulating EVs can be used as an analytical platform for miRNA profiles and protein content to diagnose and classify hematological malignancies and nonmalignant hematopoietic disorders [104].

In a study conducted by Caivano, serum EVs were harvested from various kinds of hematological neoplasm including, MM, AML, chronic lymphocytic leukemia (CLL), Hodgkin's lymphoma (HL), non-Hodgkin's lymphoma (NHL), MPNs, Waldenstrom's macroglobulinemia (WM), MDS, as well as healthy individuals as the control group. According to their results, blood EVs showed enhancement in most patients with hematological neoplastic disorders compared to healthy subjects [105]. In supporting these findings, in AML, EVs carry important diagnostic markers, such as FMS-like tyrosine kinase 3 (FLT3)- internal tandem duplication (ITD) and nucleophosmin 1 (NPM1) mRNAs [106]. In addition to their role in diagnosis, cancer cell-derived EVs contribute to AML tumorigenesis. In this regard, plasma levels of EVs are significantly higher in newly diagnosed AML patients than in healthy controls [107].

In newly diagnosed patients with AML, serum EVs were enriched with myeloblastic markers, such as CD33, CD34, and CD117, as well as genes involved in MHC class I-related genes (MICA and MICB) and the TGFβ1 pathway. EVs-derived TGFβ1 (EVs-TGFβ1) levels were considerably elevated in most patients with AML. Additionally, the plasma EVs-TGFβ1 range readily distinguishes AML patients those from in early and late remission states. EVs-TGFβ1 levels are inversely correlated with chemotherapeutic responses. These data address the importance of plasma EVs-TGFβ1 amounts as an indirect evaluation method for leukemic blasts in the BM, hence, EVs-TGFβ1 alteration could be a potential diagnostic marker in these kinds of patients [78,108]. Some patients with MM develop multidrug resistance (MDR). In a recent study, Krishnan et al. (2020) used liquid biopsy to monitor MDR and disease progression in MM. EVs differ in the expression of protein biomarkers, including P-glycoprotein (P-gp) as an MDR protein, stem cell marker (CD34), plasma cell marker (CD138), and phosphatidylserine (PS). $CD138^{+}$ circulating EVs gradually increased with disease progression. Patients with aggressive MDR exhibit an increase in specific EVs populations containing stem cell markers and P-glycoproteins ($CD138\text{-}P\text{-}gp^{+}CD34^{+}$). Dual-positive ($CD138^{-}P\text{-}gp^{+}CD34^{+}$) cells can serve as a screening marker for aggressive and unresponsive forms of the disease. This liquid biopsy can address the clinical need for diagnosing MDR and treatment failure in MM [109]. Similarly, Harshman et al. (2016) reported that CD44 is highly enriched in newly diagnosed MM patients [110].

Belov et al. (2016) provided evidence by antibody microarray of the surface protein content of plasma EVs isolated from patients with CML, displaying strong expression (CD19, CD31, CD44, CD55, and CD62L), moderate expression of CD5, CD82, HLA (-A, -B, –C), and HLA-DR, and low levels of CD21, CD49c, and CD63. None of these markers was distinguished on plasma EVs from the healthy individuals with age- and gender-matched conditions [111]. In a similar study by Luca et al. (2017), 131 newly diagnosed CLL cases were analyzed. Compared with healthy controls, there was an increased number of CLL EVs in the serum, particularly in the advanced stages of the disease. In addition, a correlation was observed between $CD19^{+}$ and $CD37^{+}$ B cell-derived MV with a high tumor burden. Collectively, circulating EVs offer a prognostic biomarker in CLL [112]. There is increasing evidence that EVs-miRNAs could be used as minimally invasive biomarkers for the early detection of hematological malignancies. Hornick et al. (2016) identified a set of miRNAs highly expressed in AML exosomes [71]. Given that chemotherapy effectively eradicates peripheral AML blasts, most treatments fail to eliminate residual leukemic cells within the BM. With this notion, the detection of serum EVs-miRNAs could circumvent the need for invasive BM aspiration and reliance on the presence of leukemic blasts in the periphery. Surprisingly, leukemic blasts and marrow stromal cells were involved in serum EVs-miRNAs. As a result, development of serum EVs-miRNAs can be a novel, sensitive biomarker for early detection and tracking of AML recurrence [113]. It has been found that miR155 plays a substantial role in the pathogenesis of several hematological malignancies. Up-regulation of miR155 has been identified in some hematological malignancies. Accordingly, miR155 in serum-derived EVs may serve as a promising non-invasive diagnostic biomarker in human hematologic malignancies [114].

In addition to hematologic malignancies, EVs can also be helpful in prognostic performance for benign hematological disorders, such as sickle cell anemia. It is an inherited hemoglobin disorder caused by a point mutation. A distinctive miRNA signature was detected in plasma EVs of sickle-cell patients that distinguished them from healthy donors that coincides with the stage of the patients' disease [115]. Aplastic anemia (AA) is a non-malignant hematological disease characterized by BM hypoplasia and pancytopenia. Soomro et al. (2022) analyzed the circulating miRNA profile in the plasma samples of aplastic anemia. According to their findings, distinct miRNA signatures were identified that could serve as novel biomarkers in patients with aplastic anemia [116]. Many believe that EVs-miRNAs, as hematologic biomarkers, can play a biological role in cell-cell crosstalk between distant tissues and cells. Beyond this, deliberate secretion of protein and RNA into EVs may impede the regulation of the cell of origin.

9. Conclusion

EVs play an important role in pathophysiology, and reducing their release during disease states may provide therapeutic benefits. Despite the existence of several molecular mechanisms involved in EVs release, wide suppression of EVs release would not be a beneficial therapeutic approach, considering the role EVs play in maintaining homeostasis in the body. To develop more targeted and disease-specific strategies, a nuanced understanding of EVs-recipient cell affinity, cargo incorporation, and biogenesis is needed. The identification of EVs surfaces that regulate target cell specificity and uptake routes is crucial for mapping the role of EVs in hematopoiesis regulation. Additionally, classifying these surface mediators may aid in harnessing the potential of EVs to deliver therapeutics within the BM or blocking cancer-derived EVs action to enhance the treatment of hematological disease. In addition, little is known about intracellular events following EVs uptake. To realize the therapeutic benefits of EVs, it is important to answer questions concerning the miRNA copy number per vesicle, cargo unloading, intracellular processing, and degradation of EVs. As a result, understanding how different cargoes of EVs cooperatively alter the fate of a cell, and whether EVs with the same cell origin regulate multiple types of cells within the BM niche will help in creating realistic therapeutics. In summary, EVs offer novel insights into the regulation of HSCs as well as translational opportunities to alleviate injury and malignancy. Because of the potential of EVs to regulate cell populations within the BM niche and considering their unique signatures, EVs could be a beneficial tool in the field of hematology. From this perspective, EVs can be used for diagnosis, disease monitoring, and therapeutic applications in the hematological field.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

CRediT authorship contribution statement

Ezzatollah Fathi: Writing – review & editing, Writing – original draft. **Behnaz Valipour:** Methodology. **Sevda Jafari:** Methodology. **Abdolhassan Kazemi:** Writing – review & editing. **Soheila Montazersaheb:** Writing – original draft. **Raheleh Farahzadi:** Writing – review & editing, Writing – original draft, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Raheleh Farahzadi reports financial support was provided by Tabriz University of Medical Sciences. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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