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Exosomal RNAs in macrophage polarization-mediated resilience to ischemic disease

Jun Huang[1], Yujie Wu[1], Haiyi Liu[1], Yonghui Yuan[1], Chenyang Jing[1], Yang Wang[2,3,*] and Dihan Zhu[1,*]

[1] State Key Laboratory of Natural Medicines, School of Life Science and Technology, China Pharmaceutical University, Nanjing, Jiangsu, China
[2] Key Laboratory of Xinjiang Endemic and Ethnic Diseases, Ministry of Education, Shihezi University School of Medicine, Shihezi, China
[3] NHC Key Laboratory of Prevention and Treatment of Central Asia High Incidence Diseases, Shihezi, China

* Correspondence authors; E-mails: ywang6@arizona.edu (Y.W.); dzhu@cpu.edu.cn (D.Z.).

Highlights:

- Exosome mediate macrophage polarization.
- Macrophage polarization regulates angiogenesis.
- Non-coding RNAs packaged in exosomes contribute to the macrophage induced angiogenesis.

Abstract: The polarization of macrophages towards an anti-inflammatory and/or pro-tissue repairing phenotype has shown promising potential in the treatment of ischemic diseases. Macrophages play a crucial role in promoting the growth of new blood vessels in ischemic tissue by clearing apoptotic debris caused by hypoxia, recruiting immune cells that support tissue repair, and releasing a variety of cytokines and growth factors. However, there is still a significant knowledge gap regarding the effective induction of this specific macrophage polarization. Non-coding RNAs have demonstrated promise in regulating macrophage activity, although there is a need for more efficient delivery system. Exosomes, which are cell-derived extracellular vesicles ranging from 30 nm to 200 nm in size, have emerged as promising carriers of non-coding RNAs for regulating macrophage activity. This review will discuss the important role of macrophage polarization in ischemic diseases and explore the potential of non-coding RNAs delivered by exosomes in modulating macrophage polarization.

Keywords: angiogenesis; exosomes; macrophages; polarization; non-coding RNA

1. Introduction

Ischemic disease is a significant contributor to global mortality and morbidity [1]. Its hallmark feature is the inadequate supply of blood [2]. In recent decades, therapeutic angiogenesis has emerged as a focal

point for addressing this concern [3]. Extensive research has been conducted on the protein/gene approach, the stem/progenitor cell approach, and the subsequent cell-free approach for the treatment of ischemic heart disease.

Exosomes represent a compelling option for cell-free therapeutics due to their ease of isolation and reduced immunogenicity. Exosomes, which typically range in size from 30 to 150 nanometers, are membrane-bound extracellular vesicles (EVs) that are generated in the endosomal compartment of most eukaryotic cells [4]. Exosomes are rich in a diverse array of biological elements derived from their source cells. These elements include proteins (such as adhesion molecules, cytoskeletons, cytokines, ribosomal proteins, growth factors, and metabolic enzymes), lipids (including cholesterol, lipid rafts, and ceramides), and nucleic acids (such as DNA, mRNA, and microRNA) [5]. They have exhibited significant potential in the treatment of ischemic diseases by promoting angiogenesis through biological molecule delivery [6].

Non-coding RNAs, especially microRNAs (miRNAs), have been verified for their critical roles in many diseases, including ischemic diseases [7,8]. MiRNAs are small, single-stranded, non-coding RNA molecules with 21 to 23 nucleotides [9]. They are present in plants, animals, and some viruses and play a crucial role in RNA silencing and the post-transcriptional regulation of gene expression. miRNAs function by base-pairing with complementary sequences in mRNA molecules, leading to the silencing of mRNA through processes such as cleavage of the mRNA strand, destabilization of the mRNA by shortening its poly(A) tail, or reduction in the translation of mRNA into proteins. Its molecular signaling pathway has been well characterized in promoting angiogenesis and adjusting the immune system [10].

The role of inflammation in ischemic diseases has historically been overlooked, primarily due to the medical community's focus on ischemic processes rather than inflammatory responses [11]. However, there has been a rapid shift in this perspective, with an increasing number of medical professionals directing their attention to the impact of the immune system on ischemic diseases. A comprehensive understanding of immune responses in diseases, beyond angiogenesis promotion, is crucial for the development of additional treatment strategies for ischemic diseases [12]. Recent emphasis has been placed on the significance of inflammatory cells, particularly macrophages, in ischemic diseases [13]. Macrophages, as important immune effector cells, exhibit remarkable plasticity that enables them to respond to environmental cues. Simply, they can be categorized into two groups based on their distinct functions: M1-like (pro-inflammatory) and M2-like macrophage (anti-inflammatory and tissue repair facilitating). M2-like macrophages secrete anti-inflammatory cytokines and contribute to tissue repair [14]. The presence of M2-like macrophages at the ischemic site has been demonstrated to be crucial for successful tissue repair and resilience [15]. M1-like macrophages, which are recognized as pro-inflammatory immune cells, were historically viewed as a detrimental factor in ischemic disease. However, their positive role in ischemic disease treatment has been recently underscored [16].

For the successful management of ischemic disease, the precise modulation of macrophage polarization is essential. However, there remains a substantial gap in our understanding of effectively achieving this modulation. In response to these challenges, there have been continued efforts to develop low-risk approaches for polarizing macrophages. This involves exploring the use of exosomes. Our and others' previous results give us some hints and inspiration. Our earlier study established specific miRNAs in the exosomes regulated angiogenesis and macrophage polarization [13].

This review will explore the intricate interplay between angiogenesis, macrophage polarization, and exosomes. Instead of offering a comprehensive overview of the well-documented relationship between macrophages and angiogenesis, our aim is to elucidate the response of macrophages to exosomes, their regulatory role in angiogenesis, and to construct a model that portrays the interconnectedness of angiogenesis, macrophages, and exosomes.

2. Macrophage polarization and ischemic diseases

Macrophages are considered pivotal immune effector cells due to their remarkably high heterogeneity [14]. These highly heterogeneous and multifunctional cells are defined by their capacity for phagocytosis, secretion of cytokines and various growth factors, as well as antigen presentation. Their inherent flexibility enables them to adapt their phenotype according to the microenvironment they inhabit. Historically, Macrophages were believed to originate from mononuclear cells derived from hematopoietic stem cells in the bone marrow. However, recent single-cell analyses in mice suggest that macrophages are not only derived from hematopoietic stem cells but also from embryonic sources, such as Langerhans cells and microglia [17]. Despite their shared origins, individual tissues harbor unique macrophage populations, such as osteoclasts in bone and Kupffer cells in the liver. Recent studies exploring the development of tissue-specific macrophages have revealed that the majority of these macrophages originate from embryonic sources, although their self-renewal mechanism and relationship with monocytes remain uncertain [18].

Macrophages exhibit considerable plasticity in their capacity to undergo activation in response to environmental stimuli. While conventional understanding posited two distinct populations of activated macrophages—classically activated macrophages (M1) stimulated by pro-inflammatory conditions (such as lipopolysaccharide, LPS) and alternatively activated macrophages (M2) stimulated by anti-inflammatory conditions (such as IL-4)—recent research has contested this dichotomy [19]. It is now recognized that macrophages exist along a continuum of phenotypes between stereotypical M1 and M2 populations. In specific contexts, macrophages have been observed to concurrently express pro- and anti-inflammatory cytokines, diverging from the traditional M1/M2 classification. Moreover, these polarization states are not fixed, as macrophages can dynamically modulate their phenotype in response to the surrounding microenvironment. A particular group of macrophages is infiltrated in tumor tissue; they are called tumor-associated macrophages (TAM) [20,21]. These Macrophages are employed by tumor cells to help tumors escape immune surveillance, invade, and promote angiogenesis; these macrophages often show the M2-like phenotype. However, recent studies suggested that the phenotype of TAMs may switch according to tumor development, more M1-like at the beginning of the tumor development, then switching to an M2-like phenotype [22].

Upon exposure to LPS and/or inflammatory mediators such as interferon (IFNγ) or tumor necrosis factor (TNFα), macrophages adopt the pro-inflammatory phenotype (M1) [23]. These M1 macrophages secrete pro-inflammatory cytokines such as interleukins (IL-1, IL-6, and IFNγ), nitric oxide (NO), and TNFα, and display heightened phagocytic behavior, augmenting their phagocytic capacity for debris, pathogens, and apoptosed cells [19]. Conversely, exposure to cytokines such as IL-4 or IL-10 prompts macrophages to assume an anti-inflammatory M2 phenotype, leading to the secretion of anti-inflammatory cytokines such as IL-10, and IL-4, as well as mediators associated with tissue remodeling, including epidermal growth factor (EGF) and transforming growth factor β (TGF-β) [24].

The derivation of M2 macrophages and the characterization of the 'pro-angiogenic' macrophage remain subjects of active inquiry and deliberation. While prior understanding ascribed angiogenic signals to M2 macrophages, recent evidence suggests a more intricate interplay between macrophages and blood vessels, introducing a heightened level of complexity in our understanding of macrophage-vessel interactions. A study conducted by *N. Jetten* demonstrated that the M2 polarized macrophage induces greater angiogenesis compared to other subsets by upregulating the expression of basic fibroblast growth factor (FGF2), insulin-like growth factor-1 (IGF1), chemokine (C–C motif) ligand 2 (CCL2), and placental growth factor (PGF) [15]. The inhibition of M2-induced angiogenesis was observed by using a blocking antibody of PlGF resulted in a 40% reduction in tube formation, while neutralization of FGF-2 signaling by sFGFR1-IIIc led to a nearly 75% reduction in tube formation. Yuan *et al.* [25] conducted a study using an M2 macrophage-polarized anti-inflammatory hydrogel (HTHE-M@D) in conjunction with mild heat stimulation to address diabetic foot ulcers (DFUs). The research yielded promising outcomes, demonstrating that the hydrogel facilitated the transition of macrophages from the M1 to M2 phenotype. Moreover, the hydrogel exhibited favorable attributes, including anti-inflammatory, antibacterial, antioxidant, and hemostatic effects, as well as biocompatibility. Although M2-like polarized macrophages have shown great potential in promoting angiogenesis and aiding in the treatment of ischemic diseases, it has been observed that M1-like macrophages also play a role in promoting angiogenesis. Macrophages pre-conditioned with LPS have been found to aid in the recovery from myocardial infarction by clearing more apoptotic debris, which helps to shift the pro-inflammatory environment towards an anti-inflammatory phenotype, ultimately promoting tissue repair. It was observed that mice with myocardial infarction who received LPS injections showed better recovery [26].

Moreover, extensive evidence supports the pro-angiogenic role of tumor-associated macrophages (TAMs), particularly concerning their cytokine secretion. *In vitro* studies have demonstrated that TAMs secrete a wide array of pro-angiogenic factors, including VEGF, TNFα, FGF2, IL1β, CXCL8, CoX2, plasminogen activator, PDGFβ, MMP7, MMP9, and MMP12, which are known to facilitate endothelial cell activation, proliferation, and survival [27]. Moreover, recent investigations have unveiled the noteworthy contribution of the hypoxic (low oxygen) tumor microenvironment to TAM-mediated angiogenesis [28]. TAMs preferentially accumulate in hypoxic regions of tumors, exerting a more potent pro-angiogenic influence compared to normoxic areas. This behavior is attributed to the hypoxic conditions' ability to induce a pro-angiogenic polarization of macrophages through the upregulation of specific transcription factors such as S100A family, SEMA family and chitinase-like protein family. These transcription factors, in turn, drive the expression of genes associated with cell proliferation and angiogenesis. Notably, some studies have elucidated the initiation of a pro-angiogenic response in both tumor cells and macrophages under hypoxic conditions (Figure 1) [29]. Additionally, recent studies have increasingly concentrated on the multifaceted role of the macrophage secretome, extending beyond its traditionally recognized cytokine profile. Investigations have demonstrated that this secretome is significantly enriched with exosomes, which are believed to be pivotal in mediating intercellular communication and modulating immunological responses. Furthermore, these exosomes are implicated in influencing macrophage polarization, thereby contributing to angiogenesisn. This growing body of research underscores the importance of the macrophage secretome as a key player in the regulation of immune responses and vascular dynamics.

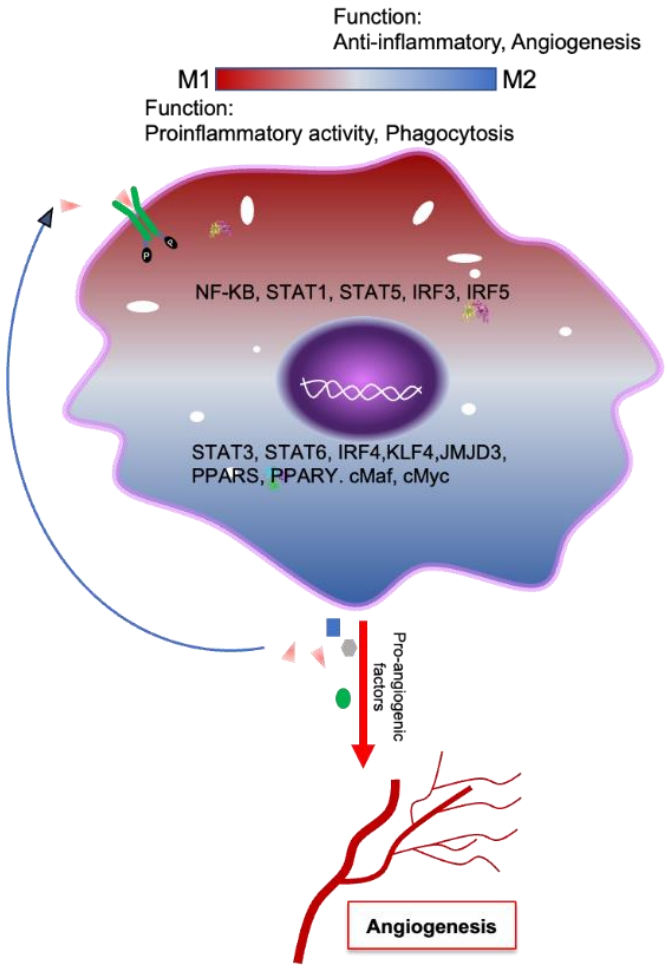

Figure 1. Both M1 and M2 polarized macrophages play a role in promoting angiogenesis. M1 macrophages demonstrate an enhanced capacity for phagocytosis, contributing to the efficient clearance of apoptotic debris resulting from hypoxic conditions. This mechanism facilitates the shifting of the microenvironment from a pro-inflammatory phase to one that is conducive to tissue repair. In contrast, M2 macrophages play a pivotal role in promoting angiogenesis through the elevated secretion of anti-inflammatory cytokines and growth factors, thereby supporting the healing process.

3. Macrophage and exosomes

Exosomes, which are small vesicles released by various cells, play a crucial role in mediating intercellular communication. Previous studies have highlighted the presence of diverse RNA species, including mRNA and miRNA, within exosomes, with *in vitro* experiments demonstrating their functional capabilities. Furthermore, protein array analysis of exosome-derived proteins has underscored the complexity of their cargo. Notably, exosomes derived from donor cells have been shown to transfer molecules to recipient cells, as evidenced by the detection of both mouse proteins and RNAs in human cells following the transfer of mouse exosomes [30]. Recent research has shed light on the potential of macrophages to engage in intercellular communication via exosome exchange [31]. These exosomes, harboring molecules derived from the donor cells, may exert influence on the phenotype or function of the recipient cells (Figure 2).

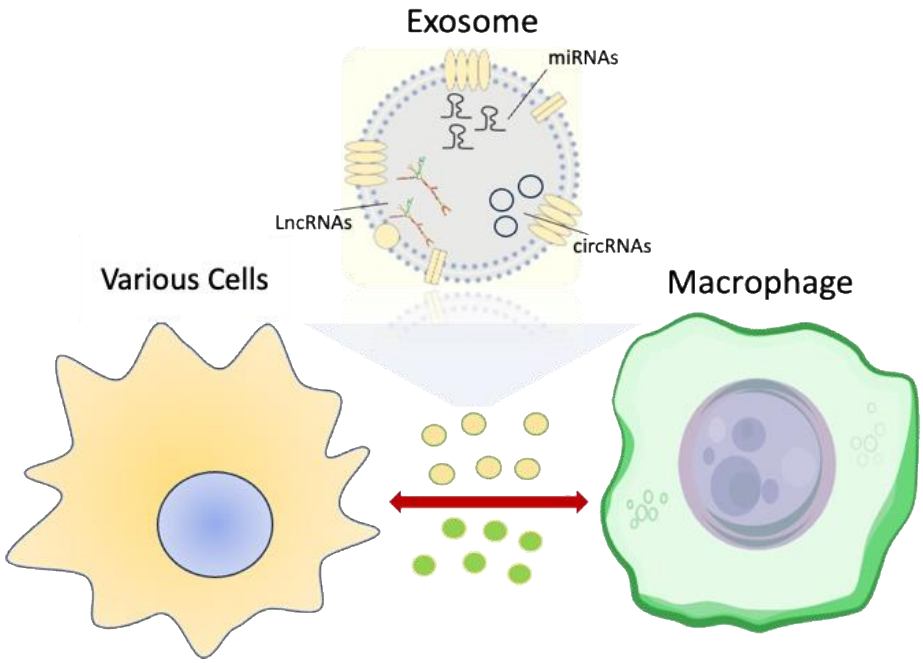

Figure 2. Exosomes mediate various cells and macrophage communication. The exosome plays a pivotal role in facilitating communication between stem cells and macrophages by transporting a diverse array of biofunctional molecules. This intercellular communication is essential for various biological processes, including tissue regeneration and immune response modulation. Through the transfer of proteins, lipids, and nucleic acids, exosomes influence cellular behaviors and promote the interplay between these distinct cell types. The intricate mechanisms underlying exosomal communication highlight their significance in both physiological and pathological contexts.

3.1. Exosomes from macrophage

Numerous studies have confirmed the release of a significant amount of exosomes by macrophages. Exosomes derived from different macrophage phenotypes carry distinct biological information, similar to that of their parental cells, and play various roles. Recent research has demonstrated that exosomes derived from macrophages encompass a diverse array of RNAs and proteins that have the potential to be conveyed to target cells, where they can execute specific functions [32]. For instance, a study conducted by Saha *et al.* highlighted the capacity of macrophage-derived exosomes containing WNTs to facilitate the rescue of intestinal stem cells and enhance survival following radiation injury [33]. This investigation delved into the role of macrophage-derived WNT, conveyed via exosomes, in the process of intestinal repair in mice. Two categories of mice were employed: Porcn-depleted mice and wild-type (WT) mice. Porcupine, a gene pivotal for WNT synthesis, was the focus of this comparative analysis. The findings revealed that while Porcn-depleted mice exhibited normal intestinal morphology, they displayed heightened sensitivity to radiation injury in comparison to their wild-type counterparts. Notably, Porcn-depleted mice could be rescued from radiation-induced injury through treatment with conditioned medium derived from wild-type bone marrow macrophages. Furthermore, upon depletion of exosomes from the macrophage conditioned medium, the medium lost its efficacy in rescuing Porcn-depleted mice from radiation-induced lethality. In summation, the study concluded that

macrophage-derived exosomes carrying WNTs are indispensable for the regenerative response of the intestine to radiation.

Exosomes from M1 macrophages enhance the effectiveness of anti-programmed cell death receptor ligand 1 (aPD-L1) in fighting cancer by prompting tumor-infiltrating M2-like TAMs to transition to the M1-like phenotype [34]. The reprogrammed M1-like macrophages secrete more pro-inflammatory cytokines, which helps in boosting the body's immune response against the tumor and leads to the inhibition of tumor growth in mice. Another study indicates that the exosomes derived from M2-like macrophages (M2-Exo) are capable of completely converting activated M1-like macrophages to M2-like phenotype by incubating the macrophages in serum‐free media containing the exosomes for 24 h [35]. The cells that received exosome treatment exhibit the distinct characteristics of M2-like macrophages, including increased expression of Arginase-1 (M2-marker) and decreased expression of iNOS (M1-marker). Additionally, M2-Exo contains factors such as CCL27, CCL11, CCL24, IL4, CXCL12, bFGF, CCL22, and MFG‐E8, that not only reprogram macrophages but also promote wound repair by inducing various cytokines and growth factors secretion such as IL4, CXCL12, and bFGF. Upon subcutaneous administration of M2-Exo near a wound, there is a noticeable decrease in M1-like macrophages and an increase in M2-like macrophages, indicating a successful switch to M2-like macrophage polarization guided by exosomes. This direct conversion of M1-like to M2-like macrophages at the wound site accelerates wound healing by enhancing processes such as angiogenesis, re-epithelialization, and collagen deposition [36].

3.2. Exosomes from stem cells

Stem cell-derived exosomes show significant potential for immune modulation, particularly in regulating macrophages. Researchers have demonstrated that adipose-derived stem cells, especially under hypoxic conditions, induce macrophage M2-like polarization, characterized by high expression of Arg-1 and CD206 [13]. The exosome-treated macrophages enhance angiogenesis in a mouse hindlimb ischemic model, thereby facilitating the restoration of blood perfusion [13]. Results further indicate that mice receiving hypoxic exosomes show better outcomes than those receiving normoxic exosomes. Additionally, using BLZ925 to disable the availability of the M-CSF receptor in the macrophages blocks macrophage M2-like polarization and attenuates the therapeutic effects of exosomes. Another study revealed that upon systemic administration in mice with collagen-induced arthritis, the exosomes harvested from metabolically engineered stem cells efficiently accumulated in the inflamed joints [37]. This resulted in a series of anti-inflammatory events through the regulation of macrophage phenotype. The engineered exosomes demonstrated a level of therapeutic efficacy that was achievable with a 10 times lower dose compared to unengineered exosomes. This suggests that the engineered exosomes have significant potential to be developed as a next-generation drug for rheumatoid arthritis due to their enhanced ability to reprogram the synovial microenvironment, achieved by inducing macrophage anti-inflammatory polarization. Exosomes originating from bone marrow-derived mesenchymal stem cells (MSCs) have demonstrated potential in ameliorating lupus nephritis through the induction of an exceptional anti-inflammatory polarization of kidney-infiltrating macrophages [38]. These exosome-educated macrophages exhibit robust anti-inflammatory capabilities by mitigating inflammation-induced apoptosis of renal cells, amplifying the secretion of anti-inflammatory cytokines, and recruiting a greater number of regulatory T cells. Notably, the exosome-educated macrophages manifest distinct

characteristics that differentiate them from traditional M2-like polarized macrophages, including the expression of CCL20. In addition to their formidable anti-inflammatory capacity, these macrophages also display a pronounced efferocytosis, enabling them to orchestrate the immune response and clear apoptotic cell debris effectively. These findings suggest that macrophages may communicate with other cells through exosomes enriched with specific biological molecules, thereby influencing gene expression and functionality in target cells. Additionally, exosomes derived from stem cells may contain molecules that can impact macrophage polarization or activity [39]. Further comprehensive studies are warranted to comprehend the implications of these discoveries fully.

The role of exosomes in macrophage polarization is a pivotal factor in modulating macrophage functions. Recent advancements in protein array technology and RNA sequencing have significantly enhanced our understanding of the underlying mechanisms involved in exosome-mediated macrophage polarization. These methodologies enable a comprehensive exploration of how exosomal content influences the polarization state of macrophages, thereby contributing to their diverse functional roles in immune responses and pathophysiological conditions.

4. Exosome-delivered non-coding RNAs in macrophage polarization and angiogenesis

In recent years, there has been increasing evidence to suggest that exosome-delivered non-coding RNAs, especially miRNAs, play a dual role in macrophage polarization and angiogenesis (Figure 3).

4.1. Exosome-delivered miRNA

MiRNAs play a crucial role in post-transcriptional regulation and are involved in various biological processes. In recent years, numerous articles have highlighted the significant role of miRNAs in regulating macrophages and angiogenesis. Exosomes have been shown to have the potential to transport biological molecules including miRNAs to ischemic sites in ischemic diseases, which can have both beneficial and detrimental effects.

MiRNA-155-5p is found to be highly expressed in M1-like macrophage-derived exosomes, and these exosomes play a role in inhibiting angiogenesis. The findings demonstrate that miRNA-155-5p directly binds to the 3' UTR of growth differentiation factor 6 (GDF6) mRNA, leading to the suppression of its protein expression. Notably, the local administration of a temperature-sensitive hydrogel, Pluronic F-127, loaded with miRNA-155-5p antagomiR, has been shown to promote angiogenesis and expedite wound healing in diabetic db/db mice by enhancing GDF6 expression [40]. Exosomes extracted from human umbilical cord mesenchymal stem cells (hucMSCs) are employed and result in the macrophage M2-like polarization, thus facilitating wound healing in diabetic rats. Immunohistochemistry verified the upregulation of the pro-angiogenic cytokines and growth factors. The results show that the transplantation of hucMSCs-exo enhanced diabetic wound healing by promoting the proliferation of endothelial cells and reducing inflammatory infiltration [41]. A recent study has highlighted the pivotal role of exosomes derived from tumor cells, particularly glioblastoma stem cells (GSCs-exos), in orchestrating the remodeling of the tumor microenvironment (TME) by inducing M2 polarization of tumor-associated macrophages (TAMs). The study unveiled a significant upregulation of miR-374b-3p in both clinical glioblastoma specimens and human GSC cell lines. Notably, miR-374b-3p was found to be highly enriched in GSCs-exos and capable of being internalized by macrophages.

Mechanistically, exosomal miR-374b-3p from GSCs was shown to drive M2-like polarization of macrophages by downregulating phosphatase and tensin expression, consequently promoting the migration and tube formation of vascular endothelial cells following co-culture with exosome-treated M2-like macrophages [42].

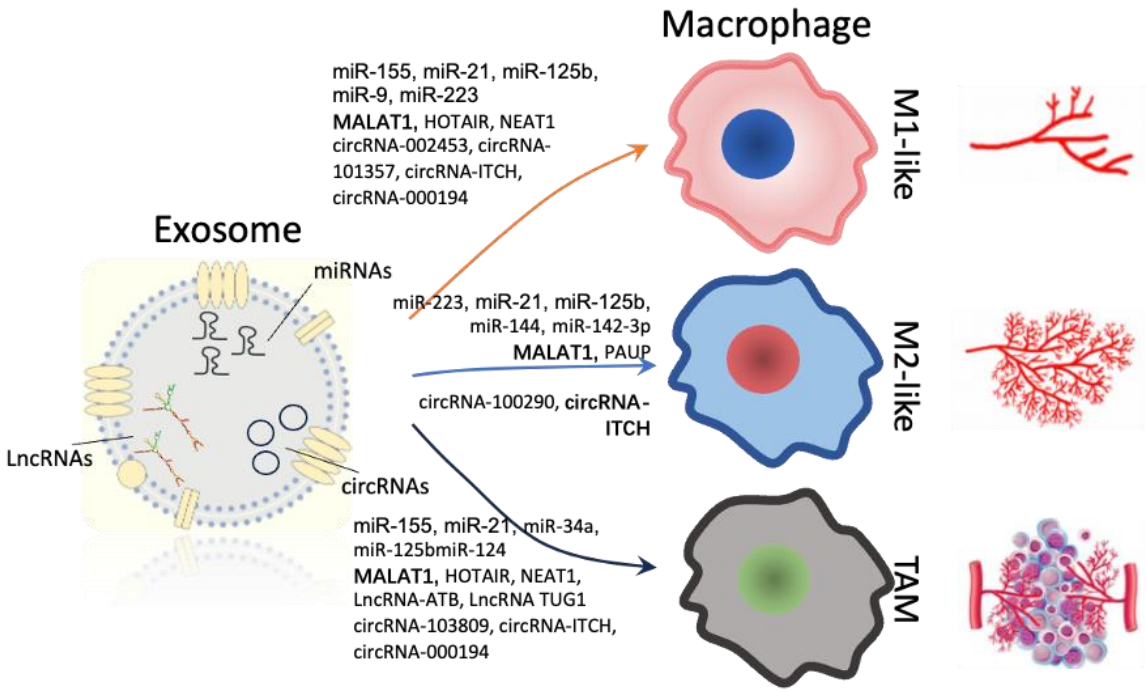

Figure 3. Exosome-delivered ncRNAs regulate macrophage polarization. Exosomes are enriched with a diverse range of non-coding RNAs (ncRNAs), including microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs). These ncRNAs play a crucial role in mediating macrophage polarization by facilitating post-transcriptional regulation, which influences cellular responses and functions in various physiological and pathological contexts. Consequently, this regulation can lead to inhibition or promotion of angiogenesis, as well as the promotion of tumor angiogenesis in respective scenarios.

4.2. Exosome-delivered other non-coding RNAs

In the realm of exosome-derived non-coding RNAs, miRNA holds a central position, while other non-coding RNAs, including long non-coding RNA (lncRNA) and circular RNA (cricRNA), are garnering escalating interest. Long non-coding RNAs are RNA transcripts of over 200 nucleotides that do not produce proteins. Some lncRNAs may encode small proteins, but they are generally defined as RNA molecules with limited or no coding capacity [43]. Circular RNA is a type of single-stranded RNA that forms a closed continuous loop, different from linear RNA. It has various properties and can arise from protein-coding genes. Some circular RNAs can code for proteins and act as gene regulators, but the biological function of most circular RNA is still unclear [44].

Both lncRNA and circRNA have demonstrated their ability to modulate macrophage and angiogenesis. The research findings indicate that exosomes derived from HUVECs, which carrying NEAT1, have been shown to promote M2-like polarization of macrophages and mitigate LPS-induced

inflammation *in vitro* [45]. This effect is achieved through the downregulation of DDX3X and NLRP3 expression. Moreover, the study demonstrated that overexpression of DDX3X in macrophages resulted in a significant increase in NLRP3 protein levels. Additionally, the knockdown of NEAT1 in exosomes partially reversed the anti-inflammatory effects observed. In a recent research study, the interaction between tumor-associated macrophage (TAM)-derived exosomes and endothelial cells in patients with epithelial ovarian cancer (EOC) was investigated [46]. The study involved isolating exosomes from TAMs present in the ascites of EOC patients and co-culturing them with human umbilical vein endothelial cells (HUVECs). The researchers observed that the exosomes significantly suppressed HUVEC migration compared to the control group by targeting the miR-146b-5p/TRAF6/NF-κB/MMP2 pathway. Interestingly, when TAM-derived exosomes were combined with EOC SKOV3-derived exosomes, they stimulated HUVEC cells and overcame the inhibition of endothelial cell migration caused by TAM-derived exosomes. Further study indicated that two long non-coding RNAs carried by SKOV3-derived exosomes remotely reversed the effect of TAMs on endothelial cells. A separate study revealed that transplants of exosomes derived from adipose-derived stem cells (ADSCs) had a significantly positive therapeutic impact on improving wound healing in diabetic mice by delivering circRNA [47]. Using high-throughput sequencing, the researchers pinpointed circRNAs that were expressed abnormally in endothelial progenitor cells (EPCs) under high glucose (HG) conditions. Their findings indicated that the overexpression of circ-Snhg11 inhibited HG-induced endothelial cell damage and the polarization of M1-like macrophages. Additionally, they identified miR-144–3p and HIF-1α as downstream targets of circ-Snhg11. The results illustrate that overexpressing circ-Snhg11 from ADSCs exosomes suppresses HG-induced endothelial cell damage and promotes M2-like macrophage polarization through the miR-144–3p/HIF-1α axis.

5. Challeges and future direction

While the application of exosome-delivered non-coding RNAs (ncRNAs) in the context of ischemic diseases has garnered significant acceptance within the research community, multiple challenges remain to be addressed. These challenges present important avenues for further investigation and advancement in the field. First, efficiently isolating pure exosomes from biological fluids or cells is difficult, as current methods can be time-consuming, inefficient, or result in contamination with other particles. Additionally, loading exosomes with specific ncRNAs, such as microRNAs or long non-coding RNAs, in sufficient quantities is challenging, as these molecules may not naturally integrate well into exosomes. Once loaded, ensuring exosome stability in the bloodstream and their ability to effectively release their cargo in the target cells remains a significant hurdle. Exosomes must also be targeted accurately to the desired cells, such as macrophages in ischemic tissues, which requires careful modification of their surface to direct them to the right locations. Moreover, exosomes can vary depending on their cellular origin, making it difficult to achieve consistent results, and there are safety concerns regarding potential immune responses or side effects. Despite these challenges, research is advancing, with promising strategies such as exosome engineering to enhance cargo loading and stability, surface modifications for better targeting, and developing better understanding of how ncRNAs influence cellular behavior. Looking ahead, personalized therapies, combining exosome treatments with other therapeutic approaches, real-time tracking of exosome movement, and rigorous clinical trials will be key to translating exosome-based ncRNA therapies into safe and effective treatments for ischemic diseases.

6. Conclusion

Ischemic diseases, especially cardiovascular diseases, are the second leading cause of mortality and morbidity worldwide [48]. Over the past few decades, many therapeutic approaches have been developed to address this issue. One promising therapy is therapeutic angiogenesis, which has been under development for over 30 years [3]. As researchers delve deeper into the field of angiogenesis, the fundamental role of the immune system is anticipated to be unveiled [49]. Macrophages, known for their versatility in various biological activities, have been repeatedly emphasized for their critical role in treating ischemic diseases [50]. Macrophage polarization gives them the ability to adapt to different requirements. Depending on the stimuli that macrophages receive, they can shift their phenotype from a pure anti-inflammatory phenotype to a pro-inflammatory phenotype in a continuum spectrum way [14]. Specific polarized macrophages have distinct functions in treating ischemic diseases [11]. Modulating macrophages to a suitable phenotype according to the diseases is proven effective in treating ischemic diseases and has achieved some success. However, inducing proper polarization of macrophages in disease is complex, some because the macrophage usually exhibits improper polarization due to the disease's abnormal microenvironment [51]. Under this situation, the exosome steps onto the center stage and displays dominance in regulating macrophage polarization in ischemic diseases through the lavish contents, especially non-coding RNAs [52].

Non-coding RNAs, such as miRNA, LncRNA, and circRNA, have been demonstrated to play pivotal roles in immune modulation and the regulation of angiogenesis [53]. A growing body of researches indicate the therapeutic potential of non-coding RNAs in the context of ischemic diseases. Exosomes containing non-coding RNAs are acknowledged as potent regulators in exosome-mediated macrophage polarization. In contrast to cytokines utilized for inducing macrophage polarization, exosomes offer numerous advantages, including enhanced safety, greater efficacy, and reduced side effects. The expanding literature in this field is progressively illuminating the prospects for treating ischemic diseases.

Abbreviation

EVs: extracellular vesicles; miRNAs: microRNAs; M1: classically activated macrophages; M2: alternatively activated macrophages; LPS: lipopolysaccharide; NO: nitric oxide; EGF: epidermal growth factor; TGF-β: transforming growth factor β; FGF2: basic fibroblast growth factor; IGF1: insulin-like growth factor-1; CCL2: chemokine (C–C motif) ligand 2; PGF: placental growth factor; TAMs: tumor-associated macrophages; WT: wild-type; aPD-L1: anti-programmed cell death receptor ligand 1; M2-Exo: exosomes derived from M2-like macrophages; MSCs: mesenchymal stem cells; lncRNAs: long non-coding RNAs; circRNAs: circular RNAs.

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Conflicts of interests

The authors indicate no potential conflicts of interest.

Authors' contribution

Conceptualization, Dihan Zhu and Yang Wang; Data acquisition, Jun Huang, Yujie Wu and Yonghui Yuan; Data analysis, Jun Huang, Yujie Wu, Yonghui Yuan, Haiyi Liu and Chenyang Jing; Data interpretation, Jun Huang, Haiyi Liu and Chenyang Jing; Resources, Dihan Zhu; Writing—original draft preparation, Dihan Zhu; Writing—review and editing, Yang Wang; Funding acquisition, Yang Wang. All authors have read and agreed to the published version of the manuscript.

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