European Journal of Medical Research

REVIEW

Open Access

Exosomes and MicroRNAs: key modulators of macrophage polarization in sepsis pathophysiology

Mohamed J. Saadh[1], Tamara Nazar Saeed[2*], Karar H. Alfarttoosi[3], Gaurav Sanghvi[4], R. Roopashree[5], Vishal Thakur[6], L. Lakshmi[7], Aziz Kubaev[8], Waam Mohammed Taher[9], Mariem Alwan[10], Mahmood Jasem Jawad[11] and Ali M. Ali Al-Nuaimi[12]

Abstract

Sepsis is a highly dangerous and complex condition that can result in death. It is characterized by a strong reaction to an infection, causing dysfunction in multiple bodily systems and a high risk of mortality. The transformation of macrophages is a vital stage in the procedure as they possess the capability to interchange between two separate types: M1, which promotes inflammation, and M2, which inhibits inflammation. The choice greatly affects the immune response of the host. This analysis underscores the rapidly expanding roles of exosomes and microRNAs (miRNAs) in regulating the trajectory of macrophage polarization during episodes of sepsis. Exosomes, extremely small extracellular vesicles, facilitate cellular communication by transferring biologically active compounds, including miRNAs, proteins, and lipids. We investigate the impact of changes in exosome production and composition caused by sepsis on macrophage polarization and function. Unique microRNAs present in exosomes play a significant role in controlling crucial signaling pathways that govern the phenotype of macrophages. Through thorough examination of recent progress in this area, we clarify the ways in which miRNAs derived from exosomes can either aggravate or alleviate the inflammatory reactions that occur during sepsis. This revelation not only deepens our comprehension of the underlying mechanisms of sepsis, but it also reveals potential new biomarkers and targets for treatment. This assessment aims to amalgamate diverse research investigations and propose potential avenues for future investigations on the influence that exosomes and miRNAs have on macrophage polarization and the body's response to sepsis. These entities are essential for controlling the host's reaction to sepsis and hold important functions in this mechanism.

Keywords Sepsis, Macrophage polarization, MicroRNA, Exosome

*Correspondence:
Tamara Nazar Saeed
tamara.nazar@alnoor.edu.iq
Full list of author information is available at the end of the article

Introduction

Sepsis is regarded by the World Health Organization (WHO) as a significant concern in terms of worldwide health. This refers to a serious state of inflammation caused by an infection, which can result in death for about 30–45% of patients who are being treated in a hospital [1, 2]. Acute respiratory distress syndrome (ARDS), a condition caused by multiple organ dysfunction syndrome, is widely regarded as the most prevalent and severe form. This has a considerable influence on the prevalence and death rates related to sepsis [3].

The role of macrophages in the body's natural defense mechanism is crucial, as they serve as important defenders and carriers of foreign materials, demonstrating their remarkable adaptability. Differentiation and polarization allow them to modulate the host's immune responses, creating a multi-faceted spectrum [4]. The alteration of macrophage polarization has a crucial impact on the outcome of sepsis [5]. When monocytes are stimulated by pathogens, they undergo a transformation into macrophages. Phagocytic cells known as macrophages eventually undergo a process of splitting into two distinct categories, each possessing specific attributes, commonly known as M1 and M2. M1 macrophages play a critical role in the production of an excessive number of inflammatory cytokines, leading to the development of cytokine storm syndrome and septic shock. M2-like macrophages are capable of producing anti-inflammatory compounds that can effectively stop the advancement of sepsis. [6].

MiRNAs are RNA molecules produced by an organism that lack the ability to generate proteins. They are typically 22 to 26 nucleotides long and serve mainly as controllers of gene expression after the genetic code has been transcribed. There is overwhelming evidence suggesting that miRNAs possess the capability to regulate a multitude of pathological mechanisms, such as cellular proliferation, metabolic processes, apoptotic pathways, and organogenesis [7]. Countless studies have substantiated the notion that miRNAs hold remarkable influence in regulating macrophage polarization, ultimately affecting the degree of inflammation [8].

Exosomes refer to a group of small, 30 to 150 nm-sized vesicles found outside of cells. These vesicles are capable of transporting biologically active substances to other cells, thereby influencing their behavior and characteristics [9].

New studies suggest that the function of macrophages is affected by the paracrine impact of the trophic elements, anti-inflammatory proteins, and exosomes that they release [10]. Exosomes, by carrying miRNAs and other cargo, contribute to intercellular communication and influence macrophage behavior, making them an important factor in sepsis pathogenesis. Once incorporated into the cell membrane, these elements contained within exosomes are released into the surrounding outside area [11]. Hence, exosomes serve a critical function in relaying messages and transferring substances across various cells and tissues [12]. Recent studies have revealed that macrophages possess the ability to transfer small RNA molecules called exosomal miRNAs, which contain genetic information, to other cells. The movement of this can greatly affect the activity of genes and the onset of illnesses [13–15].

MicroRNAs and sepsis

miRNAs have been discovered to have various functions in gene regulation, cell differentiation, and disease development. In simpler terms, ncRNAs are a type of RNA that do not code for proteins. This group comprises a diverse range of RNA molecules, including miRNAs, siRNAs, lncRNAs, piRNAs, snoRNAs, snRNAs, exRNAs, and scaRNAs. ncRNAs have a significant impact on regulating numerous biological processes and diseases, such as cancer, autoimmune disorders, heart disease, and metabolic disorders. Researchers Lee et al. and Wightman et al. recorded their finding in 1993 of a minuscule molecule composed of 22 parts, named lin-4, with a complementing sequence that is the opposite of the lin-14 gene found in Caenorhabditis elegans [16, 17]. In 2000, the crucial role of a particular form of RNA molecules was highlighted through the discovery of let-7, a small RNA molecule present in various species, which was found to possess the capability to repress genes [18–20]. In the subsequent year, Tuschl et al. came up with the term microRNA [21]. Together with other organizations, they were instrumental in unearthing a vast array of miRNAs.

According to the database at (http://www.mirbase.org), researchers have discovered around 38,600 different miRNAs in 271 different species. The human genome contains roughly 2,600 mature miRNAs, of which approximately half have been designated in miRBase V22 [22]. The FANTOM5 consortium's newly published atlas of miRNAs has unveiled that 50% of the miRNAs present in a specific human cell can be attributed to the top five most expressive miRNAs [23]. Roughly 50% of miRNAs exhibit a higher abundance in specific cell types, while approximately 25% are widely expressed across various cell types. Another 25% of miRNAs show minimal levels of expression, regardless of the cell type. Intergenic miRNAs, which are certain miRNAs, can be discovered in non-protein coding areas of genes, as well as in the introns of genes. miRNAs originate from both canonical and non-canonical pathways [24–26] (Fig. 1). The first step in the conventional pathway involves creating a full pri-miRNA that can be quite long, ranging anywhere from hundreds to thousands of nucleotides [27, 28].

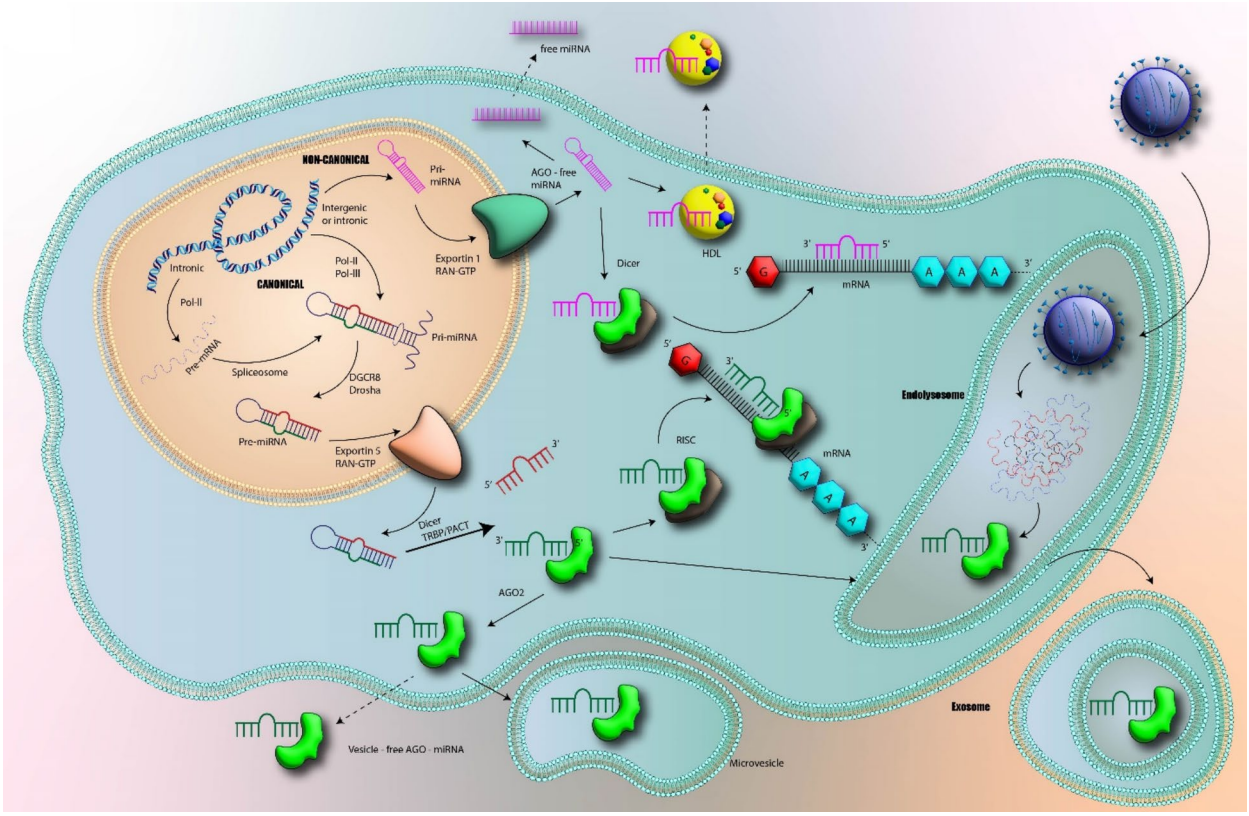

Fig. 1 Production of miRNA can happen through two different routes: the traditional and non-traditional pathways. As part of the standard process, primary microRNAs are converted into precursor microRNAs within the nucleus using the DGCR8 and Drosha enzymes. Pre-miRNA molecules can originate from the spliceosome's processing of host mRNA, resulting in miRNA integration. The movement of pre-miRNAs from the cytoplasm is highly dependent on RanGTP and is impossible without Exportin-5. Within the cytoplasm, Dicer works on them and, with the help of RNA binding proteins TRBP or PACT, they eventually become fully developed miRNAs. The cutting of shRNAs in non-traditional pathways is carried out by the DGCR8/Drosha complex. Afterward, exportin-1 transports the shRNAs to the cytoplasm, where they receive further modifications from Dicer. Fully developed microRNAs attach themselves to Ago proteins, creating what is known as RISCs. These RISCs are responsible for regulating the activity of mRNAs by either silencing them or breaking them down. Stated differently, the miRNA–AGO complexes have the ability to exit the cell using two methods: through the aid of vesicles, such as exosomes or microvesicles, or as complexes that are not enveloped by vesicles. miRNAs that bind to HDLs are intentionally released. MiRNAs that do not contain AGO can also exit the cell. Commonly utilized expressions when exploring gene expression include AGO, DGCR8, HDL, miRNA, mRNA, PACT, pre-miRNA, Ran, RISC, shRNA, and TRBP

This specific form of RNA is formed through the activity of either RNA polymerase II or III. Drosha enzyme and the DGCR8 RNA-binding protein, found in the nucleus, destruct the initial form of the primary miRNA. This results in a shortened version of miRNA called pre-miRNA, measuring around 70 nucleotides [29–34].

The spliceosome aids in the conversion of host RNA transcripts or pre-mRNAs into pri-miRNAs through RNA splicing, and then the pre-miRNAs undergo further transformation [35, 36]. The manner in which they communicate is reliant on the availability and functioning of transcription factors and Pol-II [37]. The process of transferring Pre-miRNAs from the nucleus to the cytoplasm requires the collaborative action of exportin-5 protein and RanGTP molecule. To convert pre-miRNAs into functional miRNAs, a combination of cytoplasmic enzymes called Dicer, along with other helpers, such as TRBP and PACT, work together to create mature miRNAs that are around 22 nucleotides long [32, 38, 39]. The significance of miRNA-5p and miRNA-3p is due to their ability to be produced from either the 5'or 3'end of a pre-miRNA, with evidence suggesting that they can be present simultaneously in certain cases. Alternative pathways for producing miRNA, which do not follow the usual process, involve a varying mix of proteins. These pathways can be divided into two categories: those that do not require DGCR8/Drosha proteins and those that do not require Dicer proteins (Fig. 2). The mechanism behind the splitting and movement of shRNA is accomplished by the DGCR8/Drosha complex, which

then proceeds to export it into the cytoplasm through the conventional route [40, 41]. Exportin-1 has a crucial function in facilitating the transportation of pre-miRNA to the cytoplasm. However, the intricacies of miRNA generation are not the central focus of this composition. In-depth examinations offer more extensive explanations [26, 42–44].

Sepsis displays signs of initial excessive immune activity and subsequent immune suppression. In light of this, it can be inferred that miRNAs with properties that

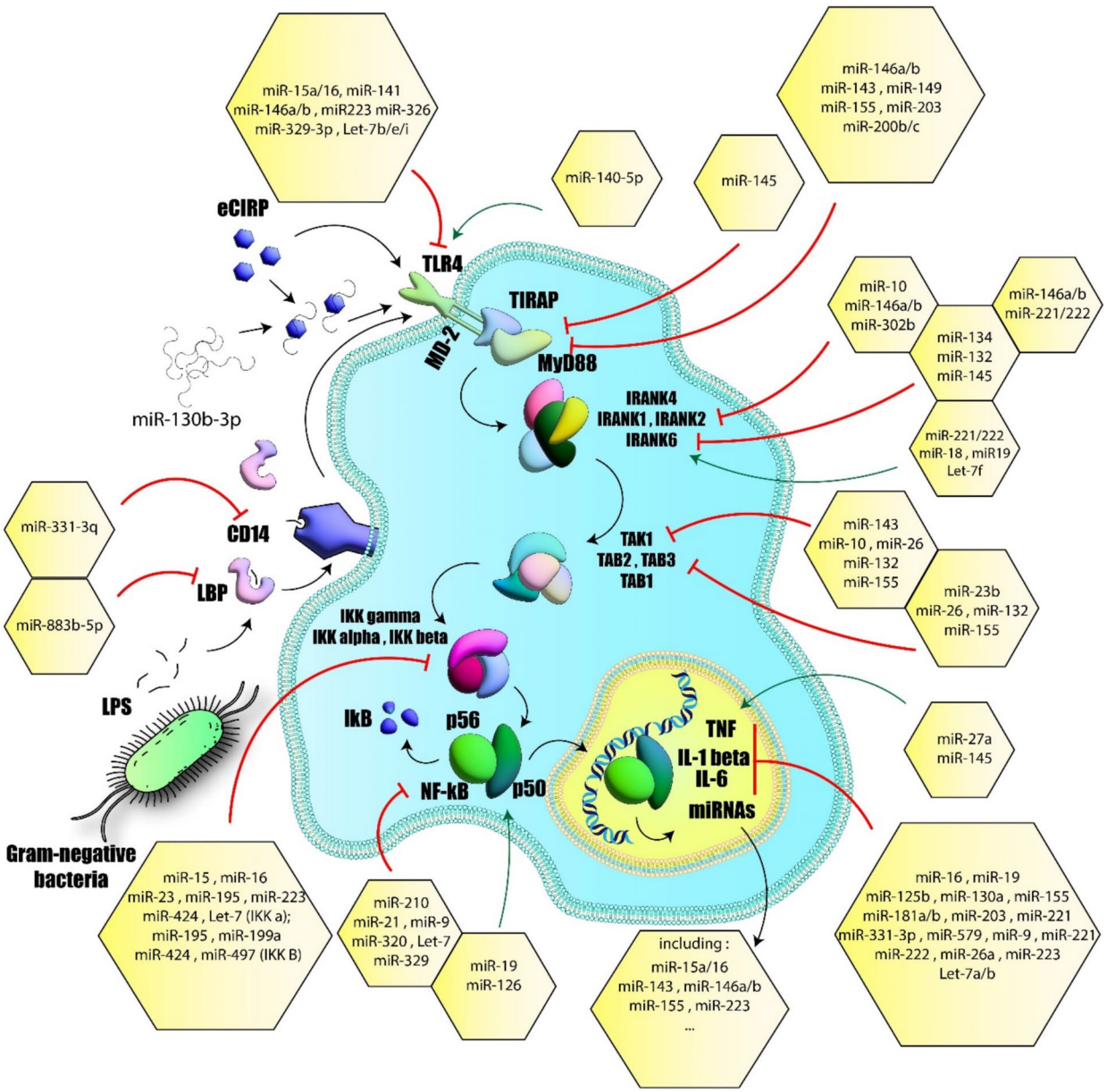

Fig. 2 Detection and communication of endotoxins heavily rely on miRNAs. This diagram depicts the mechanism by which monocytic cells recognize LPS molecules derived from Gram-negative bacteria and the resulting signaling events that take place within the cell. The main function of LPS-binding protein (LBP) is to disintegrate aggregated LPS particles into smaller elements. The merging of lipopolysaccharide (LPS) and lipopolysaccharide-binding protein (LBP) is fully transported onto CD14, a molecule that is attached to the surface of monocytic cells through a glycosylphosphatidylinositol anchor. CD14 and MD-2 collaborate to facilitate the transfer of LPS to TLR4, with the TIR domain responsible for introducing TIRAP and MyD88 into the equation. MyD88 plays a crucial role in the initial phases of activating nuclear factor-κB (NF-κB) and triggering the production of pro-inflammatory genes. The process of NF-κB pathway is responsible for controlling the generation of multiple miRNAs. To maintain simplicity, we deliberately omitted the depiction of the process, where TRIF and its pathway activate IRF and delay the activation of NF-κB, which is not influenced by MyD88

counteract inflammation could potentially be advantageous in the initial stages of sepsis, but pose a risk in the later stages of the condition. The fact that both infection and stress affect the way miRNAs are expressed has led to a considerable amount of attention being directed toward miRNAs. The stability, uncomplicated format and manifestation of miRNAs in bodily fluids offer potential for discovering novel sepsis indicators [45].

Sepsis triggers the stimulation of complement and coagulation mechanisms, as Danger-Associated Molecular Patterns (DAMPs) and Microbe-Associated Molecular Patterns (MAMPs) are present. Approximately 35% of individuals with sepsis are impacted by disseminated intravascular coagulation (DIC). In addition to causing blood clots, DIC is also linked to hemorrhaging as a result of the depletion of essential elements in the blood, such as clotting factors, anticoagulants, and platelets [46, 47]. Half of individuals with sepsis will experience a decrease in their platelet count, known as thrombocytopenia. The initiation of innate immune reactions through recognition of patterns by PRRs and cytokine receptors sets off a series of reactions in endothelial cells, resulting in an increase of adhesive molecules, alterations in the permeability of blood vessels, movement of immune cells through cell layers, harm to small blood vessels, tissue hypoxia, and, ultimately, impairment of crucial organs [48, 49]. A multitude of microRNAs produced by both internal and external (exosomes) sources control various aspects of endothelial cell activity, such as apoptosis, proliferation, migration, and inflammation [50–53]. In the case of mice suffering from sepsis, the levels of miR-155 experience a substantial boost in pulmonary endothelial cells. This leads to miR-155 specifically binding to Claudin-1, an essential protein responsible for maintaining tight junctions. As a result, the capillaries start to leak during the progression of the infection[54].

When naturally occurring, immune cells encounter small doses of Lipopolysaccharides (LPS), whether in isolation or throughout the body, they experience a brief phase of protection against LPS exposure in the future, resulting in suppression of cytokine creation. It is called endotoxin tolerance. The examination of the expression of miR-146a and miR-146b in THP-1 human monocytic cells strongly implies that these molecules could potentially aid in promoting endotoxin tolerance [55–57]. The vital role of MiR-146a in regulating the expression of the TNF gene is essential for maintaining tolerance in THP-1 cells. It plays a significant role in both the transcription and translation of this gene [57]. The existence of immune-regulating substances IL-10 and TGF-β influences the synthesis of miR-146b; however, its function is impeded by IFNγ. As a result, the immune tolerance caused by endotoxins can be reversed [56]. The concept of tolerance goes beyond simple interaction between LPS and Toll-like receptor 4 (TLR4), encompassing a wider range of implications. To put it differently, TLR2 not only contributes to the upregulation of miR-146a, but also reduces the reactivity of THP-1 cells to future exposure to Salmonella typhimurium by recognizing bacterial lipoproteins. Entry of THP-1 cells leads to a significant reduction in IRAK-1, phosphorylated inhibitory kappa B α (IκBα), and TNF production, indicating a significant decrease in these elements [58]. The ability to endure is influenced by epigenetic mechanisms, as evidenced by the careful control of miR-146a and miR-155 in unconditioned and tolerant mouse macrophages. Once activated, LPS triggers the addition of three methyl groups onto lysine 4 of histone 3, known as H3 K4 me3, serving as a cue for genes that are actively undergoing transcription. As a result, NF-κB p65 is more likely to bind to specific areas in the DNA, where the miR-146a and miR-155 genes are situated, causing their levels to rise. The most successful method for promoting the acceptance and understanding of a concept involves altering the chromatin's structure, specifically by increasing the presence of H3 K9 me3. This method also rests on the participation of CCAAT/enhancer-binding protein (C/EBP) β and p50, which are essential components of the NF-κB complex, in regulating the stimulation of miR-146a and miR-155 genes [59].

Platelets play a diverse role that surpasses just promoting blood clotting and repairing blood vessels. They serve as controllers within innate immune cells, such as PMNs, monocytes, and macrophages [48, 60, 61]. Platelets are crucial in the distribution of miRNAs, whether it be through microvesicles or exosomes, making them a vital resource for these significant substances. The previously mentioned miRNAs enter both endothelial cells and macrophages [62, 63].

Decreased expression of miR-26b in platelets is associated with elevated P-selectin protein levels and more serious outcomes, such as increased mortality, among sepsis patients [64]. To be more specific, the presence of miR-26b in mice has been found to significantly decrease the occurrence of platelet adhesion and aggregation [65]. The detection of sepsis could be improved by measuring the ratio of miR-320a to miR-127, which has been found to be elevated in platelets [66].

The genome of macrophage cells has undergone changes due to the absorption of microvesicles from platelets enriched with miR-126-3p. This has led to a notable decrease in the synthesis of cytokines, chemokines, and growth factors [63]. Moreover, the activation of platelets is closely correlated with miR-126-3p [67]. Individuals with sepsis possess exosomes that contain specific molecules, including miR15b-5p and

miR-378a-3p. These compounds possess the capability to trigger the creation of neutrophil extracellular traps (NETs), leading to potential harm to organs [68]. Furthermore, the introduction of miR-223-containing platelet microparticles has been demonstrated to effectively lower intercellular adhesion molecule 1 (ICAM-1) levels and hinder its ability to bind to peripheral blood mononuclear cells by traversing through endothelial cells. This can potentially provide defense against the detrimental vascular inflammation triggered by sepsis [69].

Numerous researches examined specific miRNAs that were either identified through previous understanding or through miRNA screenings. Although insightful for individual cases, these reductionist approaches only address a fraction of the overall function of miRNAs. Research on the effects of endotoxin, a substance commonly used to examine the response of hosts to Gram-negative bacteria, provides a clear example of this concept (Fig. 3). For the immune system to identify outside lipopolysaccharides (LPS), four essential elements—LPS binding protein (LBP), CD14, MD-2, and TLR4—must be included [70, 71]. The cell's exterior contains TLR4, which enlists the help of an adaptor protein called MyD88 to assist with its functions. The MyD88 protein initiates a series of chemical reactions that originate at IRAK1 and TRAF6, ultimately resulting in the stimulation of the NF-κB, IRFs, and MAPK signaling pathways. The transcription of immune response-related genes is solely controlled by these specific pathways. The transfer of TLR4 to the late endosome leads to a distinct form of signaling facilitated by the adaptor protein TRIF. TRIF plays a significant role in the activation of IRF3 and late stage NF-κB, essential components for the generation of type I IFNs and IFN-targeted genes. Figure 3 does not include the description of this pathway for the sake of simplicity.

More than 40 distinct types of miRNAs have been discovered, including but not limited to miR-15a, miR-16, miR-17-5p, miR-21, miR-25, miR-31, miR-98, miR-124-5p, miR-125b, miR-140-5p, miR-141, miR-146a, miR-149-5p, miR-155, miR-181c, miR-203-5p, miR-221, miR-326, miR-378, miR-448, and miR-466I. These small regulatory molecules have the ability to interfere with the detection of LPS, hindering the initiation of related communication pathways (as shown in Fig. 3). Certain microRNAs, specifically miR-15a/16, miR-17-5p, miR-25, miR-125b, miR-141, miR-326, and miR-448, have the ability to inhibit the function of TLR4. However, there is one exception in which miR-140-5p actually promotes the expression of TLR4. Several miRNAs, such as miR-9-5p, miR-19a-5p, miR-21, miR-29, miR-93, miR-98, miR-125, miR-221, miR-222, miR-223, and let-7a-5p, have been recognized as controllers of both proinflammatory and anti-inflammatory cytokines. In its essential nature, the inflammatory response regulates the amounts of miRNAs that are pro-inflammatory and those that are anti-inflammatory [72–79]. This study sheds light on the complex role of miRNAs in controlling the host's response to antimicrobial processes. This highlights the

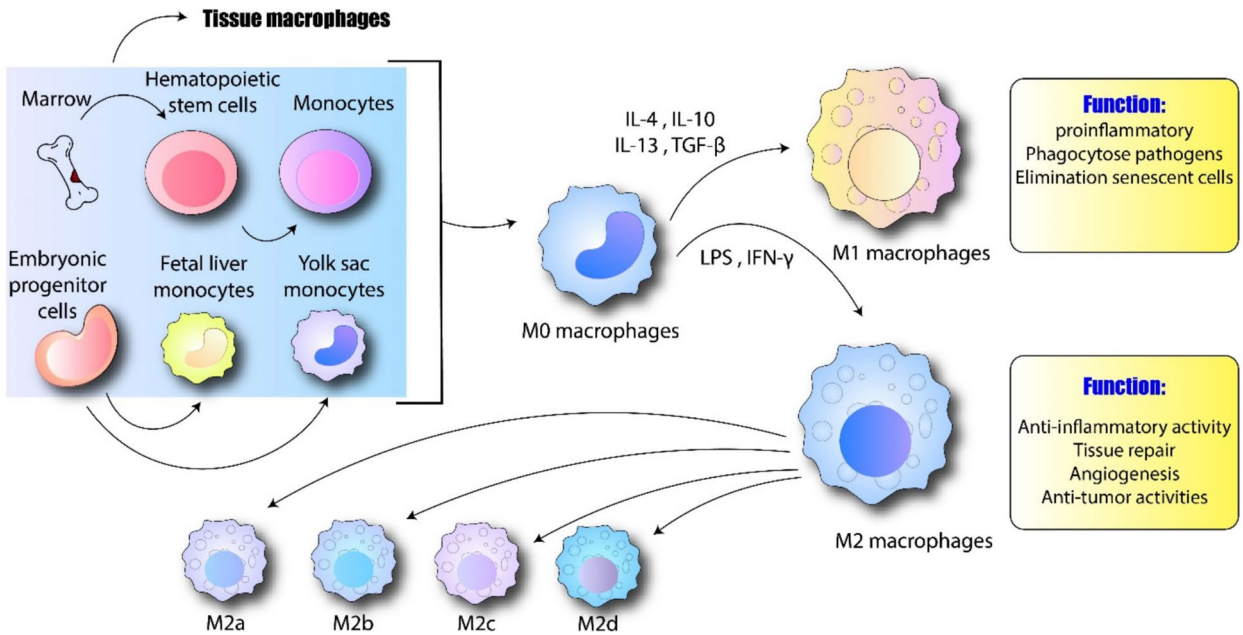

Fig. 3 Distinct features of macrophages, such as differentiation, formation, specialization, and role, are crucial for their development and functioning. One distinguishing trait of macrophages in humans is the presence of a blue coloration in the marker used to identify them

challenge of fully comprehending the overall impact of miRNAs on immune responses using a comprehensive and unified approach.

Macrophage polarization in sepsis

There are currently two dominant perspectives when it comes to explaining the beginnings of macrophages (Fig. 4). According to the initial details, it is proposed that the hematopoietic stem cells found in the bone marrow undergo a conversion into monocytes, which then travel through the bloodstream to different parts of the body. After arrival, these monocytes undergo a transformation into tissue-specific macrophages, a process which is greatly influenced by various factors including tissue damage, growth regulators in the area, and inflammation-causing proteins [80]. An alternate viewpoint suggests that progenitor cells in embryonic development transform into liver monocytes, then travel through the blood to eventually transform into yolk sac monocytes. The original cells eventually transform into larger tissue macrophages, and even embryonic macrophages are able to become part of the existing group of adult macrophages [81, 82]. The fast multiplication of immature macrophages is a critical factor in effectively eliminating waste materials during the reconstruction of tissues and eliminating surplus cells. In other words, mature macrophages are crucial for safeguarding the body as they perform important duties, including ingesting foreign

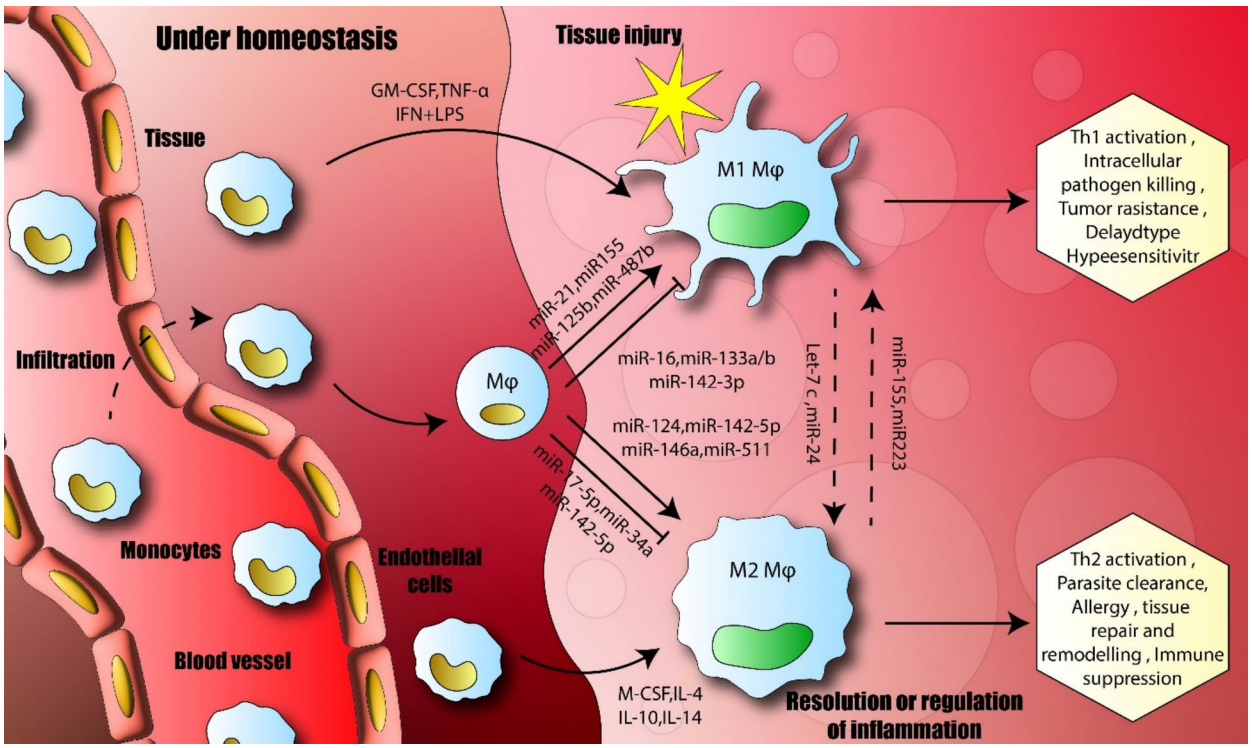

Fig. 4 Manipulation of microRNA (miRNA) to control the flexibility and differentiation of macrophages. The diagram shows how monocytes enter and transform into macrophages (MΦ) through two related but slightly different processes. Following exposure to inflammatory triggers, such as TNF-α, IFN-γ + LPS, or GM–CSF, monocytes undergo a transformation referred to as polarization, resulting in their conversion into M1 macrophages exhibiting increased and antagonistic inflammatory characteristics. As a result, certain genes (known as M1 markers) become more active and the cell displays distinct characteristics, such as type I immune response, elimination of intracellular pathogens, and protection against tumors. Certain miRNAs have been specifically chosen based on concrete evidence found in human primary macrophages, and the necessary sources for this evidence can be found in the main section of the text. In other words, higher levels of the inflammatory substances miR-155 and miR-125b could potentially serve as extra stimuli for M1 macrophages. Macrophages have the capability to undergo a conversion into a healing state known as M2, with the aid of specific cytokines, such as IL-4, IL-10, IL-13, or M-CSF. By controlling the activity of particular miRNAs, such as miR-146a and miR-511, the M2 features in MΦ are strengthened by suppressing the expression of genes that promote inflammation. These specific macrophages have a highly significant function in initiating Th2 immune reactions, eliminating parasites, decreasing ongoing inflammation, and fostering the healing and renewal of tissues. One important observation is that M1 and M2 macrophages are seen as a spectrum of two opposite cell types, instead of being two entirely separate phenotypes. Thus, tissue MΦ can demonstrate characteristics of both the aforementioned categories. The presence of let-7c and miR-24 induces a shift toward the M2 phenotype, while miR-155 and miR-223 can convert M2 macrophages into the M1 phenotype [114]

pathogens, dying or injured cells, displaying antigens, and generating immune signal molecules, such as IL-1 and TNF-α [83, 84]. Macrophages play essential roles in maintaining immune homeostasis and tissue repair across various organs. In the heart, they support tissue health by clearing waste and regulating heart rate, while in bone, they are involved in the formation and remodeling of bone tissue [85, 86]. In the liver, Kupffer cells and monocyte-derived macrophages defend against pathogens and aid tissue repair [87]. Alveolar macrophages in the lungs, generated by GM–CSF, help remove toxins, while adipose tissue macrophages regulate energy balance, although an excess can contribute to obesity [84, 88]. Macrophages in the intestines and pancreas are crucial for antigen presentation, tissue healing, and cytokine release, impacting inflammation and β-cell formation [89–91]. Perivascular macrophages in blood vessels are key in immune surveillance and maintaining vascular function [92]. In the central nervous system, microglia serve as the resident macrophages, playing a role in immune defense and maintaining brain health [93]. These diverse functions highlight the critical role of macrophages in tissue homeostasis and immune responses [83, 84]. The microscopic surroundings and signals can influence the way macrophages are organized, leading to diverse characteristics [94]. After being exposed to LPS and IFN-γ, macrophages undergo a conversion into a specific type known as M1 macrophages [95, 96]. The distinguishing characteristic of these M1 macrophages is their ability to engulf harmful microorganisms and eliminate ageing cells [97, 98]. Each specific marker found on the surface, such as TLR-2, TLR-4, CD68, CD80, CD86, CD64, MHC-II, and iNOS, plays a crucial role in recognizing and categorizing M1 macrophages present in the human body. On the other hand, CD86 and CD64 [86, 87] are not expressed in murine M1 macrophages. Various types of macrophages are essential in triggering the generation of various kinds of inflammatory molecules, including TNF-α, IL-1, IL-6, IL-12, CXCL9, CXCL10, and reactive oxygen species (ROS). Hence, this process carries the capability to stimulate inactive macrophages to transform into the M1 phenotype [99–101]. The process of generating M2 macrophages through alternative activation differs from the conventional activation method in that it relies on unique cytokines, such as IL-4, IL-10, IL-13, and TGF-β. There are four distinct types of M2 macrophages known as M2a, M2b, M2c, and M2 d. Each of these groups has a unique function in decreasing inflammation, stimulating the growth of fresh blood vessels, fighting against tumors, and assisting in the repair of tissues [94, 102]. Furthermore, the M2 subtype has been linked to alternative activation and tissue repair in both species, with the potential for therapeutic applications in human diseases. The M2 macrophages found in both mice and humans share several markers in common, including the mannose receptor, Arg1, CD206, CD163, CD209, IL1Rα, IL-1RII, FIZZ1, and Ym1/2. Nevertheless, the presence of CHIL3, a unique marker found only in humans, distinguishes them from mice. In addition, the M2 subtype has been associated with alternative activation and tissue healing in both species, suggesting possible treatment options for human diseases [94, 103–105]. I find it extremely fascinating to note that M2 macrophages have the ability to induce a transformation from unspecified macrophages to the M2 variant by releasing a diverse array of cytokines, such as IL-10, TGF-β, and CCLs 1, 17, and 18 [106]. It is crucial for the organism to reach a state of balance between the opposing states of M1 and M2 macrophages to function optimally. If there is an overabundance of M1 polarization, it can result in heightened inflammation and damage to cells [107–112]. Zhao and colleagues described a detailed computational model that describes macrophage polarization through three primary pathways: IFN-γ-driven, IL-4-driven, and hypoxia-driven. The model integrates signaling pathways, such as STAT1/IRF-1 for the M1 phenotype and STAT6/IRF-4 for the M2 phenotype, and it also includes post-transcriptional regulation by miRNAs. The computational model predicts the dynamics of macrophage polarization, the activation of key immune mediators, and the secretion of pro-inflammatory cytokines or anti-inflammatory factors [113].

MicroRNAs and macrophage polarization in sepsis

Macrophages have a vast array of potential roles which include serving as inflammatory M1 macrophages and non-inflammatory M2 macrophages. In M1 macrophages, there is a notable increase in the manifestation of miR-155. The complete elimination of mir155 and miR-155 antagomir from M1 macrophages significantly decreases the secretion of Inos, Il1b, Il6, Il12, and Tnf. Approximately 50% of the 650 genetic components comprising the M1 signature are predominantly regulated by miR-155 [115]. The presence of miR-130b-3p inhibits the activity of IRF1, preventing macrophages from transforming into the M1 phenotype. Furthermore, miR-130b-3p exhibits a potent ability to hinder the synthesis of crucial inflammatory substances, including CCL5, CXCL10, iNOS, and TNF [116]. One particular factor of note for the functioning of M2 macrophages is miR-223, which can control the actions of peroxisome proliferator-activated receptor-γ [117, 118]. Numerous miRNAs have been linked to the function and maturation of M1 macrophages. This group consists of various individual miRNAs including miR-9, miR-26a-2, miR-125a-3p, miR-125b, miR-127, miR-155-5p, miR-181a, miR-204-5p, and

miR-451. Furthermore, numerous studies have demonstrated the ability of certain miRNAs to influence the polarization and functioning of M2 macrophages. These miRNAs consist of miR-27a, miR-29b-1, miR-34a, miR-124, miR-125a-5p, miR-132, miR-143-3p, miR-145-5p, miR-146a-3p, miR-193b, miR-222, miR-223, and let-7c [119–122].

The researchers uncovered a momentous finding regarding the critical function of miR-9-5p, a type of microRNA, in initiating the activation of M1 macrophages. This was achieved through its specific binding to the NAD-dependent enzyme, SIRT1, in a study using mice with sepsis induced by cecal ligation and puncture (CLP) [123].

Several research studies have confirmed the advantageous impact of inhibiting TLR-NF-κB signaling on treating sepsis [124–130]. A possible strategy to address sepsis could involve targeting specific miRNAs that contribute to the activation of either M1 or M2 cells. The reason for this is that the TLR-NF-κB pathway has a crucial function in promoting M1 activation, which offers the possibility of effectively managing the situation [131]. To put it differently, the levels of miRNAs associated with the M2 subtype were noticeably diminished in sepsis patients compared to those with SIRS and healthy participants [132]. It has been scientifically verified that MiR-146a carries significant advantages for individuals affected by sepsis, as it effectively reduces the amounts of critical components within the TLR4-NF-κB pathway, such as IRAK1 and TRAF6. As a result, NF-κB activation is suppressed, leading to reduced occurrences of sepsis-induced heart problems, buildup of inflammatory cells, and release of inflammatory proteins [133]. According to the latest study by Benz and colleagues, the degree of miR-223 in the bloodstream cannot be relied upon as a reliable indicator for the future prospects or chances of survival for patients with sepsis in a critical condition [134]. The examination carried out on mice utilizing the cecal ligation and puncture (CLP) surgical method revealed that the levels of circulating miR-223 did not show any significant alterations following the occurrence of sepsis, at different intervals, in comparison with the control group. Individuals who were severely ill had a slightly lower level of miR-223 in their blood compared to those in good health [134]. It is worth noting that there were no noticeable variations in the levels of miR-223 between subjects with sepsis and those not affected by the condition. This contradicts the results from Wang et al., where they discovered a significant decrease in miR-223 in septic individuals in comparison with individuals with no health issues [132]. The disparity in findings from the two studies can be attributed in part to variations in the methods used during experimentation. The discrepancies noted may also be impacted by the variation in size and attributes of the groups of patients examined in these two studies.

The exact effects of macrophage polarization induced by miRNA in sepsis are still unclear, even after extensive studies on the changes in miRNA levels during the condition [124].

Wang and team reported a decline in miR-223 levels during the early stages of sepsis, but noted a gradual rise in macrophages as the condition progressed. The scientists found a clear connection between the concentrations of miR-223 and the presence of M2 macrophages in patients with sepsis [135]. After conducting experiments on mice lacking miR-223, they found that this microRNA was not crucial for triggering a strong pro-inflammatory reaction. However, it played a critical role in promoting the M2-related characteristics and abilities. The removal of miR-223 resulted in a higher severity of sepsis symptoms and ultimately, a higher death rate among the affected mice. In addition, our results suggest that the existence of IL-4, as opposed to IL-10, affects the degree of miR-223 presence in M2 macrophages. Inhibiting the activity of IL-4 using antibodies caused a reduction in the levels of miR-223 while also leading to higher levels of Nfat5 and Rasa1 and a lower percentage of M2 macrophages. As a result, the survival rates of septic mice experienced a decline. On the other hand, macrophages that were deficient in the miR-223 molecule experienced a notable reduction in their M2 polarization upon being stimulated by IL-4. Strangely, the absence of miR-223 did not impede the transformation of macrophages into the M2 phenotype caused by IL-10. Our research findings strongly support the idea that miR-223 significantly influences the shifting of macrophages from their original state to the M2 state, through its direct actions on Nfat5 and Rasa1. This emphasizes the significant role it plays in regulating the progression of sepsis. [117, 135].

Exosome and sepsis

Exosomes provide distinct methods for studying sepsis. Most of the written works have primarily concentrated on examining the existence of circulating vesicles within the blood vessels, with a specific emphasis on smaller vesicles called exosomes. The crucial role of exosomes containing significant miRNAs in promoting the healing process of various regions in the blood vessels cannot be overstated. Exosomes play a crucial role in sepsis by acting as key mediators of inflammation, contributing to the systemic inflammatory response that characterizes the condition; they carry various bioactive molecules, such as cytokines, damage-associated molecular patterns (DAMPs), and microRNAs, which can influence immune cell function, promote

organ dysfunction, and exacerbate the progression of sepsis across different stages of the disease [136, 137]. Furthermore, EVs have the capability to be isolated and analyzed for their various components, creating a potential avenue for identifying potential biomarkers and therapies.

After conducting more thorough research, it has been determined that exosomes could potentially have a significant impact on the aggravation of inflammation in cases of sepsis. Back in 2016, Balusu and his team made a groundbreaking discovery regarding a previously unknown way for the blood and brain to communicate, which becomes active in mice under conditions of inflammation. This occurs through the release of tiny particles known as exosomes containing microRNAs from the choroid plexus epithelium (CPE). The presence of systemic inflammation led to an increased abundance of exosomes and specific inflammatory microRNAs, namely, miR-146a and miR-155, in the cerebrospinal fluid (CSF) [138]. Exosomes play a vital role in the proper functioning of the brain, as they possess the remarkable capability of penetrating brain tissue and being taken in by astrocytes and microglia. Therefore, the activity of particular miRNAs is decreased and there is an increase in the expression of genes related to inflammation. After conducting a more thorough investigation into the examination of exosomes in mice, it was revealed that mice with sepsis exhibited a significant rise in the amount and dimensions of exosomes, which sharply differed from those observed in sepsis-free mice. The presence of septic exosomes significantly changed the behavior of neutrophils in the peritoneal cavity of mice [139]. The sorting of miRNAs into exosomes involves a variety of RNA-binding proteins (RBPs) and membranous proteins, such as hnRNPA2B1, play a significant role by binding to specific miRNAs, such as miR-198 and miR-17, and directing them into exosomes [140–142]. Some RBPs, such as Synaptotagmin-binding cytoplasmic RNA-interaction protein (SYNCRIP), also exhibit specificity for miRNAs enriched in exosomes, further supporting the idea that miRNA sorting is a selective, regulated process [143]. In addition, proteins such as Argonaute 2 (Ago2) are involved in sorting miRNAs into exosomes, with Ago2 phosphorylation affecting miRNA packaging and secretion [144, 145]. Y-Box Binding Protein 1 (YBX-1) selectively packages miR-133 and miR-223 into exosomes, with evidence showing that it does not affect miR-190, suggesting selective sorting by YBX-1 [146, 147]. Other proteins such as MEX3 C and Major Vault Protein (MVP) also regulate miRNA sorting into exosomes by interacting with Ago2 and other components involved in exosome biogenesis [148, 149].

Membranous proteins, such as Caveolin-1 (Cav-1) and Neural Sphingomyelinase 2 (nSMase2), play pivotal roles in exosome formation and miRNA sorting. Cav-1 is critical for the trafficking of certain miRNAs, including those bound to hnRNPA2B1, while nSMase2 regulates the secretion of miRNAs, such as miR-16 and miR-146a into exosomes [150, 151]. Vps4 A, involved in endosomal trafficking, has been implicated in sorting oncomiRs into exosomes, promoting tumor progression and metastasis [152, 153]. Despite the progress made, the exact mechanisms behind selective miRNA sorting and packaging into exosomes remain incompletely understood. More research is needed to clarify these pathways, including the proteins involved in the sorting process and their regulation in different disease states, such as cancer and heart disease.

Reithmair et al., investigated exosomal and intracellular miRNAs as diagnostic tools for sepsis. MiRNA profiles from serum exosomes, total serum, and blood cells (leukocytes, erythrocytes, platelets) of sepsis patients were characterized using next-generation sequencing and RT–qPCR. A total of 77 miRNAs were down-regulated, and 103 miRNAs were up-regulated in septic shock, with many of these miRNAs (14 in serum, 32 in exosomes, and 73 in blood cells) not previously linked to sepsis. Compartment-specific regulation of miRNAs was observed between sepsis patients and healthy volunteers. miR-199b-5p was identified as a potential early indicator for sepsis, while miR-125b-5p and miR-26b-5p were uniquely regulated in exosomes and serum, respectively. miR-27b-3p was present in all compartments. The findings suggest that exosome-derived miRNAs hold valuable information for sepsis diagnosis and survival prediction and may serve as potential targets for novel sepsis biomarkers [154].

Deng and colleagues investigated the antiapoptotic effect of circulating exosomes derived from the plasma of septic patients (Sepsis-Exos) on T lymphocytes was discovered and further investigated. Next-generation sequencing (NGS) revealed significant changes in the exosomal miRNA expression profile during sepsis, with hsa-miR-7-5p being overexpressed. Dual luciferase reporter assays showed that hsa-miR-7-5p negatively regulated the proapoptotic gene Bad, which is part of the cGMP–PKG signaling pathway. Sepsis-Exos were found to reduce the mRNA and protein levels of proapoptotic genes Bad, active Caspase-3, and Bax while increasing the expression of the antiapoptotic gene Bcl-2 via hsa-miR-7-5p, thereby inhibiting apoptosis of T lymphocytes induced by lipopolysaccharide (LPS) in vitro. In vivo, Sepsis-Exos also inhibited T lymphocyte apoptosis during sepsis, leading to a reduction in the mortality rate of septic model mice. This study provides evidence that

Sepsis-Exos contribute to reducing T lymphocyte apoptosis by directly suppressing Bad through hsa-miR-7-5p [155].

Subramani et al. (2018) found in their research that septic exosomes carry high concentrations of specific miRNAs and proinflammatory markers. To effectively combat the detrimental impact of exosomes, specific inhibition of miR-34a, miR-122, and miR-146a was employed with miRNA inhibitors, resulting in significant reduction of their negative effects. This research diverged from the conventional approach of studying inflammatory responses by examining alterations in red blood cell behavior and the influence of plasma exosomes in mice with sepsis [156]. After administering exosomes treatment from septic mice, a notable reduction in RBC deformability was observed in a study conducted on ex vivo samples following sepsis. In addition, there were notable distinctions in the composition of exosomes in sepsis-afflicted mice, characterized by a substantial spike in levels of miR-6538 and miR-2137. A thorough investigation was performed to analyze the involvement of exosomes in sepsis, utilizing a human research study to assess the quantities of miRNA in total serum, serum exosomes, and blood cells, with the aim of detecting any discrepancies among these constituents [154]. The group of investigators discovered a variety of miRNAs that exhibited varying levels of regulation in cases of sepsis. In all three compartments, miR-27b-3p was detected. Upon thorough examination of all components, it has been determined that miR-199b-5p possesses the potential to function as an initial signal for sepsis and septic shock in the blood cells. Moreover, changes in the concentrations of miR-125b-5p and miR-26b-5p were noted in both the exosomes and serum. Fully grasping the vast array of these miRNAs and their importance in various environmental settings, and comprehending the consequences of altering them for healthcare outcomes, continues to be a challenge. Real and his team achieved a significant advancement in the field of sepsis diagnosis within medical settings by effectively utilizing exosomes in 2018 [157]. The study revealed that individuals with sepsis had a prolonged existence of 35 particular miRNAs in exosomes over a span of 7 days, which differed from those without sepsis. In addition, this study demonstrated that exosomal miRNAs could be categorized based on the survival rate of patients and implied a potential connection between aberrations in the cell cycle and sepsis.

Mao and colleagues investigated the potential therapeutic ability of exosomes in septic cardiomyopathy. Their study explores the use of engineered exosomes to protect cardiac function and treat septic cardiomyopathy. It highlights three key strategies: modifying exosome surfaces, using exosomes as multifunctional drug delivery platforms, and employing plant exosome-like nanoparticles as carriers. The article emphasizes exosomes'ability to deliver small molecules, proteins, and drugs, including RNA molecules and proteins beneficial for treating septic cardiomyopathy. Despite their promise as biotherapeutic carriers, challenges remain in clinical application, such as understanding their interaction with host cells, distribution, metabolism, and long-term safety. Continued research is necessary to fully harness the potential of engineered exosomes for treating septic cardiomyopathy [158].

In their study, Zhou and colleagues (2018) adopted a different strategy in investigating the therapeutic significance of exosomes in sepsis [159]. EPCs have been extensively proven to be effective in repairing injured tissues located far from the source through their ability to migrate and primarily release exosomes loaded with beneficial components. The researchers discovered that septic mice who were treated with EPC exosomes experienced enhanced survival rates, decreased leakage in their lungs and kidneys, and improved liver and kidney function. The research revealed that the EPC exosomes contained a significant quantity of miRNA, specifically, miR-126-5p and miR-126-3p. These two miRNAs are well-documented to be present in high levels in endothelial cells. Studies carried out on endothelial cells in a laboratory setting have produced significant findings, suggesting that the coexistence of miR-126-5p and miR-126-3p within exosomes can effectively reduce the expression of inflammatory biomarkers induced by LPS. The accuracy of these results was validated by the suppression of miR-126-5p and −3p, effectively nullifying the beneficial impacts of EPC exosomes.

Exosome and macrophage polarization in sepsis

In the study, researchers utilized a mouse model to illustrate the detrimental impact of lipids on non-alcoholic fatty liver disease (NAFLD). Their findings showed that a diet high in fat and cholesterol triggered the production of hepatocyte exosomes carrying miR-192-5p. This underscores the importance of lipids in playing a role in the development of NAFLD. An extraordinary finding has revealed the vital function of this mechanism in promoting the activation of M1 macrophages. The research findings showed a crucial involvement of miR-192-5p in inducing an inflammatory M1 state in macrophages through modulation of the Rictor/Akt/FoxO1 pathway. In conclusion, the study determined that miR-192-5p found in exosomes plays a significant role in triggering M1 macrophages, leading to liver inflammation in NAFLD [160]. The demonstrable effectiveness of miR-192-5p in blocking the M1 macrophage polarization was evident in

a study on gouty arthritis. The study utilized crystals as a disease trigger [161]. The injection of the MiR-192-5p imitator greatly influenced the RAW264.7 macrophages, which were initially in a state of polarization toward M1 due to their exposure to IFN-γ and LPS. This led to a significant reduction in the production of TNF-α and IL-1β, a decrease in iNOS levels, and a suppression of CD16/32 expression, which signifies M1 activation. Suppressing miR-192-5p significantly impeded the development of M1 macrophages, resulting in a reduction of the inflammatory response caused by GA as epiregulin was prevented [161]. The disparity in the impact of miR-192-5p on the macrophage program in the two disease models can be elucidated by examining the origins of the miRNA and the macrophages involved, as these play a crucial role in determining their effects. MiR-199a-5p, originating from HK-2 cells, targets the Klotho/TLR4 pathway through exosomes after being stimulated by human serum albumin (HSA). This leads to the successful induction of M1 phenotype polarization. This ultimately had a major impact on the progress of diabetic nephropathy [162]. In a study involving mice, researchers found that a diet rich in fats led to an excessive production of miR-9-5p in exosomes among those with non-alcoholic fatty liver disease. This may contribute to the development of lipotoxicity. As a result, there was an increase in M1 activation by specifically focusing on glutaminyl transferase 2 (TGM2) [163].

During a carefully conducted scientific study, researchers observed that exosomal miR-30 d-5p, a specific form of genetic material, produced by immune cells when exposed to a protein called TNF-α, played a crucial role in regulating the transformation of M1 macrophages and their impact on the development of a particular form of cell death called macrophage pyroptosis in cases of sepsis-related acute lung injury in mice. The researchers effectively stimulated the NF-κB signaling pathway in both simulated conditions and live mice by targeting SOCS-1 and SIRT1, ultimately achieving the desired result [164].

Neutrophils, a type of white blood cell, play a critical role in the development of sepsis-related acute lung injury or ARDS in animals [165]. At a size ranging from 30 to 150 nm, exosomes are generated by a variety of cells and then discharged into the outermost area [166]. The function they possess entails transferring various proteins and hereditary materials, such as DNA, mRNA, and microRNA (miRNA), to specific cells, which is essential for facilitating communication among cells [167]. Extensive investigation using animal subjects has conclusively demonstrated that utilizing exosomes derived from polymorphonuclear leukocytes (PMNs) has a significant impact on numerous persistent respiratory disorders, including chronic obstructive pulmonary disease (COPD), bronchopulmonary dysplasia, and asthma. Despite this, the exact way in which they affect sepsis-induced acute lung injury (ALI) remains unclear [168, 169].

Collaborating with PMNs, M1 macrophages (known for their pro-inflammatory characteristics) had a significant influence on the onset of acute lung injury caused by sepsis. Exosomes found in the blood have the power to induce the activation of M1 macrophages. Therefore, a greater number of signals are being generated to initiate the inflammatory response, including IL-1β, IL-12, IL-6, and TNF-α, among various others [170, 171]. The emphasis now is on understanding how macrophages contribute to this process and how it can be targeted for potential treatments. The current conversation is now focused on examining how sepsis-induced acute lung injury may be affected by pyroptosis, a process mediated by macrophages. Pyroptosis is a distinct type of cellular demise that is initiated by the activation of caspase-1 and results in inflammatory responses. The central aim is to decipher the function of macrophages in this process and determine possible methods of intervention for medical treatment [165, 172]. Pyroptosis, a cellular mechanism, is triggered by caspase-1, an enzyme that is activated by inflammation-inducing complexes called NLRP3 inflammasomes. By breaking down gasdermin D (GSDMD), the enzyme caspase-1 generates openings in the external layer of the cell. As a result, the cells increase in size and eventually rupture, leading to the release of the complete versions of the inflammatory proteins IL-1β and IL-18 from the cell [173, 174]. When sepsis causes acute lung injury, the body's inflammatory reaction is worsened by the release of danger signals or molecules known as danger-associated molecular patterns from macrophages undergoing pyroptosis [175].

The critical role of regulating inflammation has been widely recorded to involve the interaction between PMNs and macrophages [176–179]. The provided evidence strongly suggests the involvement of exosomes in the pathophysiology of hemorrhagic shock [178]. In the case of sepsis, macrophage pyroptosis may be triggered not only by the presence of bacteria, but also by the formation of NETs [180]. Despite their significant involvement in the development of acute lung injury in sepsis patients, the effects of PMN-released exosomes on macrophages and the mechanisms behind them are not fully understood. The insufficient investigations in this field contribute to the lack of clarity.

In both living organisms and laboratory settings, the stimulation of M1 macrophages occurred due to the release of exosomes (TNF-Exo) from TNF-α-stimulated PMNs [164]. The administration of TNF-Exo was found

to trigger the occurrence of pyroptosis in macrophages, primarily through the activation of the NLRP3 inflammasome and the facilitation of the NF-κB signaling pathway. Following a thorough examination, it has been confirmed that miR-30 d-5p is vital in regulating the activity of TNF-Exo through its targeted interaction with two key proteins, SOCS-1 and SIRT1, found within macrophages. In addition, the use of intravenous miR-30 d-5p suppressors resulted in a significant decrease in the activation and death rates of M1 macrophages in the lungs caused by TNF-Exo or CLP. The study effectively showed that the decrease in histological damage was directly connected to the outcome that was mentioned. This supports the significance of exosomal miR-30 d-5p, sourced from PMNs, in the progression of ALI caused by sepsis. After conducting thorough research, it was determined that the initiation of the NF-κB signaling pathway resulted in the differentiation of M1 macrophages and instigated the onset of pyroptosis within these cells. The findings suggest a novel approach for understanding the relationship between PMN-Mϕ in sepsis-induced ALI, providing potential alternative therapies for patients with sepsis [164].

Studies have identified miRNA expression profiles in M1 and M2 polarized macrophages using techniques, such as microarray and RT–qPCR. miR-9, miR-127, miR-155, and miR-125b promote M1 polarization, while miR-124, miR-223, miR-34a, let-7c, miR-132, miR-146a, and miR-125a-5p promote M2 polarization by targeting various transcription factors and adaptor proteins [122]. Thus, quantitative measurement of miRNAs could be used for prediction of macrophage polarization.

Conclusion

Exosomes and miRNAs are emerging as pivotal regulators of macrophage polarization in sepsis, influencing the balance between pro-inflammatory and anti-inflammatory responses. However, the mechanistic relationship between these molecules and their role in macrophage polarization remains insufficiently explored. This review has highlighted the significant potential of exosomes and miRNAs in modulating the immune response during sepsis, but it is evident that current research lacks a comprehensive, critical analysis of how these factors interact and influence disease progression.

To move the field forward, further investigation is needed to elucidate the complex mechanisms by which exosomes and miRNAs affect macrophage function in sepsis. Future studies should aim to identify the specific miRNAs involved, their targets, and how their dysregulation contributes to the immune dysfunction observed in sepsis. Moreover, addressing gaps in our understanding of exosome biology—such as optimal isolation methods and modification techniques—will be essential for harnessing their therapeutic potential. Future studies should also be focused on the enhancement of use of exosomes for sepsis treatment. Beside of exosome engineering for targeted delivery, combining exosome-based therapies with traditional drugs could amplify therapeutic outcomes. For example, exosome-mediated delivery of miRNA mimics or inhibitors could be paired with immune-modulating agents or antibiotics to enhance the effectiveness of treatments for sepsis or other inflammatory diseases. This could allow for more targeted and efficient therapeutic strategies, minimizing side effects typically seen with systemic drug delivery. Furthermore, for clinical translation, exosome-based therapies could be further optimized for in vivo applications. Exosome carriers can be engineered to improve their stability in the bloodstream, enhance their ability to cross biological barriers (such as the blood–brain barrier), and increase their cellular uptake. These enhancements would allow for the more effective delivery of therapeutic miRNAs to target organs and cells, especially in complex diseases, such as cancer or neurodegenerative disorders.

The use of patient-derived exosomes and animal models in research holds great promise for advancing our understanding of diseases and developing targeted therapies. However, ethical implications need to be carefully considered, particularly regarding the use of patient samples and animal models. When using patient-derived exosomes, researchers must obtain informed consent, ensuring that patients are fully aware of how their biological samples will be used, including potential therapeutic applications. Privacy and confidentiality of patient information should also be maintained in accordance with ethical guidelines and regulations.

In the case of animal models, ethical concerns arise regarding the treatment of animals and their humane use in experiments. Animal welfare protocols must be followed, and the principle of the 3Rs (Replacement, Reduction, and Refinement) should be applied to minimize animal suffering and the number of animals used in experiments.

Moreover, the standardization of exosome isolation techniques is a critical issue. Variability in isolation methods can lead to inconsistent results, making it difficult to compare findings across studies. Establishing standardized protocols for exosome isolation is essential to ensure reproducibility and reliability of research, as well as to enhance the credibility of findings that could potentially translate into clinical applications. Clear guidelines and ethical oversight are necessary to ensure that both patient-derived exosomes and animal

models are used responsibly and with respect for ethical standards.

While the current body of research offers valuable insights, there is a need for a more integrated approach that combines cutting-edge technologies and a deeper understanding of molecular interactions. Novel experimental models and a focus on translational research could provide critical information for developing miRNA-based therapies or exosome-mediated interventions. By exploring these avenues, we could improve the diagnosis, prevention, and treatment of sepsis, ultimately leading to better clinical outcomes.

Abbreviations

AGO	Argonaute
Ali	Acute lung injury
Arg1	Arginase 1
ARDS	Acute respiratory distress syndrome
CCL5	C–C motif chemokine ligand 5
CD	Cluster of differentiation
CLP	Cecal ligation and puncture
COPD	Chronic obstructive pulmonary disease
CPE	Choroid plexus epithelium
C/EBP	CCAAT/enhancer-binding protein
DAMPs	Damage-associated molecular patterns
DGCR8	DiGeorge syndrome critical region 8
DIC	Disseminated intravascular coagulation
ECM	Extracellular matrix
EV	Extracellular vesicles
FIZZ1	Found in inflammatory zone 1
GM–CSF	Granulocyte–macrophage colony-stimulating factor
H3 K4 me3	Histone 3 lysine 4 trimethylation
HIF	Hypoxia-inducible factor
HIV	Human immunodeficiency virus
hnRNPs	Heterogeneous nuclear ribonucleoproteins
ICAM-1	Intercellular adhesion molecule 1
IFN-γ	Interferon gamma
IRF	Interferon regulatory factor
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MAMPs	Microbe-associated molecular patterns
MEX3 C	Mex-3 homolog C
miRNA	MicroRNA
NAFLD	Non-alcoholic fatty liver disease
NETs	Neutrophil extracellular traps
Nfat5	Nuclear factor of activated T cells 5
NFS1	Cysteine desulfurase
NF-kB	Nuclear factor kappa B
nSMase2	Neural sphingomyelinase 2
PACT	Protein activator of the interferon-induced protein kinase
PI3 K	Phosphoinositide 3-kinase
PTEN	Phosphatase and tensin homolog
Rasa1	Ras GTPase-activating protein 1
RISC	RNA-Induced silencing complex
RT–qPCR	Reverse transcription–quantitative polymerase chain reaction
SOCS	Suppressors of cytokine signaling
STAT1	Signal transducer and activator of transcription
SYNCRIP	Synaptotagmin-binding cytoplasmic RNA-interaction protein
TGF-β	Transforming growth factor beta
TLR4	Toll-like receptor 4
TNF-α	Tumor necrosis factor alpha
TRAF-F6	TNF receptor-associated factor 6
Vps4 A	Vacuolar protein sorting-associated protein 4
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

Acknowledgements
Not applicable.

Author contributions
Mohamed J. Saadh, Karar H. Alfarttoosi, Gaurav Sanghvi, R. Roopashree, Vishal Thakur, L. Lakshmi, Aziz Kubaev, Waam Mohammed Taher, Mariem Alwan, Mahmood Jasem Jawad, Ali M. Ali Al-Nuaimi: Investigation; methodology (equal); writing—original draft (equal). Tamara Nazar Saeed: Conceptualization (equal); funding acquisition (lead); investigation (lead); resources; supervision; writing—review and editing. All authors confirmed the final version of manuscript.

Funding
Not applicable.

Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Faculty of Pharmacy, Middle East University, Amman 11831, Jordan. [2]Department of Medical Laboratory Technics, College of Health and Medical Technology, Alnoor University, Mosul, Iraq. [3]College of Pharmacy, Ahl Al-Bayt University, Kerbala, Iraq. [4]Department of Microbiology, Faculty of Science, Marwadi University Research Center, Marwadi University, Rajkot, Gujarat 360003, India. [5]Department of Chemistry and Biochemistry, School of Sciences, JAIN (Deemed to Be University), Bangalore, Karnataka, India. [6]Centre for Research Impact & Outcome, Chitkara University Institute of Engineering and Technology, Chitkara University, Rajpura 140401, Punjab, India. [7]Department of Nursing, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India. [8]Department of Maxillofacial Surgery, Samarkand State Medical University, 18 Amir Temur Street, 140100 Samarkand, Uzbekistan. [9]College of Nursing, National University of Science and Technology, Dhi Qar, Iraq. [10]Pharmacy College, Al-Farahidi University, Baghdad, Iraq. [11]Department of Pharmacy, Al-Zahrawi University College, Karbala, Iraq. [12]Gilgamesh Ahliya University, Baghdad, Iraq.

Received: 26 January 2025 Accepted: 6 April 2025
Published online: 17 April 2025

References

1. Fleischmann C, Scherag A, Adhikari NK, Hartog CS, Tsaganos T, Schlattmann P, et al. Assessment of global incidence and mortality of hospital-treated *Sepsis*. Current estimates and limitations. Am J Respir Crit Care Med. 2016;193(3):259–72.
2. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for *Sepsis* and *Septic* shock (Sepsis-3). JAMA. 2016;315(8):801–10.
3. Moreira J. Severe *Sepsis* and *Septic* shock. N Engl J Med. 2013;369(21):2063.
4. Chen X, Liu Y, Gao Y, Shou S, Chai Y. The roles of macrophage polarization in the host immune response to *Sepsis*. Int Immunopharmacol. 2021;96: 107791.
5. Yang W, Tao K, Zhang P, Chen X, Sun X, Li R. Maresin 1 protects against lipopolysaccharide/d-galactosamine-induced acute liver injury by inhibiting macrophage pyroptosis and inflammatory response. Biochem Pharmacol. 2022;195: 114863.

6. Grailer JJ, Haggadone MD, Sarma JV, Zetoune FS, Ward PA. Induction of M2 regulatory macrophages through the β2-adrenergic receptor with protection during endotoxemia and acute lung injury. J Innate Immun. 2014;6(5):607–18.
7. Deiuliis JA. MicroRNAs as regulators of metabolic disease: pathophysiologic significance and emerging role as biomarkers and therapeutics. Int J Obes (Lond). 2016;40(1):88–101.
8. Liu G, Abraham E. MicroRNAs in immune response and macrophage polarization. Arterioscler Thromb Vasc Biol. 2013;33(2):170–7.
9. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science. 2020. https://doi.org/10.1126/science.aau6977.
10. Hu F, Lou N, Jiao J, Guo F, Xiang H, Shang D. Macrophages in pancreatitis: mechanisms and therapeutic potential. Biomed Pharmacother. 2020;131: 110693.
11. Tatischeff I. Current search through liquid biopsy of effective biomarkers for early cancer diagnosis into the rich cargoes of extracellular vesicles. Int J Mol Sci. 2021. https://doi.org/10.3390/ijms22115674.
12. Colombo M, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu Rev Cell Dev Biol. 2014;30:255–89.
13. Zhu J, Liu B, Wang Z, Wang D, Ni H, Zhang L, et al. Exosomes from nicotine-stimulated macrophages accelerate atherosclerosis through miR-21-3p/PTEN-mediated VSMC migration and proliferation. Theranostics. 2019;9(23):6901–19.
14. Chamberlain CS, Kink JA, Wildenauer LA, McCaughey M, Henry K, Spiker AM, et al. Exosome-educated macrophages and exosomes differentially improve ligament healing. Stem Cell. 2021;39(1):55–61.
15. Li Z, Wang Y, Li S, Li Y. Exosomes derived from M2 macrophages facilitate osteogenesis and reduce adipogenesis of BMSCs. Front Endocrinol (Lausanne). 2021;12: 680328.
16. Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993;75(5):843–54.
17. Wightman B, Ha I, Ruvkun G. Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. Cell. 1993;75(5):855–62.
18. Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, et al. The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. Nature. 2000;403(6772):901–6.
19. Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, et al. Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature. 2000;408(6808):86–9.
20. Hammond SM, Bernstein E, Beach D, Hannon GJ. An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature. 2000;404(6775):293–6.
21. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. Science. 2001;294(5543):853–8.
22. Alles J, Fehlmann T, Fischer U, Backes C, Galata V, Minet M, et al. An estimate of the total number of true human miRNAs. Nucl Acid Res. 2019;47(7):3353–64.
23. de Rie D, Abugessaisa I, Alam T, Arner E, Arner P, Ashoor H, et al. An integrated expression atlas of miRNAs and their promoters in human and mouse. Nat Biotechnol. 2017;35(9):872–8.
24. Lee Y, Jeon K, Lee JT, Kim S, Kim VN. MicroRNA maturation: stepwise processing and subcellular localization. Embo j. 2002;21(17):4663–70.
25. Li Z, Rana TM. Therapeutic targeting of microRNAs: current status and future challenges. Nat Rev Drug Discov. 2014;13(8):622–38.
26. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of MicroRNA biogenesis, mechanisms of actions, and circulation. Front Endocrinol (Lausanne). 2018;9:402.
27. Vishnoi A, Rani S. miRNA biogenesis and regulation of diseases: an updated overview. MicroRNA profiling: methods and protocols. New York: Springer; 2022. p. 1–12.
28. Leitão AL, Enguita FJ. A structural view of miRNA biogenesis and function. Non-coding RNA. 2022;8(1):10.
29. Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH, et al. MicroRNA genes are transcribed by RNA polymerase II. Embo j. 2004;23(20):4051–60.
30. Borchert GM, Lanier W, Davidson BL. RNA polymerase III transcribes human microRNAs. Nat Struct Mol Biol. 2006;13(12):1097–101.
31. Denli AM, Tops BB, Plasterk RH, Ketting RF, Hannon GJ. Processing of primary microRNAs by the Microprocessor complex. Nature. 2004;432(7014):231–5.
32. Han J, Lee Y, Yeom KH, Kim YK, Jin H, Kim VN. The Drosha-DGCR8 complex in primary microRNA processing. Gen Dev. 2004;18(24):3016–27.
33. Kim H, Lee YY, Kim VN. The biogenesis and regulation of animal microRNAs. Nat Rev Mol Cell Biol. 2024. https://doi.org/10.1038/s41580-024-00805-0.
34. Bofill-De Ros X, Vang Ørom UA. Recent progress in miRNA biogenesis and decay. RNA Biol. 2024;21(1):36–43.
35. Li M, Yu H, Zhou B, Gan L, Li S, Zhang C, et al. JANUS, a spliceosome-associated protein, promotes miRNA biogenesis in *Arabidopsis*. Nucl Acid Res. 2024;52(1):420–30.
36. Zhou B, Yu H, Xue Y, Li M, Zhang C, Yu B. The spliceosome-associated protein CWC15 promotes miRNA biogenesis in *Arabidopsis*. Nat Commun. 2024;15(1):2399.
37. Lin SL, Chang D, Wu DY, Ying SY. A novel RNA splicing-mediated gene silencing mechanism potential for genome evolution. Biochem Biophys Res Commun. 2003;310(3):754–60.
38. Lee Y, Hur I, Park SY, Kim YK, Suh MR, Kim VN. The role of PACT in the RNA silencing pathway. Embo j. 2006;25(3):522–32.
39. Redfern AD, Colley SM, Beveridge DJ, Ikeda N, Epis MR, Li X, et al. RNA-induced silencing complex (RISC) Proteins PACT, TRBP, and Dicer are SRA binding nuclear receptor coregulators. Proc Natl Acad Sci U S A. 2013;110(16):6536–41.
40. Vardapour R. Mutations in the DROSHA/DGCR8 microprocessor complex in high-risk blastemal Wilms tumor. Würzburg: Universität Würzburg; 2022.
41. Pidíková P, Chovancová B, Mravec B, Herichová I. The 24-h pattern of dgcr8, drosha, and dicer expression in the rat suprachiasmatic nuclei and peripheral tissues and its modulation by angiotensin II. Gen Physiol Biophys. 2022. https://doi.org/10.4149/gpb_2022033.
42. Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol. 2014;15(8):509–24.
43. Treiber T, Treiber N, Meister G. Publisher correction: regulation of microRNA biogenesis and its crosstalk with other cellular pathways. Nat Rev Mol Cell Biol. 2019;20(5):321.
44. Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet. 2010;11(9):597–610.
45. Benz F, Roy S, Trautwein C, Roderburg C, Luedde T. Circulating MicroRNAs as biomarkers for *Sepsis*. Int J Mol Sci. 2016. https://doi.org/10.3390/ijms17010078.
46. van der Poll T, Shankar-Hari M, Wiersinga WJ. The immunology of *Sepsis*. Immunity. 2021;54(11):2450–64.
47. Hotchkiss RS, Moldawer LL, Opal SM, Reinhart K, Turnbull IR, Vincent JL. *Sepsis* and *septic* shock. Nat Rev Dis Prim. 2016;2:16045.
48. Kerrigan SW, Devine T, Fitzpatrick G, Thachil J, Cox D. Early host interactions that drive the dysregulated response in *Sepsis*. Front Immunol. 2019;10:1748.
49. Dewitte A, Lepreux S, Villeneuve J, Rigothier C, Combe C, Ouattara A, et al. Blood platelets and sepsis pathophysiology: a new therapeutic prospect in critically [corrected] ill patients? Ann Intensiv Care. 2017;7(1):115.
50. Kuosmanen SM, Kansanen E, Sihvola V, Levonen AL. MicroRNA profiling reveals distinct profiles for tissue-derived and cultured endothelial cells. Sci Rep. 2017;7(1):10943.
51. Krammer TL, Mayr M, Hackl M. microRNAs as promising biomarkers of platelet activity in antiplatelet therapy monitoring. Int J Mol Sci. 2020. https://doi.org/10.3390/ijms21103477.
52. Zhong L, Simard MJ, Huot J. Endothelial microRNAs regulating the NF-κB pathway and cell adhesion molecules during inflammation. Faseb j. 2018;32(8):4070–84.
53. Shu Z, Tan J, Miao Y, Zhang Q. The role of microvesicles containing microRNAs in vascular endothelial dysfunction. J Cell Mol Med. 2019;23(12):7933–45.
54. Etzrodt V, Idowu TO, Schenk H, Seeliger B, Prasse A, Thamm K, et al. Role of endothelial microRNA 155 on capillary leakage in systemic inflammation. Crit Care (London England). 2021;25(1):76.
55. Nahid MA, Pauley KM, Satoh M, Chan EK. miR-146a is critical for endotoxin-induced tolerance: implication in innate immunity. J Biol Chem. 2009;284(50):34590–9.

56. Renzi TA, Rubino M, Gornati L, Garlanda C, Locati M, Curtale G. MiR-146b mediates endotoxin tolerance in human phagocytes. Med Inflamm. 2015;2015: 145305.
57. El Gazzar M, Church A, Liu T, McCall CE. MicroRNA-146a regulates both transcription silencing and translation disruption of TNF-α during TLR4-induced gene reprogramming. J Leukoc Biol. 2011;90(3):509–19.
58. Quinn EM, Wang JH, O'Callaghan G, Redmond HP. MicroRNA-146a is upregulated by and negatively regulates TLR2 signaling. PLoS ONE. 2013;8(4): e62232.
59. Doxaki C, Kampranis SC, Eliopoulos AG, Spilianakis C, Tsatsanis C. Coordinated regulation of miR-155 and miR-146a genes during induction of endotoxin tolerance in macrophages. J Immunol. 2015;195(12):5750–61.
60. Mandel J, Casari M, Stepanyan M, Martyanov A, Deppermann C. Beyond hemostasis: platelet innate immune interactions and thromboinflammation. Int J Mol Sci. 2022. https://doi.org/10.3390/ijms23073868.
61. Graham SM, Liles WC. Platelets in sepsis: beyond hemostasis. Blood. 2016;127(24):2947–9.
62. Laffont B, Corduan A, Plé H, Duchez AC, Cloutier N, Boilard E, et al. Activated platelets can deliver mRNA regulatory Ago2•microRNA complexes to endothelial cells via microparticles. Blood. 2013;122(2):253–61.
63. Laffont B, Corduan A, Rousseau M, Duchez AC, Lee CH, Boilard E, et al. Platelet microparticles reprogram macrophage gene expression and function. Thromb Haemost. 2016;115(2):311–23.
64. Szilágyi B, Fejes Z, Póliska S, Pócsi M, Czimmerer Z, Patsalos A, et al. Reduced miR-26b expression in megakaryocytes and platelets contributes to elevated level of platelet activation status in *Sepsis*. Int J Mol Sci. 2020. https://doi.org/10.3390/ijms21030866.
65. Peters LJF, Baaten C, Maas SL, Lu C, Nagy M, Jooss NJ, et al. MicroRNA-26b Attenuates Platelet Adhesion and Aggregation in Mice. Biomedicines. 2022. https://doi.org/10.3390/biomedicines10050983.
66. Vieira Corrêa PCM, Carneiro DM, da Silva Valente LDS, Diogo FM, Lamarão LM, da Silva Maués JH, et al. Detection of *Sepsis* in platelets using MicroRNAs and membrane antigens. Genes (Basel). 2021. https://doi.org/10.3390/genes12121877.
67. Garcia A, Dunoyer-Geindre S, Zapilko V, Nolli S, Reny JL, Fontana P. Functional validation of microRNA-126-3p as a platelet reactivity regulator using human haematopoietic stem cells. Thromb Haemost. 2019;119(2):254–63.
68. Jiao Y, Li W, Wang W, Tong X, Xia R, Fan J, et al. Platelet-derived exosomes promote neutrophil extracellular trap formation during septic shock. Crit Care (London England). 2020;24(1):380.
69. Szilágyi B, Fejes Z, Rusznyák Á, Fenyvesi F, Pócsi M, Halmi S, et al. Platelet microparticles enriched in miR-223 reduce ICAM-1-dependent vascular inflammation in *Septic* conditions. Front Physiol. 2021;12: 658524.
70. Heumann D, Roger T. Initial responses to endotoxins and gram-negative bacteria. Clin Chim Acta. 2002;323(1–2):59–72.
71. Roger T, Froidevaux C, Le Roy D, Reymond MK, Chanson AL, Mauri D, et al. Protection from lethal gram-negative bacterial sepsis by targeting toll-like receptor 4. Proc Natl Acad Sci U S A. 2009;106(7):2348–52.
72. Chen CZ, Schaffert S, Fragoso R, Loh C. Regulation of immune responses and tolerance: the microRNA perspective. Immunol Rev. 2013;253(1):112–28.
73. Mehta A, Baltimore D. MicroRNAs as regulatory elements in immune system logic. Nat Rev Immunol. 2016;16(5):279–94.
74. Kumar Kingsley SM, Vishnu BB. Role of MicroRNAs in the development and function of innate immune cells. Int Rev Immunol. 2017;36(3):154–75.
75. Zhou X, Li X, Wu M. miRNAs reshape immunity and inflammatory responses in bacterial infection. Signal Transduct Target Ther. 2018;3:14.
76. Wu CJ, Lu LF. MicroRNA in immune regulation. Curr Top Microbiol Immunol. 2017;410:249–67.
77. Arenas-Padilla M, Mata-Haro V. Regulation of TLR signaling pathways by microRNAs: implications in inflammatory diseases. Cent Eur J Immunol. 2018;43(4):482–9.
78. O'Neill LA, Sheedy FJ, McCoy CE. MicroRNAs: the fine-tuners of Toll-like receptor signalling. Nat Rev Immunol. 2011;11(3):163–75.
79. Ghafouri-Fard S, Khoshbakht T, Hussen BM, Taheri M, Arefian N. Regulatory role of non-coding RNAs on immune responses during *Sepsis*. Front Immunol. 2021;12: 798713.
80. Ahlback A, Gentek R. Fate-mapping macrophages: from ontogeny to functions. Method Mol Biol. 2024;2713:11–43.
81. Ginhoux F, Jung S. Monocytes and macrophages: developmental pathways and tissue homeostasis. Nat Rev Immunol. 2014;14(6):392–404.
82. Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. Science. 2012;336(6077):86–90.
83. Cox N, Pokrovskii M, Vicario R, Geissmann F. Origins, biology, and diseases of tissue macrophages. Annu Rev Immunol. 2021;39:313–44.
84. Lazarov T, Juarez-Carreño S, Cox N, Geissmann F. Physiology and diseases of tissue-resident macrophages. Nature. 2023;618(7966):698–707.
85. Suku M, Forrester L, Biggs M, Monaghan MG. Resident macrophages and their potential in cardiac tissue engineering. Tissue Eng Part B Rev. 2022;28(3):579–91.
86. Seyfried AN, Maloney JM, MacNamara KC. Macrophages orchestrate hematopoietic programs and regulate HSC function during inflammatory stress. Front Immunol. 2020;11:1499.
87. Elchaninov AV, Fatkhudinov TK, Vishnyakova PA, Lokhonina AV, Sukhikh GT. Phenotypical and functional polymorphism of liver resident macrophages. Cells. 2019. https://doi.org/10.3390/cells8091032.
88. Hartl D, Tirouvanziam R, Laval J, Greene CM, Habiel D, Sharma L, et al. Innate immunity of the lung: from basic mechanisms to translational medicine. J Innate Immun. 2018;10(5–6):487–501.
89. Zhang Y, Ouyang D, Chen YH, Xia H. Peritoneal resident macrophages in tumor metastasis and immunotherapy. Front Cell Dev Biol. 2022;10: 948952.
90. Lisco G, Giagulli VA, De Pergola G, Guastamacchia E, Jirillo E, Triggiani V. Pancreatic macrophages and their diabetogenic effects: highlight on several metabolic scenarios and dietary approach. Endocr Metab Immun Disord Drug Target. 2023;23(3):304–15.
91. Zhang H, Wang X, Zhang J, He Y, Yang X, Nie Y, et al. Crosstalk between gut microbiota and gut resident macrophages in inflammatory bowel disease. J Transl Int Med. 2023;11(4):382–92.
92. Lapenna A, De Palma M, Lewis CE. Perivascular macrophages in health and disease. Nat Rev Immunol. 2018;18(11):689–702.
93. Prinz M, Masuda T, Wheeler MA, Quintana FJ. Microglia and central nervous system-associated macrophages-from origin to disease modulation. Annu Rev Immunol. 2021;39:251–77.
94. Boutilier AJ, Elsawa SF. Macrophage Polarization States in the Tumor Microenvironment. Int J Mol Sci. 2021. https://doi.org/10.3390/ijms22136995.
95. Peng Y, Zhou M, Yang H, Qu R, Qiu Y, Hao J, et al. Regulatory mechanism of M1/M2 macrophage polarization in the development of autoimmune diseases. Med Inflamm. 2023;2023(1):8821610.
96. Cutolo M, Campitiello R, Gotelli E, Soldano S. The role of M1/M2 macrophage polarization in rheumatoid arthritis synovitis. Front Immunol. 2022;13: 867260.
97. Wang A, Kang X, Wang J, Zhang S. IFIH1/IRF1/STAT1 promotes sepsis associated inflammatory lung injury via activating macrophage M1 polarization. Int Immunopharmacol. 2023;114: 109478.
98. Eshghjoo S, Kim DM, Jayaraman A, Sun Y, Alaniz RC. Macrophage polarization in atherosclerosis. Genes. 2022;13(5):756.
99. Yu MY, Jia HJ, Zhang J, Ran GH, Liu Y, Yang XH. Exosomal miRNAs-mediated macrophage polarization and its potential clinical application. Int Immunopharmacol. 2023;117: 109905.
100. Yao Y, Xu XH, Jin L. Macrophage polarization in physiological and pathological pregnancy. Front Immunol. 2019;10:792.
101. Watanabe Y, Fukuda T, Hayashi C, Nakao Y, Toyoda M, Kawakami K, et al. Extracellular vesicles derived from GMSCs stimulated with TNF-α and IFN-α promote M2 macrophage polarization via enhanced CD73 and CD5L expression. Sci Rep. 2022;12(1):13344.
102. Wang LX, Zhang SX, Wu HJ, Rong XL, Guo J. M2b macrophage polarization and its roles in diseases. J Leukoc Biol. 2019;106(2):345–58.
103. Zhao W, Ma L, Deng D, Zhang T, Han L, Xu F, et al. M2 macrophage polarization: a potential target in pain relief. Front Immunol. 2023;14:1243149.
104. Zhao W, Ma L, Deng D, Zhang T, Han L, Xu F, et al. M2 macrophage polarization: a potential target in pain relief. Front Immunol. 2023;14:1243149.

105. Tsai CF, Chen GW, Chen YC, Shen CK, Lu DY, Yang LY, et al. Regulatory effects of quercetin on M1/M2 macrophage polarization and oxidative/antioxidative balance. Nutrients. 2022;14(1):67.
106. Mulder R, Banete A, Basta S. Spleen-derived macrophages are readily polarized into classically activated (M1) or alternatively activated (M2) states. Immunobiology. 2014;219(10):737–45.
107. Liu L, Guo H, Song A, Huang J, Zhang Y, Jin S, et al. Progranulin inhibits LPS-induced macrophage M1 polarization via NF-κB and MAPK pathways. BMC Immunol. 2020;21(1):32.
108. He Y, Gao Y, Zhang Q, Zhou G, Cao F, Yao S. IL-4 switches microglia/macrophage M1/M2 polarization and alleviates neurological damage by modulating the JAK1/STAT6 pathway following ICH. Neuroscience. 2020;437:161–71.
109. Ou L, Zhang A, Cheng Y, Chen Y. The cGAS-STING pathway: a promising immunotherapy target. Front Immunol. 2021;12: 795048.
110. Ying H, Fang M, Hang QQ, Chen Y, Qian X, Chen M. Pirfenidone modulates macrophage polarization and ameliorates radiation-induced lung fibrosis by inhibiting the TGF-β1/Smad3 pathway. J Cell Mol Med. 2021;25(18):8662–75.
111. Wang Y, Wan R, Peng W, Zhao X, Bai W, Hu C. Quercetin alleviates ferroptosis accompanied by reducing M1 macrophage polarization during neutrophilic airway inflammation. Eur J Pharmacol. 2023;938: 175407.
112. Liu X, Wang M, Jiang Y, Zhang X, Shi C, Zeng F, et al. Magnetic resonance imaging nanoprobe quantifies nitric oxide for evaluating M1/M2 macrophage polarization and prognosis of cancer treatments. ACS Nano. 2023;17(24):24854–66.
113. Zhao C, Mirando AC, Sové RJ, Medeiros TX, Annex BH, Popel AS. A mechanistic integrative computational model of macrophage polarization: Implications in human pathophysiology. PLoS Comput Biol. 2019;15(11): e1007468.
114. Self-Fordham JB, Naqvi AR, Uttamani JR, Kulkarni V, Nares S. MicroRNA: dynamic regulators of macrophage polarization and plasticity. Front Immunol. 2017;8:1062.
115. Jablonski KA, Gaudet AD, Amici SA, Popovich PG, Guerau-de-Arellano M. Control of the inflammatory macrophage transcriptional signature by miR-155. PLoS ONE. 2016;11(7): e0159724.
116. Guo Q, Zhu X, Wei R, Zhao L, Zhang Z, Yin X, et al. miR-130b-3p regulates M1 macrophage polarization via targeting IRF1. J Cell Physiol. 2021;236(3):2008–22.
117. Ying W, Tseng A, Chang RC, Morin A, Brehm T, Triff K, et al. MicroRNA-223 is a crucial mediator of PPARγ-regulated alternative macrophage activation. J Clin Invest. 2015;125(11):4149–59.
118. Qu R, Peng Y, Zhou M, Xu S, Yin X, Qiu Y, et al. MiR-223-3p attenuates M1 macrophage polarization via suppressing the Notch signaling pathway and NLRP3-mediated pyroptosis in experimental autoimmune uveitis. Eur J Pharmacol. 2023;960: 176139.
119. Roy S. miRNA in macrophage development and function. Antioxid Redox Signal. 2016;25(15):795–804.
120. Graff JW, Dickson AM, Clay G, McCaffrey AP, Wilson ME. Identifying functional microRNAs in macrophages with polarized phenotypes. J Biol Chem. 2012;287(26):21816–25.
121. Zhang Y, Zhang M, Zhong M, Suo Q, Lv K. Expression profiles of miRNAs in polarized macrophages. Int J Mol Med. 2013;31(4):797–802.
122. Essandoh K, Li Y, Huo J, Fan GC. MiRNA-mediated macrophage polarization and its potential role in the regulation of inflammatory response. Shock. 2016;46(2):122–31.
123. Ma W, Zhang W, Cui B, Gao J, Liu Q, Yao M, et al. Functional delivery of lncRNA TUG1 by endothelial progenitor cells derived extracellular vesicles confers anti-inflammatory macrophage polarization in sepsis via impairing miR-9-5p-targeted SIRT1 inhibition. Cell Death Dis. 2021;12(11):1056.
124. Essandoh K, Fan GC. Role of extracellular and intracellular microRNAs in sepsis. Biochim Biophys Acta. 2014;1842(11):2155–62.
125. Tsujimoto H, Ono S, Efron PA, Scumpia PO, Moldawer LL, Mochizuki H. Role of toll-like receptors in the development of sepsis. Shock. 2008;29(3):315–21.
126. Cristofaro P, Opal SM. The toll-like receptors and their role in septic shock. Expert Opin Ther Target. 2003;7(5):603–12.
127. Savva A, Roger T. Targeting toll-like receptors: promising therapeutic strategies for the management of sepsis-associated pathology and infectious diseases. Front Immunol. 2013;4:387.
128. Weighardt H, Holzmann B. Role of Toll-like receptor responses for sepsis pathogenesis. Immunobiology. 2007;212(9–10):715–22.
129. Foley NM, Wang J, Redmond HP, Wang JH. Current knowledge and future directions of TLR and NOD signaling in sepsis. Mil Med Res. 2015;2:1.
130. Salomão R, Martins PS, Brunialti MK, Fernandes Mda L, Martos LS, Mendes ME, et al. TLR signaling pathway in patients with sepsis. Shock. 2008;30(Suppl 1):73–7.
131. Lawrence T, Natoli G. Transcriptional regulation of macrophage polarization: enabling diversity with identity. Nat Rev Immunol. 2011;11(11):750–61.
132. Wang JF, Yu ML, Yu G, Bian JJ, Deng XM, Wan XJ, et al. Serum miR-146a and miR-223 as potential new biomarkers for sepsis. Biochem Biophys Res Commun. 2010;394(1):184–8.
133. Gao M, Wang X, Zhang X, Ha T, Ma H, Liu L, et al. Attenuation of cardiac dysfunction in polymicrobial *Sepsis* by MicroRNA-146a Is mediated via targeting of IRAK1 and TRAF6 expression. J Immunol. 2015;195(2):672–82.
134. Benz F, Tacke F, Luedde M, Trautwein C, Luedde T, Koch A, et al. Circulating microRNA-223 serum levels do not predict sepsis or survival in patients with critical illness. Dis Mark. 2015;2015: 384208.
135. Wang X, Zhang H, Guo R, Li X, Liu H, Wang Z, et al. MicroRNA-223 modulates the IL-4-medicated macrophage M2-type polarization to control the progress of sepsis. Int Immunopharmacol. 2021;96: 107783.
136. Gong T, Liu Y-T, Fan J. Exosomal mediators in sepsis and inflammatory organ injury: unraveling the role of exosomes in intercellular crosstalk and organ dysfunction. Mil Med Res. 2024;11(1):24.
137. Bird A, Tomescu O, Oyola S, Houpy J, Anderson I, Pincavage A. A curriculum to teach resilience skills to medical students during clinical training. MedEdPORTAL. 2020;16:10975.
138. Balusu S, Van Wonterghem E, De Rycke R, Raemdonck K, Stremersch S, Gevaert K, et al. Identification of a novel mechanism of blood-brain communication during peripheral inflammation via choroid plexus-derived extracellular vesicles. EMBO Mol Med. 2016;8(10):1162–83.
139. Xu J, Feng Y, Jeyaram A, Jay SM, Zou L, Chao W. Circulating plasma extracellular vesicles from *Septic* mice induce inflammation via MicroRNA- and TLR7-dependent mechanisms. J Immunol. 2018;201(11):3392–400.
140. Groot M, Lee H. Sorting mechanisms for MicroRNAs into extracellular vesicles and their associated diseases. Cells. 2020. https://doi.org/10.3390/cells9041044.
141. Wu B, Su S, Patil DP, Liu H, Gan J, Jaffrey SR, et al. Molecular basis for the specific and multivariant recognitions of RNA substrates by human hnRNP A2/B1. Nat Commun. 2018;9(1):420.
142. Villarroya-Beltri C, Gutiérrez-Vázquez C, Sánchez-Cabo F, Pérez-Hernández D, Vázquez J, Martin-Cofreces N, et al. Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. Nat Commun. 2013;4:2980.
143. Santangelo L, Giurato G, Cicchini C, Montaldo C, Mancone C, Tarallo R, et al. The RNA-binding protein SYNCRIP is a component of the hepatocyte exosomal machinery controlling MicroRNA sorting. Cell Rep. 2016;17(3):799–808.
144. McKenzie AJ, Hoshino D, Hong NH, Cha DJ, Franklin JL, Coffey RJ, et al. KRAS-MEK signaling controls Ago2 sorting into exosomes. Cell Rep. 2016;15(5):978–87.
145. Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci U S A. 2011;108(12):5003–8.
146. Lin F, Zeng Z, Song Y, Li L, Wu Z, Zhang X, et al. YBX-1 mediated sorting of miR-133 into hypoxia/reoxygenation-induced EPC-derived exosomes to increase fibroblast angiogenesis and MEndoT. Stem Cell Res Ther. 2019;10(1):263.
147. Chen YR, Sekine K, Nakamura K, Yanai H, Tanaka M, Miyajima A. Y-box binding protein-1 down-regulates expression of carbamoyl phosphate synthetase-I by suppressing CCAAT enhancer-binding protein-alpha function in mice. Gastroenterology. 2009;137(1):330–40.
148. Buchet-Poyau K, Courchet J, Le Hir H, Séraphin B, Scoazec JY, Duret L, et al. Identification and characterization of human Mex-3 proteins, a novel family of evolutionarily conserved RNA-binding

proteins differentially localized to processing bodies. Nucl Acid Res. 2007;35(4):1289–300.
149. Chung JH, Eng C. Nuclear-cytoplasmic partitioning of phosphatase and tensin homologue deleted on chromosome 10 (PTEN) differentially regulates the cell cycle and apoptosis. Cancer Res. 2005;65(18):8096–100.
150. Kosaka N, Iguchi H, Yoshioka Y, Takeshita F, Matsuki Y, Ochiya T. Secretory mechanisms and intercellular transfer of microRNAs in living cells. J Biol Chem. 2010;285(23):17442–52.
151. Lee H, Li C, Zhang Y, Zhang D, Otterbein LE, Jin Y. Caveolin-1 selectively regulates microRNA sorting into microvesicles after noxious stimuli. J Exp Med. 2019;216(9):2202–20.
152. Jackson CE, Scruggs BS, Schaffer JE, Hanson PI. Effects of Inhibiting VPS4 Support a General Role for ESCRTs in Extracellular Vesicle Biogenesis. Biophys J. 2017;113(6):1342–52.
153. Bishop N, Woodman P. ATPase-defective mammalian VPS4 localizes to aberrant endosomes and impairs cholesterol trafficking. Mol Biol Cell. 2000;11(1):227–39.
154. Reithmair M, Buschmann D, Märte M, Kirchner B, Hagl D, Kaufmann I, et al. Cellular and extracellular miRNAs are blood-compartment-specific diagnostic targets in sepsis. J Cell Mol Med. 2017;21(10):2403–11.
155. Deng JN, Li YQ, Liu Y, Li Q, Hu Y, Xu JQ, et al. Exosomes derived from plasma of septic patients inhibit apoptosis of T lymphocytes by down-regulating bad via hsa-miR-7-5p. Biochem Biophys Res Commun. 2019;513(4):958–66.
156. Subramani K, Raju SP, Chu X, Warren M, Pandya CD, Hoda N, et al. Effect of plasma-derived extracellular vesicles on erythrocyte deformability in polymicrobial sepsis. Int Immunopharmacol. 2018;65:244–7.
157. Real JM, Ferreira LRP, Esteves GH, Koyama FC, Dias MVS, Bezerra-Neto JE, et al. Exosomes from patients with septic shock convey miRNAs related to inflammation and cell cycle regulation: new signaling pathways in sepsis? Critic Care (London England). 2018;22(1):68.
158. Mao L, Liu S, Chen Y, Huang H, Ding F, Deng L. Engineered exosomes: a potential therapeutic strategy for septic cardiomyopathy. Front Cardiovasc Med. 2024;11:1399738.
159. Zhen J, Chen W. MiR-142 inhibits cecal ligation and puncture (CLP)-induced inflammation via inhibiting PD-L1 expression in macrophages and improves survival in septic mice. Biomed Pharmacother. 2018;97:1479–85.
160. Liu XL, Pan Q, Cao HX, Xin FZ, Zhao ZH, Yang RX, et al. Lipotoxic hepatocyte-derived exosomal MicroRNA 192–5p activates macrophages through Rictor/Akt/Forkhead box transcription factor O1 signaling in nonalcoholic fatty liver disease. Hepatology. 2020;72(2):454–69.
161. An L, Yin F. MiR-192-5p suppresses M1 macrophage polarization via epiregulin (EREG) downregulation in gouty arthritis. Tissue Cell. 2021;73:101669.
162. Jia Y, Zheng Z, Xue M, Zhang S, Hu F, Li Y, et al. Extracellular vesicles from albumin-induced tubular epithelial cells promote the M1 macrophage phenotype by targeting Klotho. Mol Ther. 2019;27(8):1452–66.
163. Liu H, Niu Q, Wang T, Dong H, Bian C. Lipotoxic hepatocytes promote nonalcoholic fatty liver disease progression by delivering microRNA-9-5p and activating macrophages. Int J Biol Sci. 2021;17(14):3745–59.
164. Jiao Y, Zhang T, Zhang C, Ji H, Tong X, Xia R, et al. Exosomal miR-30d-5p of neutrophils induces M1 macrophage polarization and primes macrophage pyroptosis in sepsis-related acute lung injury. Critic Care (London England). 2021;25(1):356.
165. Liu L, Sun B. Neutrophil pyroptosis: new perspectives on sepsis. Cell Mol Life Sci. 2019;76(11):2031–42.
166. Thébaud B, Stewart DJ. Exosomes: cell garbage can, therapeutic carrier, or trojan horse? Circulation. 2012;126(22):2553–5.
167. Li M, Zeringer E, Barta T, Schageman J, Cheng A, Vlassov AV. Analysis of the RNA content of the exosomes derived from blood serum and urine and its potential as biomarkers. Philos Trans R Soc Lond B Biol Sci. 2014. https://doi.org/10.1098/rstb.2013.0502.
168. Vargas A, Roux-Dalvai F, Droit A, Lavoie JP. Neutrophil-derived exosomes: a new mechanism contributing to airway smooth muscle remodeling. Am J Respir Cell Mol Biol. 2016;55(3):450–61.
169. Genschmer KR, Russell DW, Lal C, Szul T, Bratcher PE, Noerager BD, et al. Activated PMN exosomes: pathogenic entities causing matrix destruction and disease in the lung. Cell. 2019;176(1–2):113-26.e15.
170. Bazzan E, Turato G, Tinè M, Radu CM, Balestro E, Rigobello C, et al. Dual polarization of human alveolar macrophages progressively increases with smoking and COPD severity. Respir Res. 2017;18(1):40.
171. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity. 2014;41(1):14–20.
172. Galluzzi L, Bravo-San Pedro JM, Vitale I, Aaronson SA, Abrams JM, Adam D, et al. Essential versus accessory aspects of cell death: recommendations of the NCCD 2015. Cell Death Differ. 2015;22(1):58–73.
173. Bordon Y. Mucosal immunology: inflammasomes induce sepsis following community breakdown. Nat Rev Immunol. 2012;12(6):400–1.
174. Pu Q, Gan C, Li R, Li Y, Tan S, Li X, et al. Atg7 deficiency intensifies inflammasome activation and pyroptosis in *Pseudomonas Sepsis*. J Immunol. 2017;198(8):3205–13.
175. Chan FK, Luz NF, Moriwaki K. Programmed necrosis in the cross talk of cell death and inflammation. Annu Rev Immunol. 2015;33:79–106.
176. Fan J, Li Y, Vodovotz Y, Billiar TR, Wilson MA. Neutrophil NAD(P)H oxidase is required for hemorrhagic shock-enhanced TLR2 up-regulation in alveolar macrophages in response to LPS. Shock. 2007;28(2):213–8.
177. Fan J, Malik AB. Toll-like receptor-4 (TLR4) signaling augments chemokine-induced neutrophil migration by modulating cell surface expression of chemokine receptors. Nat Med. 2003;9(3):315–21.
178. Jiao Y, Li Z, Loughran PA, Fan EK, Scott MJ, Li Y, et al. Frontline Science: Macrophage-derived exosomes promote neutrophil necroptosis following hemorrhagic shock. J Leukoc Biol. 2018;103(2):175–83.
179. Warnatsch A, Ioannou M, Wang Q, Papayannopoulos V. Inflammation. Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. Science. 2015;349(6245):316–20.
180. Chen L, Zhao Y, Lai D, Zhang P, Yang Y, Li Y, et al. Neutrophil extracellular traps promote macrophage pyroptosis in sepsis. Cell Death Dis. 2018;9(6):597.

Publisher's Note