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Emerging role of exosomes in ulcerative colitis: ²Targeting NOD-like receptor family pyrin domain containing 3 inflammasome

Li X *et al.* Emerging role of exosomes in ulcerative colitis

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Abstract

Ulcerative colitis (UC) is a chronic recurrent inflammatory bowel disease. Despite ongoing advances in our understanding of UC, its pathogenesis is yet unelucidated, underscoring the urgent need for novel treatment strategies for patients with UC. Exosomes are nanoscale membrane particles that mediate intercellular communication by carrying various bioactive molecules, such as proteins, RNAs, DNA, and metabolites. The NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome is a cytosolic tripartite protein complex whose activation induces the maturation and secretion of proinflammatory cytokines interleukin-1 β (IL-1 β) and IL-18, triggering the inflammatory response to a pathogenic agent or injury. Growing evidence suggests that exosomes are new modulators of the NLRP3 inflammasome, with vital roles in the pathological process of UC. Here, recent evidence is reviewed on the role of exosomes and NLRP3 inflammasome in UC. First, the dual role of exosomes on NLRP3 inflammasome and the effect of NLRP3 inflammasome on exosome secretion are summarized. Finally, an outlook on the directions of exosome-NLRP3 inflammasome crosstalk research in the context of UC is proposed and areas of further research on this topic are highlighted.

Key Words: Ulcerative colitis; Exosomes; Inflammasome; Evidence; Therapeutics

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Core Tip: Clarifying the regulatory circuits that control the abnormal immune state of the intestinal mucosa is essential for understanding ulcerative colitis (UC) pathogenesis and clinical management. The role of exosomes and NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasomes in UC has been continuously highlighted in recent years. In this review, the dual role of exosomes on NLRP3 inflammasome and

the effect of NLRP3 inflammasome on exosome secretion are summarized. Furthermore, an outlook on the directions of exosome-NLRP3 inflammasome crosstalk research in the context of UC is proposed and areas of further research on this topic are highlighted.

INTRODUCTION

Ulcerative colitis (UC) is characterized by chronic, remitting, and recurrent mucosal inflammation^[1]. Although its cause is not well understood, current evidence suggests innate and adaptive immunity play critical roles in its pathogenesis^[2]. The events leading to UC involve disrupting the intestinal mucosal barrier, bringing the luminal microbial community and the mucosal immune system into direct contact^[3]. Subsequently, innate immune cells, such as macrophages and dendritic cells, rapidly recognize microorganisms or their products entering the lamina propria from the intestinal lumen and transmit signals, awakening the innate defenses and the adaptive immune system^[4]. A long-term feature of UC is inflammation maintained by various inflammatory mediators produced by activated immune cells, including proinflammatory cytokines and chemokines^[5-8]. Another characteristic is enterocyte apoptosis sustained by several inflammatory cells, which prevents mucosal healing^[2]. Considering these points, we can assume that clarifying the regulatory circuits that control the abnormal immune state of the intestinal mucosa is essential for understanding UC pathogenesis and clinical management.

⁵ The NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome mediates the inflammatory cascade *in vivo* and is a critical regulator in inflammatory bowel disease development^[9]. Its activation promotes pyroptosis and ⁴ caspase-1-dependent secretion of interleukin-1 β (IL-1 β) and IL-18, leading to a sustained inflammatory response in the intestinal mucosa^[10]. Since these two proinflammatory cytokines are present in released exosomes, one possible pathway for their unconventional secretion may occur through endosome release^[11-14]. Exosomes are nanoscale membrane-derived particles that mediate intercellular communication by

carrying many bioactive molecules, including proteins, RNAs, DNA, and metabolites^[15,16]. They also carry out numerous functions, such as releasing cytokines and inhibiting or promoting inflammasome activation, depending on the transported molecules^[17,18]. Increasing evidence suggests that crosstalk between exosomes and inflammasomes has a critical role in inflammatory diseases^[19]. Therefore, systematically exploring this crosstalk in UC should have beneficial implications for the prevention and treatment.

NLRP3 INFLAMMASOME

Composition and distribution of NLRP3 inflammasome

Inflammasomes are cytosolic multiprotein complexes that initiate inflammatory cascade responses by identifying damage-associated molecular patterns (DAMPs), cellular distress signals of the host, ¹³ pathogen-associated molecular patterns (PAMPs), and conserved components of infectious agents^[20]. T and B lymphocytes, macrophages, antigen-presenting cells, and granulocytes all express the NLRP3 inflammasome^[21]. It represents the most classical inflammasome subtype consisting of the NLRP3 receptor, ⁶ apoptosis-associated speck-like protein (ASC) adapter, and caspase-1 effector proteins^[22]. The NLRP3 receptor protein is composed of 3 domains: a C-terminal leucine-rich repeat domain, ² an N-terminal pyrin domain (PYD), and a central nucleotide-binding and oligomerization domain^[23]. The ASC adapter contains several domains: 2 transactivation structural domains, the pyrin structural domain linked to the upstream NLRP3 receptor, and the caspase recruitment domain (CARD) connected to the downstream caspase-1^[24,25].

Activation of NLRP3 inflammasome

The innate immune system senses ¹⁰ exogenous (PAMPs) or endogenous (DAMPs) danger signals by recognizing them with various pattern recognition receptors, such as Toll-like receptors and NOD-like receptors. During its involvement in the inflammatory response, NLRP3 inflammasome provides a molecular model that can be stimulated by

many DAMPs (aluminum adjuvants, ATP, uric acid crystals, and β -amyloid peptides) and PAMPs (microbial toxins, viral RNA, and bacterial surface components). Currently, canonical, non-canonical, and alternate routes can all activate the NLRP3 inflammasome^[26] (Figure 1).

Canonical NLRP3 inflammasome activation

In most cells, canonical NLRP3 inflammasome activation involves priming and activation steps. The priming step is initiated by a signal from the ligand bound to the pattern recognition receptor and promotes ¹¹transcription of pro-IL-18, pro-IL-1 β , and NLRP3 via NF- κ B-dependent pathway^[27-30]. The activation step leads to NLRP3 assembly and is promoted by various DAMPs or PAMPs through multiple molecular and cellular events, such as lysosomal disruption, mitochondrial DNA production, mitochondrial dysfunction, reactive oxygen species (ROS) release, and ion flux (Ca²⁺ influx and K⁺/Cl⁻ efflux). The activated NLRP3 inflammasome induces cleavage and activation of caspase-1 via CARD-CARD and PYD-PYD interactions^[30]. ¹Subsequently, the activated caspase-1 recruits and cleaves the proinflammatory cytokines pro-IL-18 and pro-IL-1 β , allowing their maturation and release^[30]. In addition, it cleaves the pyroptotic substrate gasdermin D (GSDMD), enabling its translocation to the cell membrane, where it forms pores and triggers inflammatory programmed cell death called pyroptosis^[31].

Non-canonical NLRP3 inflammasome activation

⁷Human caspases 4 and 5, as well as murine caspase 11, are needed for non-canonical NLRP3 inflammasome activation. In this pathway, these caspases recognize and are activated by cytosolic lipopolysaccharide (LPS) from endocytosed gram-negative bacteria or, more often, their outer membrane vesicles^[32]. The activated caspases catabolize GSDMD, leading to pyrolysis and promoting ¹²the release of mature IL-18 and IL-1 β ^[33,34]. In addition to LPS, another signal called 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (PAPC) activates the non-canonical pathway. This molecule

is abundant in membranes of mammalian cells and is oxidized by ROS released from damaged or dead cells. The oxidized PAPC binds caspase-11 and caspase-4, initiating activation or inhibition of the NLRP3 inflammasome depending on the cell type^[35].

Alternative NLRP3 inflammasome activation

Alternative activation of the NLRP3 inflammasome possesses cell- and species-specific characteristics^[36]. For example, the TLR4-TRIF-RIPK1-FADD-CASP8 axis activates an alternative inflammasome upstream of NLRP3 in porcine and human monocytes, but this activation response is absent in murine monocytes^[34,36]. Interestingly, the alternative activation lacks typical features for canonical and non-canonical activation, such as ASC speckle formation, K⁺ efflux, or pyroptosis induction^[34].

The role of NLRP3 inflammasome in UC

Susceptibility to UC significantly increases with single nucleotide polymorphisms rs10925019 and rs10754558 in the coding region of the NLRP3 gene^[37,38]. Similarly, predisposition to inflammatory bowel disease correlates with polymorphisms affecting receptors downstream of NLRP3, including interleukin 1 receptor-like 1 and 2, interleukin 1 receptor type 1 and 2, and interleukin 18 receptor 1^[39]. Disease activity of UC is associated with increased levels of inflammasome activation markers NLRP3, caspase-1, and ASC^[40,41]. A similar effect is also observed in mice with colitis, where the upregulated markers positively correlate with disease severity and pathological damage^[42,43]. Conversely, mice with colitis lacking NLRP3 or caspase-1 show significantly less severe pathology compared with wild-type mice with colitis^[44,45]. Furthermore, NLRP3 promotes intestinal mucosal inflammation *in vitro*^[46]. These findings demonstrate that NLRP3 inflammasome activity participates in UC pathogenesis and suggest that treating the disease may rely on regulating the NLRP3 inflammasome activation or its downstream cytokine effectors.

A small-molecule inhibitor of the NLRP3 inflammasome called MCC950 significantly reduces the secretion of IL-18 and IL-1 β in mice, attenuating the inflammatory cascade

response evoked by NLRP3 inflammasome activation^[47]. Carboxyamidotriazole, wogonoside, or oroxylin A are other small-molecule compounds that also alleviate experimental colitis but with a mechanism that inhibits the NLRP3 inflammasome activation^[48-50]. Although pharmacological inhibition of inflammasome overactivation benefits animals with UC, therapies targeting inflammasomes remain limited. Recent evidence suggests that dietary compounds or medicinal herbs reduce colonic inflammation in mice and, in some cases, even in patients with UC by targeting different inflammasome modulators to inactivate inflammasomes in the colon^[51]. Thus, strategies for treating UC may involve using bioactive substances purified from food or traditional medicines to regulate inflammasome activity.

EXOSOMES

Biogenesis, biology, function, and regulation of exosomes

Exosomes are endosome-derived extracellular vesicles commonly found in body fluids, including sweat, blood, and urine, and characterized by a phospholipid bilayer, small vesicle morphology, and a diameter from 30 to 150 nm^[15,16]. They mediate intercellular communication by carrying numerous biologically active molecules, such as DNA, RNAs, proteins, and metabolites, and their bioactive molecular composition depends on the cell type releasing them^[15,16]. Notably, exosomes contain two classes of proteins: conserved and specific. While the make-up of specific proteins is determined by the cell type releasing the exosome and is subject to change from varying physiological conditions acting on the cell, that of the conserved proteins is constant, rendering them exosome markers. Noteworthy examples are programmed cell death 6 interacting protein, tumor susceptibility gene 10, **members of the heat shock protein family HSP60, HSP70, and HSP90, and** antigens CD9, CD63, CD81, and CD82^[15,16]. Exosome biogenesis requires uptake, secretion, cargo sorting, and formation, achieved through the classical or direct pathways^[52]. Whereas most cells utilize the classical, or exocytic, pathway of exosome biogenesis, T cells employ a direct pathway as a quick mechanism that generates exosomes directly from the plasma membrane^[53] (Figure 2).

Since released exosomes contain crucial molecules for transferring information between cells, they are implicated in the cancer microenvironment^[54] and the pathogenesis of various illnesses, including autoimmune^[55], cardiac^[56], neurological^[57], and liver disorders^[58]. Furthermore, because exosomes collected from sick populations have different RNA profiles than exosomes collected from healthy ones^[59-61], they are potential diagnostic and therapeutic biomarkers for many diseases^[62,63].

Exosomes and UC

Exosomes are thought to play an immunomodulatory function owing to their involvement in immune synapse formation and antigen presentation^[64,65]. Because UC is an immune disease, and the saliva of patients with UC contains large amounts of exosomal proteins, the role of exosomes in UC is unquestionable^[66-68]. Indeed, animal experiments confirm that exosomal proteins are associated with proteasomal activity and inflammatory response, suggesting that some, such as saliva-derived exosomal proteasome 20S subunit alpha 7, can be used as an ideal biomarker for UC diagnosis^[68]. Other potential UC biomarkers are exosome micro RNAs, with enhanced levels in individuals with UC. For instance, elevated levels of gut-derived miR-29b in the plasma of individuals with UC not only help diagnose the disease but also an impaired cardiac function *via* miR-29b-mediated extraintestinal inhibition of vital proteins, such as brain-derived neurotrophic factor^[69]. Similarly, small GTPases that regulate exosome secretion also have increased levels in UC, such as ¹⁴RAB27A, member RAS oncogene family³ and RAB27B, member RAS oncogene family. The number of RAB27A- and RAB27B-positive immune cells in the intestinal mucosa of individuals with active UC is significantly higher than that of healthy patients, indicating that exosome-mediated immune regulation is involved in the pathological process of UC^[70].

Currently, the role of various sources of exosomes in UC is being widely explored (Table 1)^[71-105]. Mesenchymal stem cell (MSC) therapy is a cutting-edge one for treating various diseases, due to the strong immunomodulating and immunosuppressive properties of MSCs, and stem cell-derived exosomes may have a beneficial effect on UC,

according to newly available evidence^[71-91]. The ameliorative effects of MSC-derived exosomes on UC are regulated in multiple ways, including inhibition of inflammatory responses, regulation of immune cell homeostasis, improvement of intestinal flora structure, and inhibition of oxidative stress, ultimately leading to repair of intestinal mucosal damage and restoration of intestinal barrier function. Similarly, dendritic cell-derived exosomes were also found to have a reparative effect on intestinal injury in UC by inhibiting pathways associated with inflammation^[92-94]. In addition, it was found that encapsulating triptolide with DC cell-derived exosomes could not only reduce the toxicity of the drug, but also accurately deliver the drug to the therapeutic target to induce immunosuppression in UC mice, providing a new perspective for immunosuppressive treatment of UC^[95]. However, macrophage-derived exosomes do not always provide a benefit to UC. Some exosomal molecules, such as miR-590-3p produced by M2 macrophages, reduce mucosal damage and promote epithelial cell repair in mice with colitis^[96]. However, others, such as exosome miR-21a-5p produced by M1 macrophages, exacerbate UC by inhibiting E-cadherin and activating type 2 innate lymphoid cells^[97]. Furthermore, limited evidence suggests that gut-derived and serum-derived exosomes are beneficial in UC^[98,100], whereas visceral adipose-derived exosomes aggravate UC^[99]. Surprisingly, emerging evidence has recently suggested that human or bovine milk-derived exosomes express a favorable benefit in animals with colitis by decreasing oxidative stress and inflammation, indicating a new route for the development of therapeutic approaches for UC^[103-105].

EXOSOMES AND NLRP3 INFLAMMASOME CROSSTALK

Effects of exosomes on NLRP3 inflammasome

According to recent evidence, cells utilize exosome secretion to regulate NLRP3 inflammasome activation, suppressing inflammation and promoting damage repair (Table 2)^[71-73,106-147]. Since most findings originate from research on various stem cell-derived exosomes, knowledge of how they regulate the NLRP3 inflammasome activation in differentiated cells remains limited. Nonetheless, the available evidence

indicates that exosomes suppress the NLRP3 inflammasome mainly by regulating the pathways upstream of NLRP3, especially TLR-related ones and those related to oxidative stress. For example, exosome release lowers ROS production, reducing ROS levels available for the NLRP3 inflammasome activation^[107,112,126]. In addition, exosomes help protect mitochondria from damage induced by oxidative stress states, possibly by exosome-carried mitochondrial proteins^[112]. Abundant findings also suggest that exosomes regulate the activation of NLRP3 inflammasome by directly binding to NLRP3^[71,121,130,137].

We have so far learned that stem cell-derived exosomes repress the NLRP3 inflammasome activation but will see that those from other cell types, including cancer, epithelial, immune, and endothelial cells, appear to promote it (Table 2). For instance, exosomal miR-30d-5p released by polymorphonuclear neutrophils induces macrophage pyroptosis and M1 macrophage polarization *via* the NF- κ B pathway, promoting sepsis-associated acute lung injury^[138]. Similarly, tumor-derived exosomal tripartite motif containing 59 protein induces proteasomal degradation of abhydrolase domain containing 5 lipolytic co-activator in macrophages. Consequently, this event reprograms macrophages into cells with tumor-promoting function and activates the NLRP3 inflammasome, mediating the IL-1 β release and stimulating lung cancer progression^[139]. When exposed to photooxidative blue light, retinal pigment epithelium-derived exosomes exacerbate potentially harmful oxidative responses by activating the NLRP3 inflammasome^[140]. In hepatic ischemia-reperfusion injury, serum exosome levels rise significantly, freely crossing the blood-brain barrier due to their small size and stimulating pyroptosis of hippocampal and cortical tissues^[141]. By triggering NLRP3-dependent pyroptosis in alveolar macrophages, plasma-derived exosomes help cause lung damage brought on by pancreatitis^[142]. Exosomes in patients with COVID-19 increase inflammasome activity in distant endothelial cells, enhancing immunopathogenesis of the disease^[143]. In addition, plasma-derived exosomes induce pyroptosis in intestinal epithelial cells *via* NLRP3 inflammasome activation in individuals with intestinal Behçet's syndrome^[144].

In summary, the above evidence suggests that exosomes play a dual role in NLRP3-mediated inflammatory response by attenuating or enhancing the inflammasome activity. The differences in how exosomes affect the inflammasome activity may depend on the cell type producing the exosomes and the specific circumstances of their release. Importantly, modulating the NLRP3 inflammasome activity by targeting exosomes is emerging as a promising strategy to combat inflammatory diseases^[145-147].

Effects of NLRP3 inflammasome on exosomes

Some NLRP3 inflammasome activators also stimulate extracellular vesicle secretion, suggesting inflammasome activation enhances extracellular vesicle secretion^[148]. After exposure to ATP, macrophages secrete exosomes carrying the major histocompatibility complex class II proteins^[149]. Moreover, macrophages isolated from mice lacking the genes encoding the ASC adapter or NLRP3 cannot release these exosomes after exposure to ATP, indicating exosome release requires components of the NLRP3 complex^[149]. Similarly, inflammasome activation increases exosome secretion caused by a viral infection or exposure to LPS/ATP^[149]. We have seen previously that the release of mature IL-1 β largely depends on the NLRP3 inflammasome activation. When synovial fibroblasts are treated with exogenous IL-1 β , they show a significant increase in exosome secretion compared with the untreated control cells, implying IL-1 β stimulates exosome release^[150]. Although a few recent studies demonstrate that exosome secretion is induced by NLRP3 inflammasome activation, evidence supporting this claim is insufficient and requires additional confirmation^[19].

Exosome-inflammasome crosstalk in UC

In inflammatory states, such as UC, MSCs have immunomodulating and homeostatic effects and may repair intestinal damage^[151]. Increasing evidence indicates that MSCs maintain immunosuppressive signals through paracrine mediators instead of cell-to-cell contact and that paracrine processes predominantly mediate the therapeutic role of MSC-derived exosomes^[71,152]. Although we know little about how MSC-derived

exosomes suppress colonic inflammation, recent evidence suggests that crosstalk between exosomes and NLRP3 inflammasome constitutes the mechanism^[71-73,92]. Thus, the roles of exosome-NLRP3 inflammasome crosstalk in inflammatory diseases are gaining much attention^[19].

In mice with colitis, exosomes from human umbilical cord MSCs carrying miR-378a-5p significantly alleviate colonic inflammation and promote mucosal repair^[71]. Mechanically, these exosomes inhibit the NLRP3 inflammasome activation, preventing caspase-1 cleavage and the IL-18 and IL-1 β secretion and decreasing pyroptosis^[71]. Similarly, exosomes from bone marrow MSCs containing miR-539-5p alleviate colitis by directly targeting the NLRP3-caspase-1 pathway to inhibit pyroptosis^[72]. Moreover, hair follicle-derived MSCs inhibited pyroptosis by releasing exosomes in a paracrine manner, which ultimately exerted an alleviating effect in mice with colitis^[73]. Other examples involving exosomes with small RNA cargo are dendritic cells-derived exosomes transporting miR-146a which exert a therapeutic effect by directly targeting the NLRP3-caspase-1 pathway to inhibit intestinal inflammation in mice with colitis^[92] and human umbilical cord MSC-derived exosomes transferring miR-203a-3p.2 that reduce pyroptosis of macrophages caused by caspase-1 or -4^[74].

Given these points, we can conclude that crosstalk between exosomes and the NLRP3 inflammasome holds promise for developing novel treatment strategies (Figure 3). Despite the scarcity of available evidence, the connection between MSC-derived exosomes with anti-inflammatory activity and the NLRP3 inflammasome offers a fresh viewpoint on using this system as a therapy for UC in the clinical setting.

CONCLUSION

Since exosomes and the NLRP3 inflammasome play vital roles in UC, they are explored as potential new targets for preventing and treating the disease, attracting considerable attention. Importantly, crosstalk between exosomes and the NLRP3 inflammasome and its emerging therapeutic benefit is gaining increasing interest in biomedicine.

Exosomes are upstream components of the NLRP3 inflammasome pathway and attenuate or enhance the NLRP3 inflammasome activation. Based on the available data, MSC-derived exosomes repress the NLRP3 inflammasome activation in receptor cells, alleviating the inflammatory response. Therefore, these exosomes are therapeutically valuable and in stark contrast to most of those derived from non-stem cells that promote the NLRP3 inflammasome activation and exacerbate tissue inflammation. Potent effectors of the crosstalk are micro RNAs that repress the NLRP3 inflammasome activation and prevent pyroptotic cell death or promote the opposite effect, depending on the cell type releasing the exosomes and the external factors triggering exosome release. However, this contrasting effect of exosomes on the NLRP3 inflammasome and the factors that decide on its direction is supported by limited evidence. Similarly, evidence is lacking about the regulatory role of the NLRP3 inflammasome activation in exosome release. Thus, although crosstalk between exosomes and the NLRP3 inflammasome undoubtedly has a central role in UC research, further studies are necessary to elucidate it.

In conclusion, the therapeutic potential of exosomes has gained much attention since these vesicles transfer biologically active cargo between cells and could deliver drugs to treat diseases. However, because exosomes originating from different sources and exposed to specific intervention conditions have unique cargo composition and properties, selecting those most suitable for therapeutic use represents a challenge requiring substantial effort for clarification. Moreover, encapsulation and targeted delivery of drugs (*e.g.*, biologics and small molecule drugs) through exosomes is a novel approach that both reduce drugs toxicity and improve efficacy. Therefore, large-scale prospective clinical trials exploring therapeutic efficacy and adverse events of exosomes in UC will be the focus of upcoming studies on the basis of sufficient basic research evidence.

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