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New insight into the role of exosomes in idiopathic membrane nephropathy

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Abstract: Exosomes, nanoscale extracellular vesicles (EVs) derived from the invagination of the endosomal membrane, are secreted by a majority of cell types. As carriers of DNA, mRNA, proteins, and microRNAs, exosomes are implicated in regulating biological activities under physiological and pathological conditions. Kidney-derived exosomes, which vary in origin and function, may either contribute to the pathogenesis of disease or represent a potential therapeutic resource. Membranous nephropathy (MN), an autoimmune kidney disease characterized by glomerular damage, is a predominant cause of nephrotic syndrome. Notably, MN, especially idiopathic membranous nephropathy (IMN), often results in end-stage renal disease (ESRD), affecting approximately 30% of patients and posing a considerable economic challenge to healthcare systems. Despite substantial research, therapeutic options remain ineffective at halting IMN progression, underscoring the urgent need for innovative strategies. Emerging evidence has implicated exosomes in IMN's pathophysiology; Providing a fresh perspective for the discovery of novel biomarkers and therapeutic strategies. This review aims to scrutinize recent developments in exosome-related mechanisms in IMN and evaluate their potential as promising therapeutic targets and diagnostic biomarkers, with the hope of catalyzing further investigations into the utility of exosomes in MN, particularly IMN, ultimately contributing to improved patient outcomes in these challenging disease settings.

Abbreviations

EVs	Extracellular vesicles
MN	Membranous nephropathy
ESRD	End-stage renal disease
NS	Nephrotic syndrome
IMN	Idiopathic membranous nephropathy
PMN	Primary membranous nephropathy
SMN	Secondary membranous nephropathy
EM	Electron microscopy
GBM	Glomerular basement membrane
GFB	Glomerular filtration barrier
NSAIDs	Nonsteroidal anti-inflammatory drugs
MVBs	Multivesicular bodies
ILVs	Intraluminal vesicles
MSCs	Mesenchymal stem cells
ESCRT	Endosomal sorting complexes required for transport
PLA2R	M-type phospholipase A2 receptor
THSD7A	Thrombospondin type-1 domain-containing 7A
Treg	regulatory T
DAF	Decay accelerating factor
MCP	Membrane cofactor protein
CR1	Complement receptor 1
GFR	Glomerular filtration rate
LIMP-2	Lysosome membrane protein 2
MUC3A	Mucin 3A

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Introduction

Membranous nephropathy (MN) is a leading etiology of adult nephrotic syndrome (NS) and a significant contributor to

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primary glomerulonephritis [1,2]. Approximately 80% of MN patients suffer from idiopathic membranous nephropathy (IMN) or primary membranous nephropathy (PMN), which is devoid of an evident secondary cause. This renal disorder is more prevalent among the middle-aged and elderly demographics, even though it can manifest across all age brackets [2,3]. MN is characterized by edema, severe proteinuria, hypoalbuminemia, and hyperlipidemia. However, significant hematuria is rarely observed [4,5]. Clinically, approximately one-third of patients progress to ESRD within 5–10 years, while another third remain in a state of microalbuminuria and the remaining third experience spontaneous remission [6–8]. The disease exhibits distinctive pathological features under optical microscopy, including diffuse glomerular lesions with thickened capillary walls, eosinophilic deposits on the epithelial lateral surface, and "spike" (stage II) or "chain ring" (stage III) changes. Immunofluorescence microscopy reveals fine granular deposits of IgG and complement C3 along the glomerular capillary wall. Electron microscopy (EM) examination reveals electron-dense deposits beneath the epithelium and extensive fusion of podocyte foot processes. Ehrenreich and Churg proposed a histomorphological classification for stages I through IV based on immunological deposits and glomerular basement membrane (GBM) thickness observed by EM [9]. Secondary membranous nephropathy (SMN) is a complex and multifactorial condition that affects the kidneys. It is characterized by inflammation and damage to the glomeruli, which are tiny structures in the kidney responsible for filtering waste products from the blood. There are several factors that can contribute to the development of SMN, including autoimmune diseases such as lupus or rheumatoid arthritis, infections like hepatitis B or C, malignancies such as lymphoma or leukemia, drug use including nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics and anticonvulsants, and exposure to heavy metals like mercury or lead. Yet, SMN forms nearly 20% of IMN cases, signifying its significant prevalence [10–13]. The inconsistent patient outcomes in IMN pose therapeutic challenges, emphasizing the pressing need for advanced biomarkers to accurately predict disease trajectory and outcomes [14].

Exosomes are extracellular vesicles that play a significant role in intercellular communication by encapsulating proteins and nucleic acids, such as single-stranded DNAs, double-stranded DNAs, mitochondrial DNAs, mRNAs, and microRNAs. Ranging from 40 to 160 nm in size, they serve as important mediators of cell-to-cell communication [15,16], which originate from small intraluminal vesicles within multivesicular bodies (MVBs), undergo fusion with the cell's plasma membrane to release intraluminal vesicles (ILVs) into the intercellular space [17]. Exosome biogenesis facilitates the transportation of various receptors, proteins, genetic materials, and lipids to target cells. Recent studies have investigated the components of urinary exosomes originating from various nephron segments and their correlation with renal physiology and pathophysiology, including serum/plasma/blood, renal tubular cells, renal tissues, glomerular endothelial cells, mesenchymal stem cells (MSCs), urinary stem cells, and macrophages in kidney diseases are being explored [18–20]. Given these findings, the central research question guiding this review is: "How do exosomes contribute to the pathogenesis, diagnosis, and potential therapeutic interventions of IMN?" By focusing on this central theme, we aim to synthesize and critically assess the current knowledge on the multifaceted roles of exosomes in IMN.

The Biogenesis of Exosomes

In the late 1960s, Bonucci and Anderson reported that chondrocytes secrete small vesicles measuring 100 nm, marking the initial documentation of small, secreted vesicles [21,22]. Later on in 1981, Trams et al. utilized transmission electron microscopy to scrutinize exosomes [23]. Six years later, Pan et al. were able to provide a more precise characterization of exosomes through their analysis of reticulocyte maturation processes in sheep [24]. However, initially these cells were dismissed as mere "waste" and their potential role was not immediately recognized by researchers.

Exosomes are currently recognized as lipid bilayers with a width ranging from 40–160 nm. Exosome formation begins within the endosomal system of the cell. When a portion of the plasma membrane undergoes invagination, early endosomes are formed. Within the early endosomes, there are further invaginations of the limiting membrane, leading to the formation of ILVs inside. As more ILVs accumulate within an early endosome, it matures into a MVBs or late endosome [25,26]. MVBs ultimately merge with the plasma membrane, liberating ILVs into the extracellular milieu. When ILVs are released this way, they are termed exosomes. In addition, the production of exosomes is primarily regulated by the endosomal sorting complexes required for transport (ESCRT), machinery, which consists of four distinct components: ESCRT-0, ESCRT-I, ESCRT-II and ESCRT-III [27,28]. Moreover, MVBs undergo relocalization to the plasma membrane under the guidance of small RabGTPase proteins [29]. This protein family also participates in the recycling of endosomes, trafficking of multivesicular bodies, and transit of intracellular vesicles [30] (Fig. 1).

Beginning with the nucleus as the epicenter of cellular information, the synthesis of proteins and lipids is initiated in the endoplasmic reticulum. As these molecules mature and undergo post-translational modifications, they traverse through the trans-Golgi network, emphasizing the seamless continuum of intracellular trafficking. Concurrently, the cell's energy metabolism is anchored by the depicted mitochondrion. The structural scaffold, maintained by actin filaments, not only provides architectural stability but also coordinates with embedded cell surface proteins to mediate endocytosis. This uptake of extracellular materials swiftly transitions into a systematic and orchestrated sorting within the endosomal compartments. With the critical guidance of the ESCRT machinery, select cargoes are compartmentalized into intraluminal vesicles within the multivesicular body (MVB). These MVBs, guided by the navigational cues from RabGTPase proteins and poised amidst the cellular waste management pathways of lysosomes, eventually align with

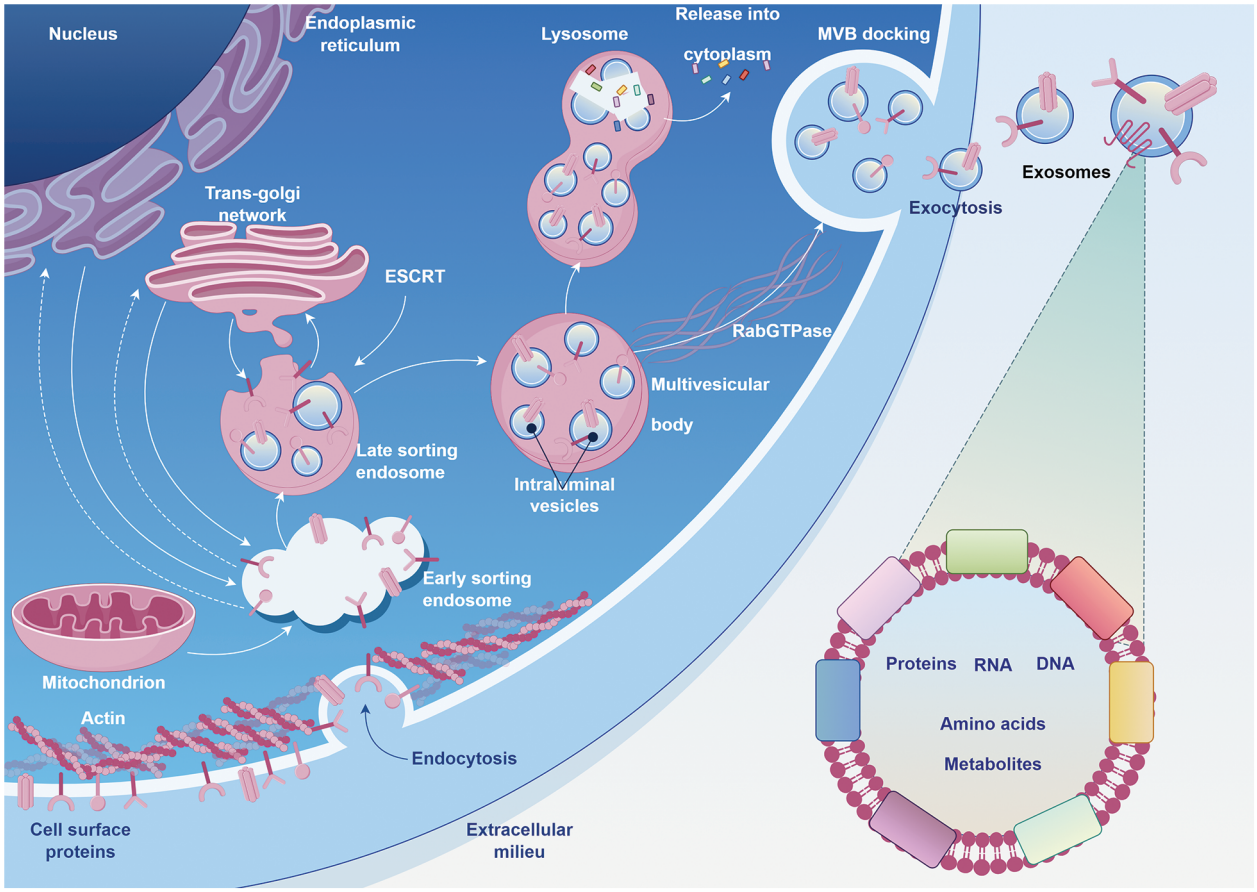

FIGURE 1. The journey of exosome formation, maturation, and release within a cell.

the plasma membrane. In a harmonized finale, the MVBs fuse, releasing exosomes—cellular messengers replete with proteins, RNA, DNA, amino acids, and metabolites—into the extracellular space, symbolizing the cell's mastery in intercellular communication and adaptative signaling.

Exosomes carry a multifaceted molecular cargo, encompassing DNA, RNA, proteins, lipids, microRNAs, small RNAs and non-coding RNAs that are subject to variation based on the originating cell [31]. These cargoes are strictly regulated by the parent cell and serve as a conduit for transmitting specific cellular information to acceptor cells [15]. Despite their heterogeneity in terms of size, composition, and biological origin, exosomes have attracted significant attention in research due to their potential roles in diagnosing and treating various diseases such as neurodegenerative disorders, metabolic diseases, and cancer [32–36]. Surface engineering of exosomes for targeted drug delivery has also been explored, showcasing promising strategies for the diagnosis and treatment of diseases [37–39].

The isolation and extraction of exosomes constitute pivotal initial steps in exploring their functions. Various sophisticated techniques have been developed for exosome isolation and purification, including immunoaffinity capture, density gradient ultracentrifugation, size exclusion chromatography, polymer precipitation, and ultracentrifugation (Fig. 2) [40]. For instance, immunoaffinity capture leverages the specific binding of antibodies to exosomal surface proteins for isolation, density gradient ultracentrifugation separates exosomes based on their buoyant densities, size exclusion chromatography employs a gel filtration technique to isolate exosomes based on their size, polymer precipitation induces the aggregation of exosomes, facilitating their subsequent centrifugation, ultracentrifugation is a common technique that separates exosomes by spinning a liquid sample at high speed. Each technique offers distinct advantages and potential drawbacks, necessitating meticulous consideration in accordance with the research objectives. While certain methods may yield greater exosome purity, others may prove more efficient or less labor-intensive. Therefore, selecting an appropriate isolation and extraction method is crucial to successfully studying exosome functions.

Exosomes are Involved in the Pathogenesis of IMN

In patients with IMN, the development of autoimmunity occurs due to circulating autoantibodies binding to glomerular podocyte antigens. This binding is the most crucial mechanism for the development of IMN. Currently, it is known that autoantibodies bind to antigens such as M-type phospholipase A2 receptor (PLA2R), thrombospondin type-1 domain-containing 7A (THSD7A), and other podocyte antigens, forming basement membrane subepithelial deposits and activating complement [41–43]. This process ultimately damages podocytes, leading to proteinuria [44]. Autoantibodies are generated against both endogenous and exogenous antigens in the early stages of IMN pathogenesis. These antibodies specifically recognize

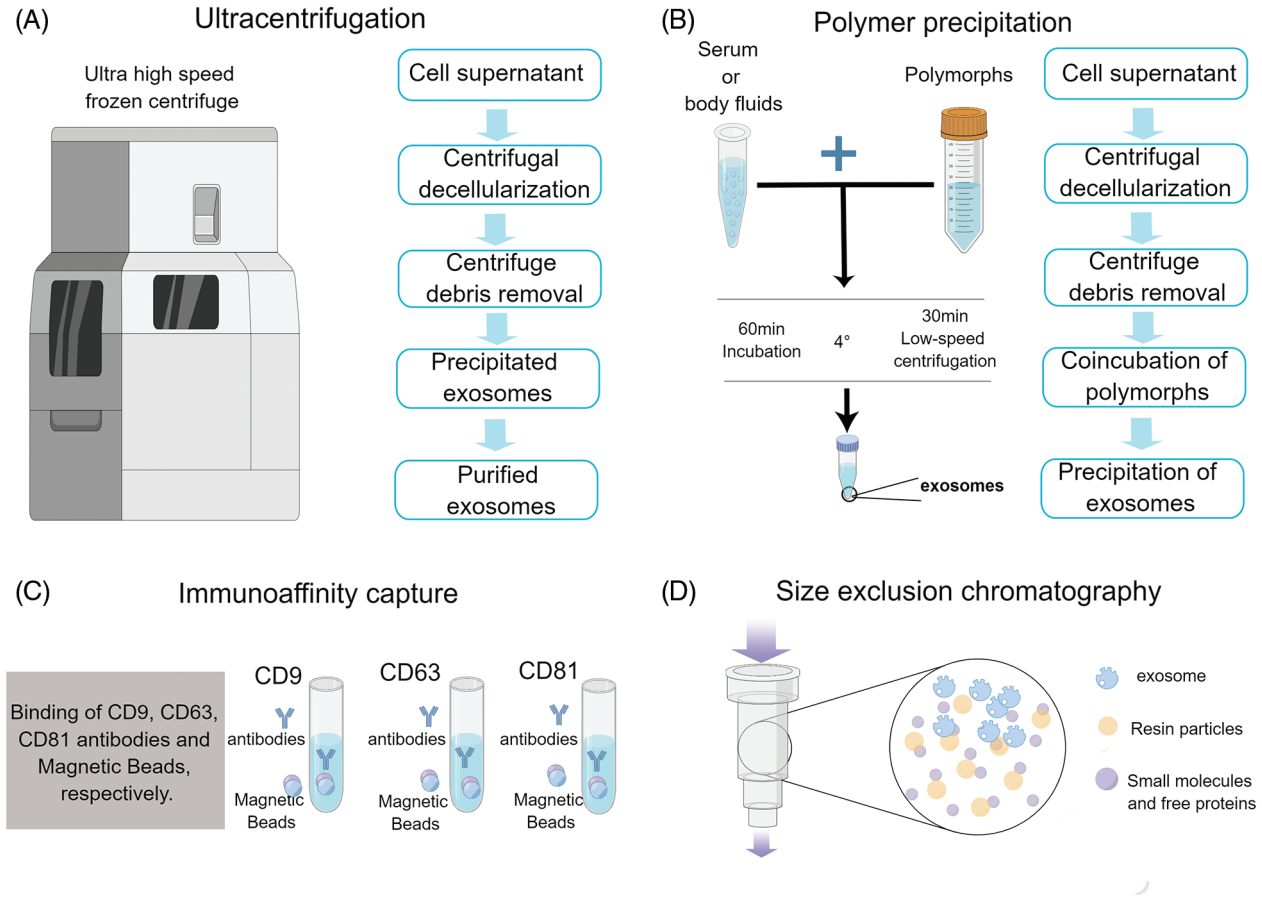

FIGURE 2. Icons show the extraction method of exosomes.

antigens that are inherently expressed on podocytes in IMN, leading to the formation of subepithelial immune complexes upon binding with endogenous antigens [45]. Although the subepithelial deposition of circulating immune complexes may potentially serve as a disease-initiating mechanism in IMN, its confirmation in patients remains elusive [46]. From previous research, the development of autoantibodies can span years or even decades prior to the clinical manifestation of disease, rendering it a formidable challenge to investigate the initial immunological responses and reduction in immunological tolerance in IMN (proteinuria or edema) [47]. Levels of anti-PLA2R antibodies typically rise a few months before a diagnosis of idiopathic membranous nephropathy in patients. The development of clinical symptoms may hinge on a multitude of factors, including the quantity of immune complexes deposited in the subepithelial space, the antibody's ability to traverse the GBM, specific epitope-binding capacity, and IgG subclass composition, all in addition to circulating anti-PLA2R antibody levels. However, it remains uncertain whether these characteristics vary depending on the implicated antigens and associated autoantibodies [48].

Although the exact mechanism remains elusive, exosomes are believed to play a pivotal role in the pathogenesis of IMN. These roles include: exosome-mediated transfer of antibodies or antigens, facilitation of intercellular communication, immunomodulation, inflammatory response, and podocyte injury. Notably, these effects are believed to reinforce each other.

While IMN is caused by autoantibodies binding to podocyte antigens, the mystery lies in how these autoantibodies penetrate the subepithelial area, given that the glomerular filtration barrier (GFB) is highly selective and virtually impenetrable to antibodies. Mario Schiffer's team has tackled this issue and discovered that patients with IMN have elevated levels of GEC-derived miR-192-5p and podocyte-derived miR-378a-3p in their urine and glomeruli, while glomerular NPNT is reduced. In a paracrine manner, GECs use exosomes containing miR-192-5p to inhibit podocyte NPNT transmission. Diminished NPNT induces structural modifications in the GBM, characterized by intensified luminosity, division and stratification, particularly in the atypical inner layer resembling the ultrastructure of advanced MN. The reduction of NPNT leading to increased GBM permeability may exert a pivotal role in IMN pathophysiology by facilitating podocyte antigen presentation and enabling autoantibodies to infiltrate into the subepithelial region [49] (Fig. 2). At this juncture, exosomes appear to assume a 'treacherous' role in the progression of the ailment.

Exosome isolation techniques vary depending on the source and intended use of these extracellular particles. Some common methods include (A) Schematic representation of the exosome isolation procedure using ultracentrifugation. The process begins with the collection of cell supernatant, followed by a centrifugal decellularization step to remove whole cells. Subsequently, centrifugal debris removal is performed to eliminate cellular fragments and other

impurities. This leads to the accumulation of precipitated exosomes at the bottom. After a final purification step, purified exosomes are obtained, ready for downstream analyses or applications. (B) Schematic representation of the exosome isolation procedure using polymer precipitation. The workflow starts with the extraction of the cell supernatant. This is followed by centrifugal decellularization to exclude whole cells. The next phase involves centrifuge debris removal, eliminating cellular fragments and potential contaminants. Afterward, the sample undergoes coincubation with polymers, facilitating the aggregation and subsequent precipitation of exosomes. (C) Schematic representation of the exosome isolation procedure using immunoaffinity capture. This method employs specific antibodies targeted against exosomal markers such as CD9, CD63, and CD81. Initially, the sample is exposed to immobilized antibodies that recognize these markers. Upon binding, the exosomes are selectively captured while non-targeted components are washed away. This results in a highly specific collection of exosomes that can be eluted and utilized for downstream analyses. (D) Schematic representation of the exosome isolation procedure using size exclusion chromatography. A mixture containing exosomes is loaded onto a chromatography column packed with porous beads. As the sample travels through the column, larger particles are excluded from entering the pores and elute earlier, while smaller particles permeate into the beads and are delayed. Exosomes, having a specific size range, are then selectively eluted at a distinct fraction, allowing for their effective separation from other components. Each method has its own unique set of advantages and limitations that must be carefully considered when selecting an appropriate protocol.

In another aspect, podocytes may secrete exosomes to interact with other cells, such as immune cells, resulting in immune modulation, complement activation and other processes. For instance, it has been reported that dendritic cells can release exosomes containing antigen-presenting characteristics and peptide-MHC complexes which facilitate the activation of both dendritic cells and T cells [50]. However, there is insufficient literary evidence to substantiate whether this holds true for foot cells. Furthermore, T cells play a pivotal role in the development of autoimmunity. Several studies conducted on individuals with IMN have demonstrated alterations in T cell subpopulations [51]. Patients with IMN exhibit an elevated frequency of T helper 17 cells and a decreased frequency of regulatory T (Treg) cells in their serum, as compared to healthy individuals. However, this imbalance can be rectified through appropriate proteinuria treatment [52,53]. Another clinical report has revealed that the severity of IMN is closely associated with the number of follicular helper T cells, as well as plasma cells and regulatory B cells [54–56]. Intriguingly, a vast body of literature attests to the direct or indirect activation of T cells by exosomes. For instance, one study revealed that tumor-derived exosomes can downregulate CD3 in primary activated T cells and induce apoptosis in $CD8^+$ T cells [57]. A separate investigation revealed that during homologous interactions between T cells and dendritic cells, exosomes secreted by the latter into the extracellular milieu specifically target activated T cells within that microenvironment [58]. Furthermore, it has been reported that exosomes possess the ability to present antigens to dendritic cells and bind with TLR2/3, thereby initiating dendritic cell activation through the NF-κB signaling pathway. This ultimately leads to an imbalance in $CD4^+$ T lymphocyte differentiation [59].

In addition to that, complement activation plays a crucial role in the pathophysiology of MN. Complement components, including C1q, C3, C4d and C5b-9, are frequently detected within the immunological deposits of MN patients alongside proteins from both classical and alternative complement pathways [60]. This has been demonstrated in a rat model of Heymann nephritis, where immunological deposits triggered the activation of complement system components, including C3 and C5b-9 deposition [61,62]. Currently, mounting evidence suggests the pivotal role of exosomes in this process. For example, a study on Diabetic Retinopathy (DR) revealed that plasma-borne exosomes carrying IgG trigger the classical complement pathway. Interestingly, an increase in these IgG-laden exosomes was observed in diabetes, exacerbating the pathological damage associated with DR [63]. In a separate investigation, it has been revealed that exosomes derived from tumors can activate the complement system via a calcium-sensitive pathway, demonstrating a concentration-dependent effect. This activation subsequently enhances the metastatic dissemination of the tumor [64]. Exosomes secreted by platelets, leukocytes, and red blood cells collectively act as mediators of the pathogenic aspects of complement activation. They can induce complement activation on their parental cells or explicitly accumulate complement on their own surface [65–67]. This behavior is expected to be more prevalent in individuals with complement-mediated diseases rather than those who are healthy. Under physiological conditions, the excessive activation of complement on exosomes derived from blood is regulated by various complement regulators, such as decay accelerating factor (DAF), membrane cofactor protein (MCP), CD59, and complement receptor 1 (CR1) [68].

Lastly, the impact of exosomes on MN development and progression is multifaceted and extends beyond mere facilitation of autoantibody formation. One crucial role they play is in antigen delivery. By carrying and presenting specific antigens, exosomes can trigger localized inflammation within the glomeruli. This inflammation can trigger a cascade of immunological reactions, leading to modifications in the glomerular basement membrane and culminating in the formation of subepithelial deposits, which are characteristic of MN. This process not only contributes to the initial stages of MN but also has the potential to drive its progression. In the presence of persistent inflammation and formation of immune complexes, renal damage can intensify, resulting in a decline in renal function and the progressive nature of MN. Additionally, exosomes may also play a role in regulating glomerular filtration by means of signaling pathways that are mediated by these tiny vesicles, which could potentially increase the glomerular filtration rate (GFR). The glomerular filtration rate (GFR) is of paramount importance to the proper functioning of the kidneys, and any deviations

from this critical parameter have been linked to the onset of nephrotic syndrome, a common affliction among those with MN. Exosome-mediated mechanisms facilitating an increase in GFR may potentially exacerbate proteinuria, a significant clinical manifestation of MN. Additionally, these exosomes may transport molecules capable of modulating the permeability of glomerular capillaries, indirectly influencing the filtration process and worsening the severity of nephrotic syndrome.

Consequently, a thorough grasp of the diverse roles exosomes play in MN pathogenesis, encompassing aspects such as antigen presentation, immunomodulation, inflammatory response, and podocyte injury, among others, could offer fresh perspectives on disease mechanisms and uncover potential therapeutic avenues. Nevertheless, more extensive research is crucial to conclusively determine the depth of exosome participation in MN and evaluate their viability as therapeutic targets.

Roles of Exosomes in the Diagnosis of IMN

To this day, renal biopsy remains the diagnostic gold standard for IMN. Its unparalleled accuracy provides a definitive diagnosis, but the procedure comes with its own set of challenges. The invasive nature of a renal biopsy, combined with its specific puncture requirements and potential complications, makes it a less than ideal choice for routine diagnosis, especially for monitoring disease progression or therapeutic responses.

With the identification of PLA2R and THSD7A as target antigens for IMN, diagnosis has been refined to align with the disease's underlying pathology [69]. PLA2R and THSD7A exhibit a remarkable degree of specificity towards IMN [43,70,71]. As an illustration, a study revealed that IMN was present in 129 out of 132 patients with anti-PLA2R antibody-positive status following renal biopsies [72]. In a meta-analysis of 15 trials, the sensitivity and specificity of anti-PLA2R antibody detection were found to be 78% and 99%, respectively [73]. Similarly, antibodies against THSD7A have not been detected in healthy individuals or patients with other renal and systemic diseases, demonstrating 100% specificity for MN damage. However, a previous study found that among a group of 42 patients with PLA2R antigen-positive immune complexes in renal biopsies, 10 individuals had no detectable blood antibodies. In a separate investigation, the presence of anti-PLA2R antibody was only detected in 73 out of 217 IMN patients (33.6%) in Japan [74]. According to these studies, while anti-PLA2R antibodies may persist in some individuals for years without manifesting any significant clinical symptoms, in other patients their levels escalate and become detectable only a few months prior to an MN diagnosis. One possible explanation for this phenomenon is that despite the strong affinity of anti-PLA2R antibody towards its antigen, it remains unrecognized until all binding sites are occupied [75]. Therefore, it serves as a poignant reminder that despite the efficacy of anti-PLA2R and anti-THSD7A antibodies in IMN diagnosis, limitations persist.

Exosomes, especially those derived from urine, present a promising non-invasive method for IMN diagnosis. Their stability, combined with the ease of collection, offers significant advantages over traditional methods. The cost-effectiveness of exosome-based tests, given their non-invasive nature, makes them an attractive option for routine diagnosis and monitoring. Exosomes remain stable at 4°C for 24 h prior to storage at −80°C, while those derived from urine maintain stability for up to 12 months when stored under similar conditions, as reported by Lv et al. Remarkably, even after undergoing five freeze-thaw cycles, exosomal miRNAs remain detectable [76,77]. Urinary exosomes, accumulating evidence suggests, hold great potential as noninvasive indicators for the diagnosis of IMN [78,79]. For example, proteomic studies have offered valuable insights into the role of proteins in urinary exosomes. One notable protein is lysosome membrane protein 2 (LIMP-2), which is found in abundance in the urinary microvesicles of IMN patients. This presence correlates with an increased expression of LIMP-2 in the glomerulus. Additionally, the co-localization of LIMP-2 and IgG on the glomerular basement membrane has been observed, pointing towards the significance of urinary exosomes in biomarker discovery. Such findings have established the *de novo* expression of LIMP-2 in the glomeruli affected by IMN [80].

Interestingly, MicroRNAs (miRNAs) carried by urinary exosomes have been the focus of several studies. The researchers have discovered that IMN patients exhibit increased levels of miR-192-5p and miR-378a-3p in their urine and glomeruli, accompanied by a corresponding reduction in glomerular NPNT. By delivering exosomes carrying miR-192-5p, glomerular endothelial cells have inhibited podocyte NPNT through paracrine signaling. This interaction has resulted in structural alterations within the GBM, particularly affecting its inner layer (Fig. 3) [49]. Furthermore, a high-throughput sequencing analysis has revealed that miRNAs present in urine exosomes are the most efficacious biomarkers for distinguishing IMN in adults. The authors discovered significant disparities in the urinary exosome miRNA expression profile between individuals with IMN and those who are healthy. MiR-9-5p exhibited a close correlation with various clinical indicators of IMN, including serum albumin, triglycerides, and β2-microglobulin, among others. Additionally, MiR-30b-5p demonstrated a positive correlation with anti-phospholipase A2 receptor antibodies. Therefore, miR-30b-5p and miR-9-5p may serve as novel non-invasive biomarkers for IMN [81]. The results of another high-throughput sequencing analysis have revealed significant differences in the expression patterns of urinary exosomal miRNAs and repetitive region-derived sRNAs between patients with IMN and healthy individuals [82].

On glomerular podocytes, the endogenous antigens phospholipase A2 receptor 1 (PLA2R1) and thrombospondin type 1 domaincontaining protein 7A (THSD7A) are expressed, whereas, GEC blocks podocyte NPNT via paracrine transmission of exosomes containing miR-192-5p. Reduced NPNT as a result of GBM permeability may permit autoantibodies to enter the subepithelial region. As revealed for PLA 2R1 and THSD7A, primary membranous nephropathy (MN) is initiated by the

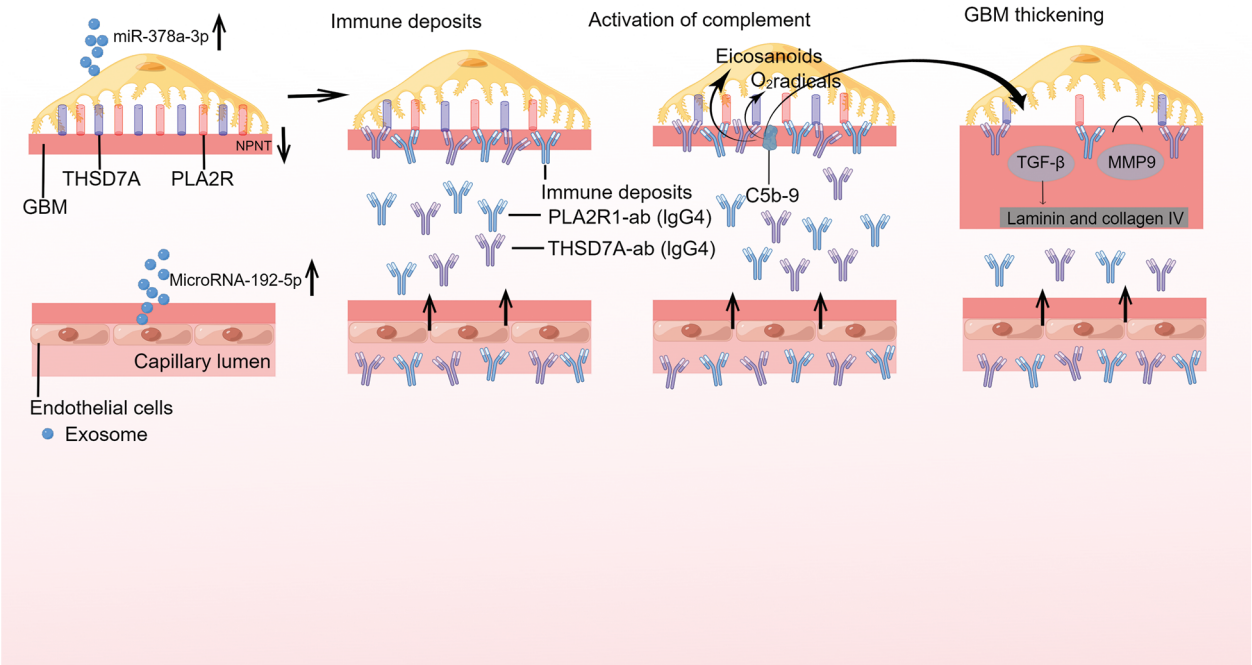

FIGURE 3. Exosomes are involved in the pathogenesis of primary membranous nephropathy.

binding of circulating antibodies to endogenous antigens expressed on glomerular podocytes. Antibody binding induces the production of immunological deposits in the subepithelial space and in the glomerular basement membrane (GBM), which might affect the integrity of the podocyte cytoskeleton and result in proteinuria. The presence of immune deposits activates the complement system. The activation of secondary mediators like oxygen radicals and eicosanoids is caused by the development of the terminal complement component C5b-9, commonly known as the membrane assault complex. These mechanisms result in the breakdown of the glomerular filtration barrier and initate proteinuria. These structural alterations are mediated by TGF-induced production of laminin and collagen type IV, as well as the metalloproteinase 9 (MMP9) protein turnover in the GBM.

Recent research has directed attention to the potential of circular RNAs (circRNAs) as diagnostic markers for IMN. Profiling these circRNAs in exosomes from IMN patients' serum and urine has revealed distinct and specific expression patterns. For instance, MUC3A originating from chr7:100550808|1005501062 could potentially be a diagnostic biomarker for IMN [83]. Another study pinpointed the increased expression of hsa circ 0001250 in IMN, especially in instances of high proteinuria. This circRNA might play a role in IMN's pathophysiology by targeting specific miRNAs [84] (Table 1).

Urinary exosomes, enriched with specific microRNAs and proteins, offer early detection capabilities for IMN and insights into its progression. As reflections of their parent cell's biological state, exosomes reveal insights into disease development. Changes in exosomal content can signal

TABLE 1

Summary of research diagnosis on MN-related exosomes biomarkers

Biomarkers	Sample type	Value in MN	Reference
LIMP-2	Urine	Diagnosis	[74]
miR-192-5p, miR-378a-3p	Urine	Diagnosis	[42]
miR-30b-5p, miR-9-5p	Urine	Diagnosis	[75]
MUC3A	Serum and urine	Diagnosis	[77]
Has-circ-0001250	Urine	Diagnosis	[78]
hsa-miR-1301-3p, hsa-miR-2110	Urine	Diagnosis	[76]
hsa-miR-615-3p, hsa-miR-125a-3p			
hsa-miR-27b-5p, hsa-miR-1271-5p			
hsa-miR-324-5p, hsa-miR-30b-3p			
hsa-miR-589-5p, hsa-miR-454-3p			

TABLE 2

Highlighting the pros and cons of each method in terms of accuracy, specificity, sensitivity, cost, and availability

Diagnostic method	Pros	Cons
Renal Biopsy	1. Unparalleled diagnostic precision. 2. Can differentiate between various renal diseases. 3. Detects even subtle pathological changes.	1. Expensive due to specialized equipment and expertise. 2. Requires hospital settings and might not be available in all healthcare centers. 3. Possibility of complications due to its invasive nature.
Antibody Detection (PLA2R and THSD7A)	1. High specificity towards IMN. 2. In many cases, sensitivity is high. For instance, anti-PLA2R antibody detection has 78% sensitivity. 3. Typically lower cost than renal biopsies.	1. Patients may test negative for both PLA2R and THSD7A antibodies, making diagnosis unreliable in such cases. 2. Requires laboratory infrastructure and trained personnel.
Exosome-based Diagnosis	1. Urinary exosomes stability, combined with the ease of collection, making it non-invasive. 2. Emerging research shows promise for high specificity and sensitivity.	1. Still in research phase, so comprehensive accuracy data is not yet available. 2. While potentially cost-effective in the long run, initial investments in research and technology may be high.

disease severity. While we are still learning about exosomal cargo origins and mechanisms, it is essential to discern if these changes are mere disease byproducts or active contributors to IMN. The clinical utility of exosomal biomarkers needs validation in larger patient groups, but they hold promise for non-invasive IMN diagnosis and personalized treatment. Future research should focus on understanding exosome biology and its therapeutic potential for IMN (Table 2).

Roles of Exosomes in Therapeutics Target of IMN

Despite the ability of current therapies to temper the course of IMN, a pervasive linkage remains with a high prevalence of ESRD. This saliently underscores the urgency for more efficacious interventions that could arrest or reverse the progression of IMN. Consequently, the pursuit of innovative, robust renoprotective strategies is of paramount importance. Emerging evidence advocates the potential exploitation of exosomes, given their rich reservoir of mRNAs, miRNAs, and proteins, coupled with their instrumental role in mediating cell-cell communication, as propitious therapeutic delivery vectors [85]. Exosomes, laden with RNA, may induce a transient restoration of perturbed cellular processes by transferring their cargo to recipient cells. For example, studies have demonstrated that specific miRNAs, when transported via exosomes, can modulate molecular pathways integral to the pathogenesis of IMN. However, the acquisition of a comprehensive mechanistic understanding of this process remains indispensable [86]. Moreover, exosomes derived from MSCs have unveiled considerable promise in the IMN treatment landscape, particularly owing to their inherent immunomodulatory properties. By fine-tuning immune cell functionality and attenuating inflammation, MSC-derived exosomes could facilitate the re-establishment of immune equilibrium within the kidneys, thereby potentially ameliorating symptoms and decelerating disease progression [87].

Exosomes show transformative potential for both the diagnosis and treatment of IMN. Their role in mediating cell-cell communication and their rich content of mRNAs, miRNAs, and proteins could offer novel renoprotective strategies for IMN. Moreover, understanding the mechanisms underlying exosome-mediated miRNA and circRNA regulation, through both *in vitro* and *in vivo* models, can shed light on their utility in a disease-specific context. MSCs derived exosomes, in particular, hold promise due to their inherent immunomodulatory properties.

However, harnessing this potential is not without challenges. The production of these nano-sized vesicles requires upscaling, and there is a paramount need for pure extraction without contamination from other vesicles. This necessitates robust protocols for exosome production, characterization, and administration, especially given the current lack of standardization in the field. The variable content of exosomes between individuals, influenced by factors such as genetics and environment, could affect diagnostic precision. Additionally, the accurate delivery of exosomes to target tissues, long-term safety concerns, and the cost-prohibitive nature of advanced exosomal techniques pose significant hurdles.

As we continue to explore exosomes' role in IMN, it is crucial to approach their potential with a comprehensive understanding, considering both their promising aspects and the challenges that need addressing.

Conclusions and Future Perspectives

Idiopathic Membranous Nephropathy presents a significant and intricate public health challenge that affects individuals worldwide. Despite numerous efforts, targeted therapies for IMN have yet to be discovered. Collaboratively, nephrologists and endocrinologists are exploring innovative techniques to promptly and accurately detect renal damage caused by IMN while investigating the disease's molecular basis to develop groundbreaking treatments.

Recent research on exosomes in IMN has unveiled novel aspects of the disease's pathogenesis. Exosomes play a pivotal role in facilitating intercellular communication, exerting

significant influence on both the onset and progression of IMN. Exosomes released by immune cells and podocytes can incite inflammation and inflict damage upon glomeruli, ultimately leading to the formation of immune complexes and the subsequent development of MN. Exosomes present a significant potential as both diagnostic and therapeutic targets in the management of IMN. Scientists have identified exosomal biomarkers, including specific miRNAs and proteins, which may serve as non-invasive diagnostic tools for MN. Moreover, targeting exosomes offers a promising avenue for the treatment of MN. Preliminary studies suggest that inhibiting either exosome release or uptake could reduce the severity of MN, emphasizing that therapies based on exosomes might prove effective.

However, it is of paramount importance to delve deeper into the molecular mechanisms underlying the role of exosomes in IMN. Understanding these intricacies can provide a clearer picture of the potential therapeutic applications of exosomes in treating IMN. Furthermore, comprehending the challenges in harnessing exosomes for clinical use is indispensable. While the identification of specific exosomal biomarkers linked with the development and progression of MN is crucial for creating efficacious diagnostic tools and therapies, the mechanics governing kidney exosome release and uptake remain vital areas of research for developing potent exosome-centered treatments.

In conclusion, the examination of exosomes in IMN has yielded invaluable insights into the pathogenesis of this disease, emphasizing their potential as both diagnostic and therapeutic targets. Further exploration in this realm may pave the way for revolutionary diagnostic tools and therapies, ultimately enhancing clinical outcomes for patients afflicted with IMN.

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