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Review

The role of exosome in different types of rejection in kidney transplantation; a diagnostic and prognostic approach

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Abstract

Exosomes play an important role in rejection after organ transplantation. Different cells secrete exosomes to activate different cellular-molecular signaling which ultimately leads to kidney rejection or transplantation. To shed some lights on this issue, this paper aimed at reviewing the related articles published between the years of 2005 and 2020, and retrieved from Pubmed database and Google scholar search engine using the keywords "exosome", "kidney transplantation", "diagnosis", and "rejection". The findings show that exosomes secreted by immune cells express the surface markers of the origin cell and exert their influence on the target cells according to the function of the stem cell. Generally, almost all cells involved in immune responses secrete their own exosomes, which transmit their contents to cause genetic and phenotypic changes in receptor cells. Since the secretion of exosomes from immune cells increases after kidney transplant rejection and the markers on the surface of the enlarged exosomes indicate the presence of the secreted cell, it is possible to inhibit the signaling pathways caused by the exosomes through monitoring the patient's clinical process after transplantation.

Keywords: Exosome, Kidney transplantation, Diagnosis, Rejection

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Introduction

Nowadays, transplantation of different organs is considered as an important and effective treatment which has attracted particular attention in the process of the patient's survival; however, it has a series of complications that can be life threatening for the patients (1). Kidney is one of the organs that is transplanted every year for many patients in the world. Although kidney transplantation has improved the clinical condition of patients, rejection and infection due to the use of immunosuppressive drugs may reduce patients' survival (2). Although the main diagnostic markers for diagnosing and preventing the acute and chronic rejection of kidney transplantation have not been identified, some factors seem to play important roles in rejecting kidney transplantation such as exosomes. Exosomes which are a series of extracellular vesicles secreted by many cells into urine, blood, and interstitial fluid, have been reported to increase in patients after kidney transplant rejection (3,4). Studies have shown that the presence of RNAs and proteins within exosomes is highly specific and can be used as a diagnostic marker to detect kidney transplant rejection. In addition, it has been reported that exosomes play an important role in rejecting kidney transplantation due to disruption of signaling pathways (5). Therefore, the aim of the present study is to evaluate the role of exosomes in the pathogenesis of kidney transplant rejection.

Structure and Function of Exosome

Exosomes are small membrane vesicles with an average size of 30 to 100 nm that are secreted by most cell types such as B/T lymphocytes, macrophages, dendritic cells (DCs), endothelial / epithelial cells, human embryonic kidney cells and body fluids (6). Like other vesicles, exosomes have two compartments, an aqueous core and a bilayer lipid membrane. The exosome is a regulated macromolecular machine that plays an important role in intercellular communication without direct cell-to-cell contact by transferring its contents, such as proteins, lipids, and RNAs, between cells (7). These membrane vesicles are released into the extracellular environment by the plasma membrane fusion of multivesicular bodies and cause genetic and phenotypic changes in the recipient cells by transferring their contents from the donor cells to the recipient cells. For example, RNA in exosomes derived from mouse mast cells can be transferred to human mast cells and transcribed into mouse proteins (8,9). Three possible mechanisms for the function of exosomes have been reported: 1) Exosomes fuse with the plasma membrane of the receptor cell and subsequently

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Implication for health policy/practice/research/ medical education

The identification of cellular and molecular mediators involved in the pathogenesis of renal transplant rejection could be a new therapeutic strategy for the treatment of patients with chronic kidney disease. Exosomes, as one of the main factors involved in intercellular signaling, play an important role in post-transplant rejection.

release their contents into the cytoplasm of the receptor cell, 2) Exosomes communicate with target cells through receptor-ligand interactions or binding to cell surface lipids such as phosphatidylserine (PS), and 3) Exosomes enter receptor cells through endocytosis (10). Immune cells secrete proteins and substances that bind to the receptors of surrounding cells in a variety of ways such as, releasing exosomes into their environment and altering their function. Furthermore, the small size of the exosomes allows them to travel through the endothelium and penetrate the underlying tissue (9). Depending on their origin and composition, exosomes can have immune-stimulating or immunosuppressive properties. Exosome secretion by antigen presenting cells (APCs) seems to have a potential role in initiating immune responses. Exosomes secreted by different types of immune cells show molecules on their surface that are specific to the types of cells secreting them (11).

Communication between exosomes and immune cells

a. Macrophages

The onset of macrophage-mediated inflammation signaling is a consistent finding in the exacerbation of acute renal injury (12). In damaged kidney tissue, the increase of proinflammatory macrophages leads to tissue damage, inflammation, and fibrosis of the kidney. Signaling by binding of C-C motif chemokine receptor 2 (CCR2) to its ligand, monocyte chemoattractant protein (MCP-1) causes monocytes to accumulate in damaged tissues and differentiate them into macrophages (13). MCP-1 and CCR2 are involved in a variety of kidney diseases as well as progressive kidney damages including, chronic kidney transplant rejection by activating nuclear factor kappa B (NF-kB) pathway and activator protein 1 (AP-1) (14). Therefore, inhibition of MCP-1/CCR2 interaction may be effective in preventing macrophage-induced tissue damage. Inhibition of CCR2 by propagermanium reduces macrophage infiltration and expression of TGF-β and consequently prevents inflammatory responses (15).

The findings show that mesenchymal stem cell-derived exosomes (MSCs) contain CCR2 which can play a protective role in kidney damage, because increasing the expression of CCR2 in exosomes derived from MSCs by binding to MCP-1 reduces its concentration (16). So, it can be said that exosomes secreted by resident macrophages in the kidney can also have CCR2, which binds to MCP-1 to increase inflammatory factors in the kidney. As a result, macrophage activity in the kidney can be suppressed by creating conditions that make MCP-1 unavailable, which benefits kidney function and reduces tissue damage (17,18). From a different perspective, studies in mice show that the uptake of circulating monocytes into the kidney is significantly reduced after the CCR2 knockout, which is also beneficial for kidney function and prevents further damage because inhibiting the transfer of monocytes reduces their differentiation into macrophages (19). As the findings show, the effect of CCR2 reduction or elimination on kidney is a controversial issue. We theorize that preventing the transfer of macrophages to the kidney by blocking signaling or removing CCR2 before transplantation begins conversely after transplantation, that is, increased CCR2 expression can prevent the release of MCP-1 in the recipient and prevent the onset of inflammatory responses.

Biglycan and hyaluronan are secreted by kidney cells during ischemia reperfusion injury which is an inevitable event associated with kidney transplantation, and by binding to toll-like receptor 4,2 (TLR4, 2), expressed on the surface of renal tubular epithelial cells and glomerular endothelial cells (20). They activate NF-κB and increase the production of inflammatory cytokines and MCP-1. All TLRs except TLR3 can activate NF-κB and promote inflammation through MyD88 which is one of the main reasons for transplant rejection (21). During kidney infection, TLRs activate renal macrophages by innate pattern-recognition receptors through detecting pathogen-associated molecular patterns (PAMPs), leading to the secretion of exosomes containing proinflammatory molecules from macrophages (22). Mice deficient in TLR adapter protein MyD88 show greater resistance to allograft rejection in donor kidney transplantation (21). Hypo function of TLR4 has a protective effect against kidney allograft transplantation and reduces transplant rejection, so using its inhibitor (TAK-242) can prevent the activation of MyD88 signaling and inflammatory activity during and after controlled transplantation (23). In addition, depletion of renal macrophages with clodronate and inhibition of pathway JNK improve kidney function and reduce the production of inflammatory factors (24). In contrast, cisplatin-treated macrophages (a major antineoplastic drug in damaged kidney tissue) are more sensitive to PAMPs and damage-associated molecular patterns (DAMPs), known ligands of TLRs, and cause high NF-κB activity, mitogen-activated protein kinase (MAPK) and increased secretion of inflammatory cytokines (25).

Preventing the onset of inflammatory signaling of macrophages such as NF-κB by various mediators can prevent the risk of graft rejection and reduce the use of immunosuppressive drugs (Table 1). B-arrestins are cytosolic adapter proteins known as G protein-coupled receptor negative adapters involved in the pathogenesis of various tissue disorders (26). β-arrestin-1 and β-arrestin-2 are expressed in many tissues of the body like kidneys,

and their mode of action in signaling pathways is unclear and controversial (27). These proteins attenuate NF-κB activity by binding to IκBα, an inhibitor of NF-κB (28). As a result, its increase can prevent inflammatory responses induced by NF-κB after transplantation. However, one study found that β-arrestins trigger them by interfering with various signaling pathways such as, JNK, MAPK, and NF-κB. β-arrestin-1 exacerbates renal fibrosis through Wnt1/β-catenin signaling and is associated with fibroblast activation (29). Silencing the β-arrestin-1 gene also reduces TGF-β secretion from macrophages, infiltration of macrophages, and secretion of inflammatory cytokines from macrophages. To bridge the gap, further studies on kidney transplant patients are recommended to reach a clear conclusion about the association between β-arrestins and signals involved in renal impairment.

b. Dendritic cells

Active DCs are the most effective APCs in humans and are present in lymphoid and nonlymphoid tissues, including the kidneys. Donor DCs that travel to the recipient's secondary lymphatic tissues after transplantation are a major factor in post-transplant immune responses (30). DCs surface antigen binds to T cell receptors through the TCR/CD3 complex and stimulates T cells to initiate "signal 1". Then, "Signal 2" begins when CD80 and CD86 interact with CD28 T cells on the surface of DCs. Signal 1 and Signal 2 activate the three signaling pathways calcium-calcineurin pathway, MAPK, and NF-kB. These pathways activate transcription factors that secrete many inflammatory molecules (31). IL-2 and IL-15 trigger the "signal 3" through the pathway (PI3K) phosphoinositide 3-kinase and molecular-target of rapamycin (mTOR) to initiate cell proliferation. Exosomes secreted by antigen-stimulated DCs secrete efficient and active exosomes that can lead to humoral and cellular responses (32). Cyclosporine is an immunosuppressive drug that binds to cyclophilin and inhibits calcineurin signaling and prevents DCs-induced cell proliferation (33) (Table 1).

Exosomes secreted by normal and immature DCs can increase the phagocytosis of apoptotic cells by milk fat globule EGF-8 (MFG-E8) and decrease the secretion of inflammatory cytokines (40). MFGE8 or lactadherin is one of the major proteins identified in exosomes secreted by DC cells that bind to PS in other exosomes and increase phagocytosis of apoptotic cells by macrophages (41). The presence of MFG-E8 in phagocytic cells by inhibiting the NF-κB pathway causes the secretion of anti-inflammatory cytokines like IL-10 and tumor growth factor β (TGFβ) from these cells and increases the production of regulatory T cells. Exosomes produced from DCs treated with TGF-β and IL-10 induce immune tolerance in mice undergoing skin transplantation (42). In a study, it has been reported that MFG-E8 can improve renal function (43). Given this, treating kidney transplant patients with MFG-E8 after transplantation may increase the retention of the kidney in the recipient and reduce the use of immunosuppressive drugs.

since exosomes show PS at their surface, they can be detected by phagocytes and reduced by endoplasmic reticulum uptake by endothelial cells through blocking PS by means of annexin V or Diannexin (44). Tim1 and Tim4 are two PS ligands that are expressed on the surface of activated lymphocytes or phagocytes (45). The use of PS blockers during or after kidney transplantation may reduce the transmission of exosomes secreted by connective tissue DCs and increase tissue persistence in the recipient body. Based on the importance of DC in kidney transplantation, it can be argued that removing this cell from the kidney or inactivating it before transplantation can also lead to long-term survival of connective tissue in the recipients. In general, inhibition of DCs in various ways is beneficial to the bonding process.

T-cells

Organ transplantation can activate recipient's T lymphocytes in two ways: 1. through alloantigens that are delivered directly by DCs from donor organ donors to T cell MHC, 2. Alloantigens that are received and processed by DCs receptors and indirectly stimulate the T cell (46). It seems that in the second pathway, exosomes secreted by DCs may be involved and consequently lead to the response of T cells. Since renal transplant rejection depends on the activation of the recipient's T lymphocytes, exosomes secreted by activated T-cells appear to trigger various cytokines that are important mediators in initiating immune responses. For example, significant increases in serum levels of Interferon-γ (IFN-γ) and interleukin 10 (IL-10) have been reported in patients with renal transplant rejection (47). IFN-γ production is mainly induced by NK cells and T cells under antigenic stimuli. IFN-γ increases PU.1 expression and causes myeloid differentiation which is directly related to increased rejection of myeloid cells (48). Due to the increase in IFN-γ levels in transplant recipients, the control of this cytokine prior to transplantation by a limiting factor, IFN-regulatory factor-2 (IRF-2), seems to prevent transplant rejection (49). By the way, the role of IFN-γ in the transplantation process is not well understood. Some studies have shown that IFN-γ can act as a hematopoietic inhibitor by inhibiting the overproduction of immune effector cells such as T cells and myeloid cells at the homeostasis level which seems to be in favor of preserving the grafted tissue.

Decreased ATP causes cell vulnerability and dysfunction of immune system cells. Increasing c-AMP increases IFN-γ secretion by acting on the P38/JNK/ATF-2 signaling pathway (50). IFN-γ stimulation reduces ATP in T cells and their apoptosis. When IFN-γ binds to its receptor, it activates the Janus kinase (JAK) as an ATP-dependent enzyme (STAT) signal transducer and activator of transcription through phosphorylation (51).

Table 1. Some signaling inhibitors involved in kidney function and related disorders

Drugs	Mechanism	Potential action	Ref.
B-arrestin 1	By binding to IκBα, it inhibits NF-κB activity	It can control NF-κB-induced inflammatory pathways after transplantation (Activation of fibroblasts and exacerbation of renal fibrosis)	(29)
Propagermanium	Targets glycosylphosphatidylinositol-anchored proteins and indirectly inhibit NF-κB and AP-1.	It can prevent an increase in inflammatory factors that increase the risk of transplant rejection	(15)
Pyrrolidine dithiocarbamate	Inhibition of NF-κB signaling	Reduces kidney damage, tubular necrosis and tubular inflammation due to folate or adenine overload in mice	(34,35)
Resatorvid (TAK-242)	Prevent TLR-4 signaling with binds directly to the amino acid Cys747 in the intracellular domain of TLR-4	Indirectly inhibits the activation of NF-κB and MyD88 and prevents the secretion of proinflammatory exosomes (Increased lipopolysaccharide in urine, Reduction of moderate pulmonary artery pressure)	(36)
Cyclosporine	By binding to cyclophilin, it inhibits calcineurin phosphatase and T cell activity	Inhibition of T cell activity can be very effective for the stability of the transplanted organ (nephrotoxicity, hypertension and hemolytic-uremic syndrome and diabetes mellitus after transplantation)	(33)
Ruxolitinib	Inhibiting the JAK1-2-STAT3 and Akt/mTOR signaling, Decreased MCP-1 expression in the kidney	In general, inhibition of JAK-STAT activity improves impaired renal function, Suppressed fibroblast activation and may be potentially used to treat kidney diseases.	(37)
Dkk1,2	Bind to LRP5/6 and act as a Wnt signaling inhibitor	Reduced TGf-β-induced proteinuria and fibrosis	(38)
γ-Secretase inhibitor	Inhibition of Notch signaling and TGF-β/Smad2/3 signaling pathway	Significantly reduces the level of transcripts of fibrosis markers and its use can prevent acute kidney damage	(39)

Phosphorylated STAT3 expression has been shown to increase in impaired renal epithelial cells, and genetic deletion of STAT improves renal fibrosis in rat models (52). Thus, increasing c-AMP indirectly activates the JAK/STAT pathway and can be associated with both increased T cell apoptosis and increased production of inflammatory factors due to JAK/STAT activation. Conversely, other studies have shown that IFN-γ can enhance cell proliferation. Studies have shown that a lack of IFN-γ signaling causes hematopoietic stem cells to often remain in the dormant phase and become less differentiated, a feature that can be used for effective transplantation (53).

In vitro tumor cell vesicles induce T cell apoptosis via Fas Ligand (FasL) and galectin-9, or increase differentiation toward Tregs. They can also reduce NK cell cytotoxicity by displaying NKG2D ligands (54). Similarly, exosomes in the plasma of pregnant women contain FasL, which inhibits T cell activation in vitro and reduces CD3ζ expression as a result of T cell response (55). Based on this information, it can be said that stimulation of FasL and NKG2D ligands in transplant patients suppresses the immune response by T cells in transplant patients and reduces the risk of transplant rejection.

Granulocyte colony-stimulating factor (G-CSF) is an inflammatory cytokine that induces myeloid differentiation via C/EBPβ. In addition to its effect on myeloid lineage, G-CSF also has a regulatory effect on T cells (56). Studies have shown that G-CSF can suppress T cell-induced inflammatory tolerance in a number of ways. For example, this cytokine can induce Th1 to Th2 differentiation, suppress proliferative T cell in response to allogeneic antigens, and increase apoptotic signaling in these cells (57). The function of G-CSF on T cells suggests that the use of this factor to induce tolerance in T cells can prevent rapid graft rejection. It can be argued that G-CSF injection into the patient before or after transplantation can control the immune response induced by T cells against the transplanted organ.

MyD88 is crucial for the activation and proliferation of primary T cells and the development of strong immune T cell responses. MyD88 deficiency increases Tregs cells compared to Th17 and decreases activated CD8 T cells, thus, the graft tolerance is increased. A study in mice showed that reducing MyD88 signaling led to developed donor antigen-specific tolerance (21).

In general, preventing the proliferation of T cells and inhibiting the secretion of exosomes from them, which promotes immune responses and inflammatory signaling in patients undergoing kidney transplantation, has not only a protective effect against acute and chronic allograft rejection, but also increased survival after transplantation.

B-cells

B cells as immunoregulatory cells are involved in acute and chronic graft-versus-host disease through the presentation of antigens and the production of various antibodies and cytokines. BM cells and cells in peripheral blood B can be affected by G-CSF and their function can be altered (58). Studies have shown that, in kidney injury, the expression of G-CSF in the kidney is increased while its exact mechanism on B cells is not known with certainty (59). In general, G-CSF can cause downregulated DNA replication by up-regulating the proapoptotic genes. In addition, it has been shown that injecting G-CSF into

donors can dramatically increase the apoptosis of BM B cells (60). Another finding suggests that G-CSF may be able to suppress B lymphopoiesis, but it can also increase the number of B cells in the peripheral blood (61).

G-CSF enhances the expression of the suppressor of cytokine signaling (SOCS1), the cell cycle arrest gene, in B cells SOCS1 disrupts IL-7 R signaling by negatively regulating the JAK-STAT3 signaling pathway, reducing the response of B cells to IL-7, and reducing B cell proliferation (62). Based on the aforementioned findings, it can be concluded that the use of exosomes of cells that inject G-CSF, like BM stromal cells, and transferring them to transplant patients can increase the apoptosis of B cells and prevent the proliferation and initiation of responses.

Follicular helper T cells (Tfh cells), including only Tfh2 and Tfh17, can cause B cells to proliferate by secreting large amounts of IL-21 (63). Tfh cell-derived exosomes in antibody-mediated rejection patients dramatically increase B cell proliferation and differentiation. Also, exosomes secreted by Tfh cells have surface markers namely, "CD4 +" and "CXCR5 +", which are higher in antibody-mediated rejection patients than in other patients. Unlike G-CSF, which was associated with B cell destruction, increased Tfh cell activity is in favor of B cell differentiation and proliferation, thereby increasing transplant rejection (64). As a result, it seems that using T follicular helper (Tfh) cell inhibitors (i.e. anti-CD20 monoclonal) is fruitful in the process of transplantation and further studies on how to control this cell is absolutely helpful.

Urinary exosomes

Urine is a diagnostic marker for the detection of renal dysfunction due to its close correlation with the kidney, and its molecular examination can be helpful in diagnosing acute or chronic kidney transplant rejection (65). When inflammatory reactions are activated, the amount of CD3 positive vesicles released from T cells in patients' urine increases which may be due to the release of other inflammatory cytokines including IL-6. For example, synaptotagmin-17 (SYT17), which is involved in inflammation, is a known protein in the kidney that activates IL-6 and NF-κB via STAT-3 (66). Furthermore, inflammatory factors such as IL-6, TNF-α and kidney-releasing growth factors may cause the production of SYT17. The results of study indicate that patients who express SYT17 also have higher levels of urinary vesicles after kidney transplant rejection (67). Thus, SYT17 is not only a target but also an up-regulator for IL-6, which leads to the excretion of more inflammatory vesicles after transplant rejection.

The use of immunosuppressive drugs after organ transplantation is associated with very common side effects. Cyclosporine and tacrolimus are very effective in preventing graft rejection; however, they can increase Na-K-2Cl cotransporter (NKCC2) and Na-Cl cotransporter (NCC) in patients' urinary vesicles (68). The expression of NKCC2 and NCC disrupts sodium-potassium transport channels and causes hypertension. On the other hand, it has been shown that the secretion of inflammatory cytokines like IL-6 can increase NKCC2 function (69). As a result, urinalysis of kidney transplant patients can be helpful in evaluating the effects of drugs used and controlling their clinical condition.

One of the factors observed in the urinary exosomes of transplant patients is neutrophil gelatinase-associated lipocalin (NGAL), which is produced in the distal nephron. This protein can be a sign of allograft damage and its increase is one of the biological signs of acute and chronic kidney damage (70).

In a nutshell, exosomes reflect the physiological state of kidney cells and their examination can reflect the condition of the transplanted kidney, that is, when the inflammatory response due to rejection of the kidney transplant is activated, vesicles containing inflammatory mediators is observed in the urine of the sick person.

Effect of exosome on HLA

Presence of human leukocyte antigen (HLA) is one of the reasons for transplant immunity rejection. HLA molecules are divided into two classes, HLA-I and HLA-II. Exosomes secreted by MQs, DCs, and B cells express HLA-II, but HLA-I is expressed on many cells in various organs and T cell exosomes (71). HLA-I consists of a heavy chain and a light chain β2-microglobulin (B2M) that disrupts the B2M gene by inactivating HLA-I function and reducing TNF-α. According to these results, disruption of the B2M gene could lead to a complete loss of HLA-I expression on T cells and a significant reduction in immunogenicity (72). This can reduce the use of immunosuppressive drugs and prevent post-transplant infections. In general, disturbances in the expression and structure of HLA molecules on exosomes may reduce the risk of transplant rejection in renal patients.

Exosomes secreted by DCs contain antigens that can be absorbed by the HLA dendritic receptor or delivered to T lymphocytes and activate this cell (73). HLA-II expression appears to be directly related to inflammation because exosomes secreted by intestinal epithelial cells exposed to IFN-γ exhibit more MHC II molecules. Exosomes from antigen-stimulated adult DCs also increase the rejection of male rat skin grafts by female mice and activate CD4 + T cells (74). On the other hand, membrane vesicles derived from tumor cells have been shown to have suppressive properties of the immune system (75). Transplanting these exosomes to patients after transplantation increases the likelihood of transplant survival and inhibits the activity of inflammatory cells and exosome secretion from them. Therefore, it can be said that the exosomes secreted from each cell have the characteristics and function of the mother cell, and by preventing their secretion, the effect of the mother cell on other cells or various organs can be prevented.

Exosome isolation methods

Extracellular vesicles are divided into three groups based on biogenesis, size, content, and function including 'microvesicles', 'exosomes', and 'apoptotic bodies', and no specific markers have been identified to distinguish between different types of extracellular vesicles (76). Because exosomes are found in body fluids such as blood and urine, they are ideal for use as carriers of biomarkers, allowing you to use a non-invasive "fluid biopsy" method for prognosis, diagnosis, and control response of the patients to treatment (77). Ultracentrifugation techniques such as, density gradient centrifugation, rate-zonal centrifugation, and isopycnic centrifugation are methods of isolating exosomes that separate exosomes from the extracellular matrix based on particle density, size and shape (78).

Deferential ultracentrifugation is the first method used to separate exosomes and is the gold standard method for separating exosomes (79). This method requires a large volume of sample, and the exosomes accumulate in 100 000×g spins and are washed. Exposure for more than 4 hours has been shown to cause significant mechanical damage to exosomes at 100 000×g spins. If used for less than 4 hours, it cannot cause complete separation of exosomes from other extracellular space components (80). Due to the low purity in this method and the need for a large volume of sample, it seems that it is not a suitable method for extracting exosomes (77).

Other methods for exosome separation include ultrafiltration, physical and biochemical properties (i.e. microfluidic based isolation techniques), immunoaffinity, and exosome precipitation (i.e. using polyethylene glycol and lectin) have been developed.

Acoustic nanofilter and ExoSearch chip are two methods of microfluidic based isolation techniques. Acoustic nanofilter is a special method in the development stage that has high speed (less than 30 minutes), high purity, and low sample volume (50 µL), ExoSearch chip has the ability to separate exosomes from 20 µL plasma in 40 minutes (81). The development of microfluidic based isolation techniques for the use of exosomes in clinical diagnosis, treatment, and prognosis can be helpful because they require the least amount of plasma and less time compared to other isolation methods. Also, they are affordable and require minimal expertise and training (82).

In Immunoaffinity capture-based techniques, using specific antibodies against the desired antigen at the exosome surface can isolate exosomes from a specific source and other extracellular vesicles. The main advantage of this technique over other methods is that it allows the separation of exosomes from a specific source. It can also separate exosomes from other types of extracellular vesicles if a specific marker for exosomes is identified. The immunoaffinity method leads to less production of extracted exosomes than other methods, and antibody design in this method is also costly (83,84).

However, due to the complexity of the biological fluids from which the exosomes are separated, and the similarity of the physiochemical and biochemical properties between the exosomes and other vesicles, precise separation of exosomes is difficult and usually leads to complex mixtures of extracellular vesicles and other extracellular components. On the other hand, using these methods is costly and time-consuming. Therefore, the use of exosomes in the clinical environment and the study of their contents are limited due to the lack of standard separation methods.

Conclusion

The identification of cellular and molecular mediators involved in the pathogenesis of renal transplant rejection could be a new therapeutic strategy for the treatment of patients with chronic kidney disease. Exosomes, as one of the main factors involved in intercellular signaling, play an important role in post-transplant rejection. Different cells secrete exosomes to activate different cellular-molecular signaling, which ultimately leads to kidney rejection or transplantation. The use of inflammatory signaling inhibitors can control both immune rejection and the risks associated with the transplantation due to the secretion of exosomes from immune cells.

Authors' contribution

HN is a single author of this manuscript

Conflicts of interest

The author declares that he has no competing interests.

Ethical issues

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