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Review Article

The exosome as a novel predictive/diagnostic biomarker of rejection in the field of transplantation

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ABSTRACT

Finding a non-invasive biomarker to monitor allograft status after transplantation could contribute to better control of the post-transplant status of transplant recipients and, if possible, could be used instead of invasive biopsy for proving rejection.

On the other hand, reducing the dosage of immunosuppression or stopping lifelong use of them because of their severe side effects is an important goal in order to dispose of their severe side effects. The ability of exosomes as a biomarker of rejection and as a therapeutic strategy was investigated in the human kidney, heart, and lung transplantation or in transplantation models with interesting results.

Moreover, the ability of exosome was assessed as antigen-presenting vesicles (APVs), in which exosomes can either participate in immune stimulation (semi-direct recognition) or immune suppression thereby, influence on the transplantation outcome. In this paper, authors try to provide comprehensive information about triple role of exosomes in the transplantation medicine.

1. Introduction

1.1. Challenges in transplantation

The half-life of a transplanted kidney is < 10 years. The main reason for allograft rejection is the alloimmune response that is mediated via T cells, B cells, macrophages, dendritic cells (DCs), etc. The alloimmune response against the allograft is mediated via two different pathways including direct and indirect pathways. In the direct pathway, donor DCs migrate to the regional lymph nodes (LNs) and present the entire allo-major histocompatibility complex (MHC)-peptide to recipient T cells, while in the indirect pathway recipient DCs capture alloantigen (like allo-MHC) and present it to allospecific T cells in the context of self-MHC [1].

However, it was reported that exosomes contribute to alloimmune responses against allograft. The entire allo-MHC-peptide of exosomes could be presented to the allospecific T cells via recipient DCs that is known as a semi-direct pathway [2]. Exosomes not only are involved in the third pathway of allorecognition but also can present their MHC-peptide to T cells apart from antigen-presenting cells (APCs), and thereby activate the immune system (Fig. 2).

Moreover, there is no non-invasive reliable biomarker to monitor early post-transplant status. Such a biomarker could be important for graft management and improving long-term allograft survival. Since exosomes' content and surface markers are remarkably different in patients with rejection and non-rejection ones, they could be considered as novel predictive or diagnostic biomarkers in the field of transplantation [3].

On the other hand, the exosomes' ability as a therapeutic strategy was highlighted in the several transplantation model studies [4,5]. Because of several severe side effects of immunosuppressive drugs, an exosome-based therapeutic strategy could be beneficial for the long-term survival of transplant recipients.

Hence, Exosomes as a new insight into the field of transplantation are gaining the attention of the researchers. In the current paper, we will review and discuss the triple role of exosomes (role as an antigen-presenting vesicle (APV), as a biomarker, and as a therapeutic strategy) in the transplant recipients or transplantation models to clarify their role in the field of transplantation.

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1.2. EVs nomenclature and the definition of Exosomes

Extracellular vesicles (EVs), which was described > 40 years ago [6,7], are heterogeneous membrane-enclosed vesicles. There are various nomenclatures of EVs such as tolerosomes, calcifying matrix vesicles, prostasomes, oncosomes etc. These different names derive from their origin, size, biogenesis, function, isolation methods, and field of research. For instance, calcifying matrix vesicles and tolerosomes that involve in bone formation and immune tolerance, respectively, or prostasomes and oncosomes that are released from prostate epithelium and tumor cells, respectively [8–10].

However, on the basis of origin and size, EVs divided into two generic categories: exosomes (30–120 nm) and microvesicles (100–1000 nm). They are released from the late endosome and plasma membrane, respectively and have broader utility [9,11–14]. In this paper, we will discuss the exosomes as a main type of EVs.

Exosomes, which are formed within the multivesicular bodies (MVBs) in the secreting cells, are homogeneous small membrane vesicles consisting of a lipid bilayer, transmembrane protein, and core contents that include specific and non-specific proteins, mRNAs, microRNAs (miRNAs), and long non-coding RNAs (lncRNAs) [13,15,16] (Fig. 1).

Exosomes are secreted by most of the cells, immune cells and non-immune cells, such as T cells, B cells, DCs, macrophages, fibroblasts, endothelial cells, tumor cells [17,18]. Also, exosomes are found in a wide range of biological fluids, including blood, urine, bronchoalveolar lavage fluid (BALF), ascites, saliva, breast milk, amniotic fluid, seminal fluid, synovial fluid, and cerebrospinal fluid.

The release of the exosomes in the body fluids is mediated via MVBs which could be regulated by Rab small GTPase family members such as Rab11, Rab27, Rab35, (known as major regulating intracellular trafficking) ceramide, and calcium (Fig. 1). Generally, the release of exosomes increases in response to cell activation [19] and different stress situations like oxidative stress, hypoxia, heat shock and acidic PH [13,15].

1.3. The biological function of Exosomes

Exosomes participate in both physiological, (e.g., embryo implantation, the regulatory function of semen, pregnancy, immune response modulation) and pathological processes such as the development of cancer, neurodegenerative diseases [19]. They also participate in the rejection/tolerance of kidney transplantation in recipients [3,20] as well as pathogenesis of autoimmune diseases, including rheumatoid arthritis, Sjogren's syndrome, and systemic lupus erythematosus.

Exosomes can functionally transfer their cargos such as mRNAs, miRNAs to the recipient cells [21]. Thus, regarding the transfer of the cargos and the influence of them on other cells, exosomes could be considered as a vehicle for cell-cell communication like the other types of cell-cell communication (including secretion of soluble factors [like cytokines and chemokines], and cell contact-dependent communication [such as trogocytosis, membrane nanotubes or nibbling]) [15,22].

Exosomes use a different mechanism to conduct their pathological roles. For instance, in the context of transplantation, exosomes are involved in the activation or suppression of immune response by presenting the allo-MHC-peptide to the allospecific T cells.

In the context of cancer, for example, tumor-derived exosomes are capable of transferring Fas ligand (FasL) to T cells, thereby leading to apoptosis and tumor progression [23]. Moreover, different cargos are transferred between distinct cell populations and have a diverse effect [19].

2. The influence of Exosomes on the immune response

Like immune cells, exosomes can either stimulate or suppress the immune response [21,24]. Why do exosomes have a double role in the

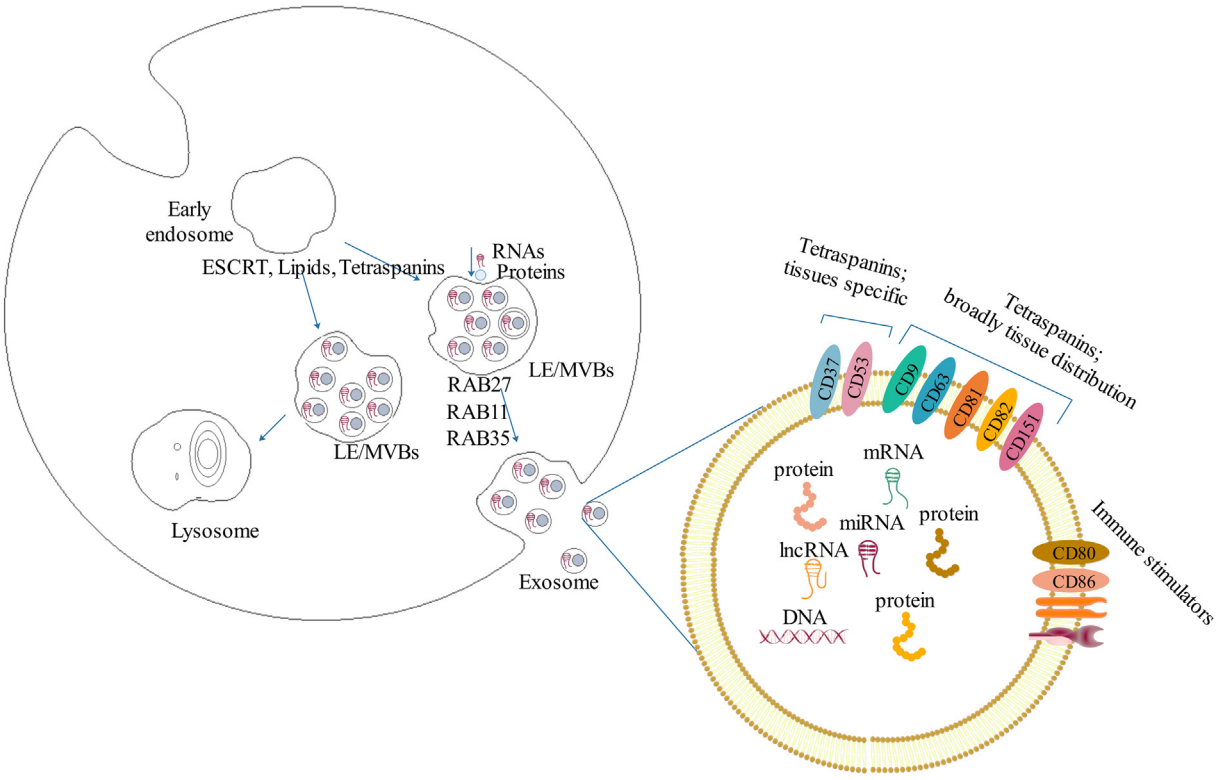

Fig. 1. Biogenesis of Exosome. An early endosome develops into the late endosome. Intraluminal vesicles are formed by inward budding of the endosomal membrane through different molecular mechanisms and consequently result in MVBs. MVBs either fuse with lysosomes or fuse with the plasma membrane (PM). If they fuse with PM, they will release their content to the extracellular milieu.

immune response? Exosomes are miniature of the cells from which are secreted [17,25], and regarding their content could be considered as a liquid biopsy [13]. Therefore, mature DCs, which could stimulate the immune response, secrete those types of exosomes that could activate T cells, thereby leading to immune response stimulation.

On the other hand, imDCs-derived exosomes, which display a low level of MHC class II/I along with adhesion and costimulatory molecules show modulatory function and suppress the immune response. Moreover, immature DCs-derived exosomes (imDex) express FasL, leading to the Fas-expressing T cells apoptosis [21]. Segura et al. (2005) highlighted the role of immature and mature DCs-derived exosomes in the transplantation model. They compared exosomes derived from lipopolysaccharide (LPS)-treated (mature) DCs with untreated (immature) ones. In vitro, they showed that exosomes produced by LPS-treated mature DCs can very strongly stimulate T cells compared with imDex. In vivo, they showed that only exosomes secreted by mature DCs were capable of triggering the T cells response [26].

Some more studies also highlighted the major role of exosomes in the alternation of the immune response. Using heart transplantation model, Liu et al. (2016) showed that although a small number of donor DCs reached lymphoid tissues, DC-derived exosomes migrated to lymphoid tissues and strongly triggered activation of T cells [27]. Moreover, Li et al. (2012) demonstrated that exosomes derived from immature DCs in combination with immunosuppressive drugs can negatively regulate the immune response in their cardiac transplantation model [28].

Moreover, the role of the EV-associated antibody was reported in graft rejection. Dieudé et al. (2015) indicated that apoptotic renal endothelial cells released apoptotic exosome-like vesicles, which were significantly different from apoptotic bodies based on their size and content. The apoptotic exosome-like vesicles produced autoantibodies and culminated in graft rejection [29].

Altogether, these data suggest a vital role of exosomes in stimulation/suppression of immune response, while the immune cells are in a small number to conduct their major role. Such ability of exosomes in deviating immune responses could be used as a therapeutic strategy.

3. Exosomes directly/indirectly activate T cells

APCs like DCs have an inevitable role in immune response stimulation. DCs play an essential role in the allograft rejection by triggering direct and indirect pathway [1]. As DCs-derived exosomes display MHC class II and costimulatory molecules, they can act as APVs through two pathways. These are a direct and semi-direct pathway.

Peche et al. (2006) as well as Segura et al. (2005) demonstrated the direct role of exosomes in the induction or the suppression of the immune response in the context of transplantation. In other words, they indicated that exosomes apart from APCs can present their MHC-peptide to T cells and activate them [26,30].

However, two studies indicated that when exosomes were captured by DCs resulted in efficient T cells stimulation, and there was thus a need for APCs to present allo-MHC-peptide derived from exosomes to T cells (semi-direct pathway) [31,32]. In the direct pathway, DCs-derived exosomes could directly present their MHC-peptide to T cells. In the semi-direct pathway, DCs-derived exosomes could be captured by recipients DCs, thereby their entire MHC-peptide could be presented to T cells through DCs, which known as cross-dressing phenomenon [33] (Fig. 2).

Herrera and co-workers in 2004 described semi-direct recognition in the transplantation model, in which using flow cytometry indicated that DCs acquired functional allo-MHC-peptide from epithelial cells as well as other DCs. These cells then presented the allo-MHC-peptide to T cells [34]. Marino et al. (2016) also confirmed the previous study in their transplantation model [35].

It is well established that direct recognition, which is mediated by DCs, is involved in the T cells stimulation and usually contribute to acute rejection [1]. However, two recent studies indicated the role of the semi-direct recognition (the potential role of exosomes) rather than the direct recognition in stimulation of the immune response against allograft.

Liu et al. (2016) in their transplantation model demonstrated that those DCs, which commonly migrate from graft to regional lymph nodes, were in a limited number to efficiently initiate an immune response but rejection happened. Thus, what was the reason? They indicated that efficient donor DCs-derived exosomes migrated to the LNs and mediated the acute rejection [27].

Moreover, using skin, heart or islet transplantation, Marino and coworker confirmed the previous study, and showed that LNs and spleen of skin-graft mice were empty from donor cells but had a number of recipient cross-dressed cell. In the heart or islet model of transplantation, they also indicated the efficient role of cross-dressed cells in triggering the T cells stimulation [35]. Here, the essential role of exosomes (semi-direct pathway) in rejection of graft in the transplantation model was emphasized. Maybe such a role of cross-dressed cells exists in human transplantation.

Altogether, these data indicate an inevitable role of exosomes as a major component that can stimulate the immune response against the allograft or negatively regulate the immune response. Besides the immune cells that play an essential role in the alloimmune response, which we reviewed in our previous paper [1], exosomes can function as immune cells (APCs) and similar to immune cells can trigger, induce or suppress the immune response.

Maybe, exosome-based therapeutic approaches help to the prevention of alloimmune response against the allograft and improve long-term survival of transplanted organs and reduce the dosage of immunosuppression drugs and consequences of them, such as infection, malignancy or cardiovascular disease after transplantation.

4. Exosome and its distinct capacities in the field of transplantation

Exosomes' content and their ability for presenting antigen (directly/indirectly) result in stimulation/suppression of immune response, and because of that exosomes are investigated by researchers from different aspects. Moreover, depending on the type of cells from which exosomes are secreted, they could be used as a therapeutic strategy. These aspects are the ability of exosomes as APVs, biomarker, and therapeutic strategy.

The first aspect is the antigen presentation ability of MHC-expressing exosomes, in which exosomes can act as APVs by two ways. These two ways were discussed (see section 3). Altogether, according to our mentioned statement, MHC-expressing exosomes play a pivotal role in the stimulation/suppression of immune response, and thereby participating in rejection/tolerance of allograft.

The second aspect refers to both exosomes' content and surface markers. There is no reliable biomarker to detect or monitor acute allograft rejection or allograft status in the early post- transplantation period [36]. Since exosomes contain specific and nonspecific proteins and nucleic acids (see section 1.2), they can provide important information about the tissues from which they are released [1,37,38]. In addition, the number of exosomes could display the metabolism of cells from which they are secreted. Exosomes also have more advantages. For example, they can protect their cargos (proteins and nucleic acids) from degradation. In general, exosomes as a biomarker could participate in early detection and monitoring of diseases (e.g. liver disease) and also can evaluate treatment response [39,40].

Moreover, they can be used as a predictive or diagnostic biomarker and also may be considered as a liquid biopsy [13,36]. It was established that graft can release exosomes into the bloodstream of recipients [38]. Rejected grafts are pathologically different from stable grafts and each of them has their own specific exosomes, which may give us key information about graft status [36]. Several studies investigated this

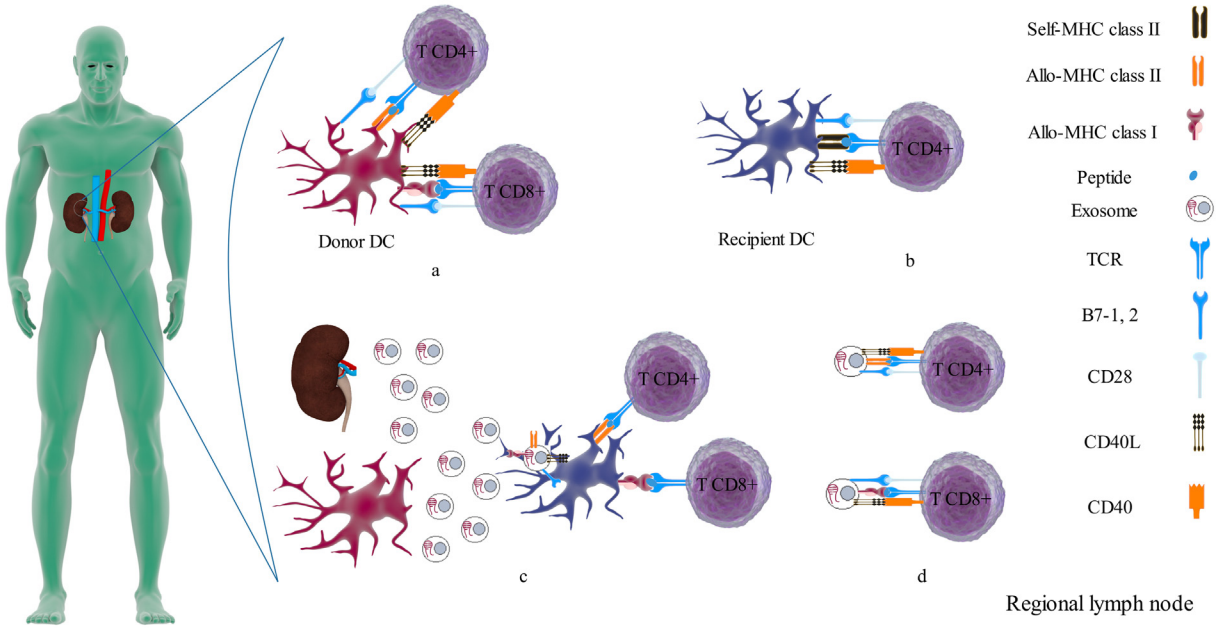

Fig. 2. Various Types of Allorecognition by T Cells. Allorecognition occurred in the regional lymph nodes. a. Direct recognition: Donor DCs migrate to the regional LNs and present their entire allo-MHC-peptide to the allospecific T cells. b. Indirect recognition: Recipient DCs migrate to the allograft and capture alloantigens (donor MHC molecule) and then enter the bloodstream to reach the LNs in order to present the alloantigen to the T cells in the context of self-MHC. c. Exosomes, which are released from allograft or donor DCs, are captured by recipient DCs and their allo-MHC is expressed on the surface of recipient DCs (known as cross-dressed cells). Cross-dressed cells present allo-MHC-peptide to the T cells. d. Apart from APCs, exosomes, which are secreted by allograft or donor DCs, can directly present their allo-MHC-peptide to the T cells.

aspect of exosomes' ability.

Using heart transplantation model, it was reported that heart exosomes profiling can detect and monitor early graft dysfunction [41]. This report was confirmed in other study and then validated in clinical settings of islet and renal transplantation [38]. Moreover, several human-based studies with different transplanted organs were done, which are discussed below.

4.1. Exosomes' content as a biomarker in human heart transplantation

The exosomal proteome was assessed in the serum of human heart transplant recipients. Patients who had either acute cellular rejection or antibody-mediated rejection (ABMR) were compared with patients without rejection. The exosomal protein content of rejected patients was significantly different from patients without rejection. Fifteen proteins were significantly different in the rejection and non-rejection subjects and of these, eight proteins, including complement activator and immunoglobulin components, were related to immune response [36].

Consistent with the previous study, Dewi et al. (2017) emphasized the role of exosomal content in detecting and monitoring of acute cellular rejection. They showed that exosomal miRNAs, including miR-142-3p, miR-92a-3p, and miR-339-3p, were significantly higher in the rejection patients compared with non-rejection controls (Table 1). Their further investigation was based on in vitro assay, in which they indicated that activated T cells released miR-142-3p-containing exosomes.

These exosomes functionally transferred miR-142-3p into the endothelial cells when they were uptaken by these cells and resulted in down-regulation of *RAB11FIP2* gene expression, which is involved in vascular permeability. Finally, in the setting of cardiac allograft rejection, they interestingly demonstrated that exosomes extracted from a serum sample of patients with acute rejection resulted in down-regulation of the *RAB11FIP2* expression in human Umbilical Vein Endothelial Cells (HUVECs) compared with exosomes from non-rejection controls, and thereby contributed to acute rejection [42].

These findings suggest that both protein and nucleotide content of exosomes could be considered as a non-invasive biomarker to discriminate patients with rejection and those without rejection.

4.2. Exosome's content as a biomarker in human lung transplantation

What about the role of exosomes in the lung transplantation? Several studies reported the role of the second aspect of exosomes' function in the lung transplantation. Gunasekaran et al. (2018) demonstrated the role of exosomes as a biomarker, which can discriminate the rejection patients from non-rejection ones. They showed that exosomes from patients with bronchiolitis obliterans syndrome (BOS [known as chronic rejection]) had significantly higher lung-associated self-antigens (SAg) (including K-a-1-tubulin [Ka1T] and collagen V [Col-V]) than a group without rejection.

It was found that pre-transplant or de novo antibodies to SAg contribute to poor allograft survival. They further evaluated whether SAg-containing exosomes could induce the antibody production. Mice immunized with exosomes from BOS patients, but not from without rejection patients, showed antibody to SAg. It suggests that only exosomes from BOS patients express SAgs and can induce humoral immune responses and lead to allograft dysfunction.

As it was mentioned above (see section 3), exosomes can act as APVs and affect immune responses. However, Gunasekaran interestingly reported that exosomes from non-rejection patients did not express MHC class II and costimulatory molecules (CD80, CD86, CD40), but adhesion molecules were expressed on both patients with and without rejection [43].

This study results in (i) exosomes are different in patients with and without rejection, thereby can be used as a predictive/diagnostic biomarker for allograft rejection. (ii) The condition of patients with stable graft function does not allow exosomes to express components of antigen presentation, thereby suppressing the first aspect of exosomes' function.

In another study done by Gunasekaran et al. (2017) it was shown that both sampling from serum and BALF had similar results (Table 1).

Table 1
The role of exosome as a predictive/diagnostic biomarker in human transplantation.

Transplanted organs	Study subjects	Exosomal markers	Biomarker (predictive/ diagnostic)	Sample type	Isolation method	References
Heart	ACR/AMR VS non-rejection	Internal proteins	Diagnostic	Serum	Kit (not exactly specified)	[36] (2018)
Heart	ACR VS non-rejection	Internal miRNAs	Diagnostic	Serum	Ultracentrifugation	[42] (2017)
Lung	BOS VS non-rejection	Surface marker	Diagnostic	Serum	Ultracentrifugation	[43] (2018)
Lung	AR/BOS VS non-rejection	Internal miRNAs/surface marker	Predictive	Serum and BALF	Serum: Kit Follow by centrifugation and exosome precipitation reagent BALF: Ultracentrifugation	[44] (2017)
Lung	ACR VS non-rejection	Internal mRNAs	Diagnostic	BALF	Ultracentrifugation	[45] (2015)
Kidney	ACR VS non-rejection	Internal proteins	Diagnostic	Urine	Ultracentrifugation	[3] (2018)
Kidney	AMR vs CMR and AMR/CMR VS non-rejection	Internal mRNAs	Predictive	Plasma	–	[20] (2017)
Kidney	ACR VS non-rejection	Surface marker	Diagnostic	Urine	Ultracentrifugation	[46] (2017)

Abbreviation. ACR: Acute cellular rejection; AMR: Antibody-mediated rejection; VS: Versus; BOS: Bronchiolitis obliterans syndrome; AR: Acute rejection; BALF: Bronchoalveolar lavage fluid; CMR: cell-mediated rejection.

Serum and BALF exosomes of patients with acute rejection or BOS presented the expression of Col-V and Kα1T whereas exosomes isolated from non-rejection patients did not. They also demonstrated the predictive role of exosomes, in which Col-V-expressing exosomes were isolated 3 and 6 months before acute rejection and BOS diagnosis, respectively.

It suggests that the graft, which will be rejected, releases exosomes expressing Col-V and then early post-transplant monitoring of exosomes may help us to prevent the allograft dysfunction after transplantation [44].In addition, it was also reported that patients with acute rejection had distinctly different exosomal mRNA or shuttle RNA population signatures compared with patients without rejection [45].

Altogether, there is an indispensable role between exosomes and allograft status. Exosomes possess both predictive and diagnostic role for graft rejection in the patients undergoing lung transplantation. Exosome-based graft monitoring early after transplantation may provide accurate results about graft function and help clinicians to make early decisions about patients and control the presumptive graft dysfunction.

4.3. Exosomes' content as a biomarker in the human kidney transplantation

Exosomes as predictive/diagnostic biomarkers were also assessed in the kidney transplant recipients. In a cross-sectional study, Lim et al. (2018) examined the exosomal proteome and highlighted the role of some of these proteins as a diagnostic biomarker for the acute T cell-mediated rejection (TCMR). They demonstrated that tetraspanin-1 (TSPAN1) and hemopexin (HPX) two of the exosomal proteins were significantly higher in patients with acute TCMR than patients without rejection.

In the interaction networks of TSPAN1 and HPX with other proteins, there was a relationship between the TSPAN or HPX and T cell immune response [3]. These two proteins showed a potential diagnostic ability for acute TCMR. However, more studies with similar demographic and clinical characteristics are required to confirm previous results.

In addition, exosomal mRNAs transcripts (*gp130*, *CCL4*, *TNFα*, *CAV1*, *DARC*, and *SH2D1B*) distinguished ABMRs patients from cellular rejection recipients. Since the blood samples were collected before the diagnosis of biopsy-proven rejection, the exosomal mRNAs transcripts displayed a predictive biomarker for allograft rejection [20] (Table 1). In general, the exosomes present a piece of key information about graft function in the transplanted subjects. Exosome-based monitoring in the early post-kidney transplantation period may help the clinicians to control patients' status better and improve long-term allograft function.

Infiltration of T cells in the kidney graft is associated with cellular rejection. T cells can infiltrate the kidney tubules and in close proximity to forming urine. Therefore, with entering the specific T cell-derived exosomes to the urine, they could be detectable in it and be considered as biomarkers of acute cellular rejection (ACR).

Park et al. (2017) indicated the role of specific T cell-exosome as a diagnostic biomarker of ACR. They showed that $CD3^+$ exosomes were remarkably higher in the patients with ACR than patients without rejection [46], suggesting that urinary specific T cell-derived exosomes could reflect infiltration of T cells in the graft, thereby they could be used instead of the invasive biopsy procedure to prove ACR. However, more studies are needed to confirm the result of this study.

Because of exosome characteristics, several exosome-based studies were done in renal transplant recipients or among patients with renal failure with a different purpose [47–50]. Altogether, these data suggest that exosomes can perfectly reflect the graft status, from which are secreted, and there are remarkably different exosomes between patients with rejection and those without rejection. However, it needs more similar exosome-based studies with large sample size in order to prove the role of exosomes as a predictive or diagnostic biomarker in transplant recipients.

5. EV biomarkers discovery

Although the role of exosomes as a biomarker was highlighted in the human heart, lung and kidney transplantation, this aspect of exosomes'

function needs more investigation. Even though there are around 150,000 biomarkers in the literature, only around 100 biomarkers are routinely used in the clinic [40].

There are several pre-analytical and analytical variables, which affect the final results. Some of these variables that alter the results in the field of EVs are time, speed, and the number of centrifugation as well as sample storage and isolation methods. For example, freezing at −20 °C have a major effect on the exosome-associated proteins compared with freezing at −80 °C. Additionally, the anticoagulant should be selected according to the downstream analysis. For instance, for RNA analysis, it is better to choose the EDTA and don't use the heparin [40,51,52].

Since platelet can become easily activated and thereby release EVs, which interfere with the isolation of EVs of interest, it is important to control platelet activation during blood collection and handling. For this purpose, the use of closed blood collection systems and needle gauge 21 are recommended [53]. Moreover, since clot formation results in the additional release of EVs, there is a need for another strategy to minimize the additional EVs, which is the use of plasma instead of serum [52].

Different isolation methods (e.g. differential centrifugation, density gradients or size-exclusion-based methods) have a diverse effect on the EVs type and purity. Since there is no acceptable standardized method for the isolation of EVs, the proper method should be chosen on the basis of research goal [39,54]. For example, the method that is used to isolate EVs from healthy individual urine cannot be used to isolate EVs from patients with nephrotic syndrome effectively. In general, the urine sample (proteinuric vs. non-proteinuric) and downstream analyses are important to choose the proper isolation method [55].

Inattention to the pre-analytical or analytical variables will be accompanied by inconsistent results, and, as a consequence, the validation studies may not be useful in the field of EVs biomarker discovery. Since validation studies are necessary for the biomarker discovery, similar independent studies must be performed. However, there is a lack of such studies and this lead failure in biomarker discovery [40].

In the end, since the results of EVs are greatly influenced by these variables, we suggest that pre-analytical variables including sample collection, handling, storage, etc., should be mentioned well especially in the EV-based studies, in order to better analysis of the results.

6. The therapeutic ability of Exosomes

In this section, the third aspect of exosome ability is reviewed in the context of transplantation model. Transplant recipients need to receive immunosuppressive drugs to accept and maintain their allograft. However, these drugs have several severe side effects such as infections, cancer, and cardiovascular disorder [1].

On the other hand, it was reported that post-transplant infections have a negative impact on patients' survival and cause to morbidity and mortality in them [56]. The goal of transplant medicine is to achieve operational/immunological tolerance, in which patients maintain their allograft for at least one year after stopping the use of immunosuppressive drugs [1]; however, this maybe takes longer than usual.

Thus, instead of immunosuppressive drugs or high dose of them another strategy such as exosome-based therapy could be considered. Several transplantation model studies indicated the role of exosomes as a therapeutic strategy and had impressive results.

Peche et al. in 2003, for the first time, evaluated the therapeutic ability of exosomes in prolongation of allograft survival on their heart transplantation model. Donor-type imDex with different doses (1, 10, 25, 100 μg) was injected into the recipient rats 14 and 7 days before transplantation. The doses of 10 and 25 μg imDex significantly prolonged the allograft survival compared with 1, 100 μg doses, and no treatment group (Table 2).

Exosome therapy prolonged allograft survival time but did not induce tolerance [4]. Therefore, in the further study, in 2006, Peche et al. injected imDex to the recipient rats after transplantation and combined with short-term LF 15–0195 (LF) as immunosuppression. Exosomes alone similar to the previous study only significantly prolonged allograft survival time and did not induce the tolerance.

However, imDex combined with LF showed long-term cardiac allograft survival in all treated recipients [30]. From these two studies, it can be concluded that the injection of exosomes alone whether before or after transplantation only result in short-term allograft survival but not long-term. However, exosomes in combination with a short course of immunosuppression lead to long-term allograft survival. Several years after, in 2011 and 2012, two studies confirm the results of Peche's studies [28,57] (Table 2).

Additionally, in an excellent study, Ma et al. (2016) evaluated both abilities of exosomes with and without the combination of donor antigen-specific regulatory T cells (Tregs) in order to assess the survival time after transplantation. They reported that imDex-treated rats with doses of 10, 40 or 80 μg had significantly higher mean survival time (MST) than untreated rats; and imDex-treated rats with a dose of a 20 μg had high MST compared with imDex-treated rats with doses of 10, 40 or 80 μg. This was consistent with the previous studies. These results emphasized the immunoregulatory role of imDex and their potential therapeutic strategy when injected into the rats. Moreover, when imDex in combination with donor antigen-specific Tregs injected into the rats, the results interestingly showed the most MST (> 100 days) [5].

Exosomes from Tregs also had the same results. Yu et al. in 2011 indicated that autologous transfer of Tregs-derived exosomes into rat models prolonged the kidney allograft survival and postponed the allograft rejection (Table 2). Moreover, in vitro assessment showed that the exosomes suppressed T cells proliferation [58].

Exosome therapy alone remarkably prolonged allograft survival compared with no treatment, but this effect was to some extent beneficial and did not induce tolerance. To improve allograft survival, imDex in combination with a short course of immunosuppression or donor antigen-specific Tregs was used and interestingly showed long-time survival of allograft.

Altogether, these data represent an indispensable role for exosomes to be used as a therapeutic strategy. This aspect of exosome ability is acceptable in intestinal, heart, liver or kidney models of transplantations. Exosome in combination with low doses of immunosuppression or donor antigen-specific Tregs had the greatest outcome among others. It could open up a new perspective to use another therapeutic strategy (exosome) rather than immunosuppression to dispose of their sever sides effect after transplantation and improve the allograft survival.

Another type of immunosuppression or immune cells with different doses combined with imDex may result in better effect. Such a strategy may lead to great results in human transplantation, reduce the dosage of immunosuppressive drugs and improve long-term allograft survival.

7. Conclusion and perspective

In this paper, we focused on three current gaps in the field of transplantation and consequently the triple role of exosomes related to these gaps. Immune response immediately damages allograft after transplantation, which is the predominant cause of graft failure. Since exosomes are similar to their parental cells, they can act as APVs; thereby stimulate or suppress the immune response. Moreover, their indispensable role in semi-direct recognition was also established. Thus, exosomes along with immune cells and sometimes more efficient than them participate in alloimmune response and affect the transplantation outcome. If the graft has immature DCs more than mature ones, it most likely results in tolerance rather than immune response activation.

On the other hand, exosomes can reflect the graft/tissues, from which are secreted, and remarkably discriminate patients with rejection and those without. Thus, exosomes may participate in the early diagnosis of the acute rejection and may use instead of the invasive and

Table 2
Exosome-based therapy and increase of allograft survival in various transplantation models.

Transplantation model	Study subjects	Exosome source	Exosome dosage	Time of injection	Allograft survival time	Type of injection	References
Intestinal-Rat	1- imDex-treatment	Donor im-DCs from bone marrow	1 μg		7.3 ± 1.0 d	penile vein (IV)	[57]*
			10 μg		9.8 ± 1.2 d		
			20 μg		14.5 ± 1.0 d		
			50 μg	7 d pre-transplantation	7.8 ± 1.2 d		
	2- Recipient imDex-treatment		20 μg		7.5 ± 1.0 d		
	3- no-treatment		–		7.0 ± 0.6 d		
Kidney-Rat	1- Treg-derived exosomes	Tregs and B cells from Mesentric lymph nodes		1 d post-transplantation	21.0 ± 2.17 d	Tail vein (IV)	[58]*
	2- Treg-derived exosomes			3 d post-transplantation	18.8 ± 2.54 d		
	3- B cell-derived exosomes		0.5 ml	5 d post-transplantation	14.1 ± 2.47 d		
	4- PBS-control			–	6.8 ± 0.94 d		
Liver-Rat	1- imDex-treatment	Donor im-DCs from bone marrow	1 μg		10 d	Caudal vein (IV)	[5]**
			10 μg		17 d		
			20 μg		37 d		
			40 μg	7 d befor, the d of, and 7 d after transplantation	25 d		
			80 μg		26 d		
	2- 20 μg-imDex cmbined with donor-specific Treg-treatment		–		> 100 d		
	3- no-treatment (physiological saline)		–		10 d		
Heart-Rat	1- imDex-treatment	Mainly donor im-DCs from bone marrow	1 μg		7 d	IsV	[4]**
			10 μg		25 d		
			25 μg	14 and 7 d befor transplantation	15.5 d		
			100 μg		9.5 d		
	2- Recipient imDex-treatment		10 μg		7 d		
	3- no-treatment		–		6 d		
Heart-Mouse	1- imDex-treatment	Donor im-DCs from bone marrow	0.1 μg		8 d	caudal vein (IV)	[28]**
			1 μg		12.5 d		
			10 μg		27 d		
			25 μg		14 d		
			50 μg	7 d befor, the d of, and 7 d after transplantation	12 d		
	2–10 μg-combined with Rapamycin		–		92 d		
	3- Recipient imDex-treatment		10 μg		8 d		
	4- no-treatment (physiological saline)		–		7.5 d		
Heart-Rat	imDex-treatment	Mainly donor im-DCs from bone marrow	10 μg		9 d	IV	[30]**
			25 μg		25.5 d		
			50 μg	The d of and 6 d after transplantation	8 d		
			100 μg		7 d		
	25 μg-imDex combined with LF as immunosuppressive-treatment		–		> 100 d		
	Recipient imDex-treatment		25 μg		8 d		
	no-treatment		–		6 d		

Abbreviation. imDex: Immature dendritic cell exosome; imDCs: Immature dendritic cells; d: Days; IV: Intravenously; PBS; Phosphate-buffered saline; LF: LF 15-0195.
* Results were shown as mean ± SD.
** Results were shown as median.

expensive biopsy procedure. Moreover, since there is no reliable biomarker to monitor patients' early post-transplantation status, exosomes could also be considered as a novel reliable predictive biomarker.

The therapeutic strategy could be the third aspect of exosomes' ability. Today, which lifelong use of immunosuppressive drugs has severe side effects, we need to use other therapeutic strategies to induce tolerance. The transplantation models presented a new outlook to the ability of exosomes as a therapeutic strategy and improvement of long-term allograft survival. Injection of exosomes into the rats can increase the survival of allograft. Moreover, exosome in combination with immunosuppression lead to long-term allograft survival. However, to the best of our knowledge exosome-based therapy in human transplantation has not been reported. Such a strategy in human transplantation may be accompanied by low doses of immunosuppressive drugs and the increased rate of recipients' survival.

It is worthwhile to note that maybe exosomes in different immunological characteristic [1] have a diverse effect. Therefore, if the immunological characteristic of recipients along with exosome therapy be assessed, it probably will help us to understand better about the pure role of exosomes in prolongation of allograft survival.

Conflict of interests

The authors declare no conflict of interest.

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