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Role of microRNAs in immunity and organ transplantation

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Organ transplantation has evolved rapidly and there is now widespread use of donated organs for the treatment of end-stage organ failure. Although the therapeutic options achieving long-term graft survival have improved, acute and chronic rejections are still a major problem. Studies to identify noninvasive biomarkers for rejection and underlying molecular events have increased significantly in the past decade, but a major breakthrough is still missing. The recent discovery of small regulatory RNA molecules (microRNAs) resulted in a new and improved understanding of the mechanisms of gene regulation and also led to the development of the first new microRNA (miRNA)-based therapies. miRNAs are endogenous, single-stranded RNAs consisting of about 19–25 noncoding nucleotides, which have an important role in regulating gene expression. Additionally, circulating miRNAs that might be useful as novel disease biomarkers were detected. Here, we summarise current knowledge about the role of miRNAs in immunology and transplantation medicine and their role as potential biomarkers. We also focus on the molecular mechanisms and therapeutic implications of the use of miRNA-based therapeutic strategies to improve long-term allograft survival.

Within the past few decades, transplantation has become standard therapy for treating failure of selected organs. Initial problems and complications included acute rejection of the transplanted organ, which was reduced as a result of the development of powerful immunosuppressant therapies. Although the therapeutic options achieving long-term graft survival have improved, acute and chronic rejections are still allograft- and life-threatening situations. The incidence of acute rejection has been reduced; however, the rates of graft survival beyond 5 years remain basically stable (Ref. 1). The task of avoiding rejection by using immunosuppressant drugs and preventing serious infections caused by immunosuppressant use in organ recipients is a difficult one. Thus, improvement of transplant organ function and

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reduction of side effects are the main aims of many experimental and clinical trials.

Although knowledge on the underlying immunomodulatory mechanisms leading to organ dysfunction, inflammation and fibrosis has increased, the treatment of acute or chronic organ rejection is far from optimal. Today, the gold standard to detect rejection of a transplanted solid organ includes organ biopsies and specific marker staining. Histological findings influence both the prognosis of the patient and the resulting therapy (Ref. 2). Rejection is an immune response of the host, and if not controlled, leads to destruction of the allograft. It is therefore of great interest to detect early signs of rejection or allograft loss. Hyperacute rejections occur within minutes to hours, but are rarely seen because of human leukocyte antigen (HLA) matching, improvements in crossmatching that detect preformed antidonor antibodies, and immunosuppressant therapy. Acute rejections occur after days to weeks and are either antibody- or T-cell-related, or include vascular-mediated rejections (Ref. 3). T cells are involved in most forms of rejection. Donor alloantigens are processed by antigen-presenting cells (APCs), which travel to lymphoid organs where the antigens are presented to T cells. The T cells differentiate into different subgroups followed by invasion and destruction of the allograft. In contrast to hyperacute or acute rejections, chronic rejection occurs months to years after transplantation, often because of insufficient immunosuppression, and leads to organ remodelling, including exaggerated fibrosis and dysfunction.

A similar process is observed in allogenic haematopoietic cell transplantation (Ref. 4). Graft-versus-host disease (GVHD) is a major complication after haematopoietic cell transplantation, where donor T cells respond to proteins on host cells such as HLAs (Refs 5, 6) or other proteins such as minor histocompatibility antigens (Ref. 7). GVHD is divided into several phases; conditioning regimens prior to transplantation upregulate a variety of inflammatory mediators, such as interleukin (IL)-1 and tumour necrosis factor (TNF)-α, as well as adhesion molecules, leading to incipient tissue damage. As part of the innate immune response, neutrophils and macrophages migrate to the damaged tissue and cause further injury. Dendritic cells capture antigens from damaged tissue and migrate to lymphoid organs, where they mature. Mature dendritic cells, which are abundant in co-stimulatory molecules, process large fragments of these antigens into smaller peptides. These peptides bind to major histocompatibility complex (MHC) molecules and are presented on the surface of dendritic cells as allopeptide–MHC complexes. Donor T-cell recognition and activation ensues, which involves interaction between the allopeptide MHC and antigen-specific T-cell receptors (TCRs) (Ref. 8). Finally, a cellular and inflammatory effector phase occurs (Refs 9, 10, 11). Here, a variety of chemokines such as CXCL2, CXCL9–CXCL11, CCL2–CCL5, CCL17 and CCL27 are upregulated, supporting the migration of T cells to allegedly hostile cell proteins that cause damage to the affected tissues. This phase is also characterised by other cytolytic mechanisms, such as the release of TNF-α, perforin, granzyme B, Fas–FasL and reactive oxygen species (Ref. 12).

Because of the significant problems that occur when a transplanted organ is rejected, post-transplant monitoring is of utmost importance and is currently carried out by recording certain functional parameters such as serum creatinine levels in the case of transplanted kidneys. A sudden change in such parameters might indicate a rejection, but requires more invasive procedures such as organ biopsies for validation. Although organ biopsies are commonly carried out, their invasive nature presents a certain hazard for the patient and recognises rejection only at a relatively advanced stage of the derailed immune process. It is therefore the aim of many research groups to establish more sensitive noninvasive methods to detect early graft impairment. For instance, changes in gene expression patterns during acute rejection have been identified, which may be used for better description of the type of rejection (Ref. 13). Other research groups have shown that the messenger RNAs (mRNAs) of circulating cells such as that of the Fas ligand may be useful in predicting organ rejection (Ref. 14). A number of other potential biomarkers such as IL-17, major histocompatibility complex class I chain-related molecule A or netrin-1 have also been shown to be sensible parameters of allograft status (Refs 15, 16, 17, 18). In cardiac allografts, a distinct chemokine and receptor gene expression

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pattern was found during early and late acute rejection (Ref. 19). Sixty endomyocardial biopsies from 24 patients at 3 months or 9-12 months after transplantation were compared. In these samples, the expression levels of IP-10, Mig, RANTES, CXCR3 and CCR5 were increased after 9–12 months, suggesting an increased intensity of inflammation in rejection at later post-transplantation periods. However, there is still a great need to develop better diagnostic and prognostic markers for organ rejection. The recent discovery of small regulatory RNAs such as microRNAs (miRNA) resulted in an improved understanding of underlying mechanisms of the control of gene expression, the development of new gene-based therapies and potential use in transplantation medicine as biomarkers.

miRNAs and their use as biomarkers and therapeutic targets

miRNAs are endogenous, small RNA molecules consisting of about 19-25 noncoding nucleotides (Ref. 20). miRNAs have an important role in regulating large parts of the genome (Ref. 21). Since the 1990s when Lee and co-workers described the first function of the miRNA lin-4, which targets lin-14 in *Caenorhabditis elegans* (Ref. 22), a variety of functions of miRNAs in essential biological processes such as cell proliferation and apoptosis have been identified (Ref. 23). miRNAs are transcribed in the nucleus by ribonuclease (RNA) polymerase II as a long primary miRNA transcript and are then processed by the enzymes ribonuclease III (Drosha) and DGCR8 into a shorter precursor miRNA (Ref. 24). Further maturing of the miRNA takes place after transportation from the nucleus to the cytoplasm through exportin-5. In the cytoplasm, precursor miRNAs are further processed by the RNase III Dicer to give mature 19-25-nucleotide RNA duplexes. Embedded in an RNA-induced silencing complex, one strand is then guided to the target mRNA to result in mRNA degradation or translational repression of target protein expression.

In this review, we summarise current knowledge on miRNAs in immunity and transplantation medicine, and their role as potential biomarkers. We also focus on the molecular mechanisms and therapeutic implications of the use of miRNA-based therapeutic strategies to improve long-term allograft survival.

miRNAs as biomarkers of organ allograft status

Organ-specific expressed miRNAs

Certain mRNA profiles in urinary and peripheral blood cells have previously been used as diagnostic parameters of acute rejections in renal allografts (Ref. 25). The occurrence of such different mRNA expression profiles supports the idea that noninvasive biomarker profiles might be capable of predicting allograft status and rejection of certain organs. Further evidence also suggests that, similar to mRNAs, miRNAs might also predict allograft status. Organ-specific expression of miRNAs in renal allograft biopsies has been previously studied (Ref. 26). A total of 365 miRNAs were analysed in seven biopsies and a smaller subset group of miRNAs were profiled in 33 biopsies of samples with acute rejection and normal controls. The study revealed that a number of mature miRNAs were able to differentiate between biopsies of acute rejection and biopsies from healthy tissue. Indeed, acute rejection and renal function could be predicted with a high level of precision using miRNA levels in the graft. The expression of miRNAs in normal human peripheral blood mononuclear cells showed a high level of miRNA(miR)-142-5p, miR-155 and miR-223, which were overexpressed in patients with acute rejection (Ref. 26). Within the organ, miR-146a/b was higher in samples with acute rejection when compared with controls. miRNA-146a/b is considered as a T-helper (Th)1-specific miRNA, which is expressed at low levels in naive T cells and upregulated during maturation to Th1 cells, but not in Th2 cells (Ref. 27) (Fig. 1). Interestingly, intragraft levels of mRNA for Th1 cytokine interferon, but not for Th2 cytokine IL-4, were higher in biopsies of organs with acute rejection when compared with control biopsies, supporting the theory of Th1 cells infiltrating the allograft during rejection. Another miRNA overexpressed in samples of acute rejection was miR-223, which is expressed on T and B cells and monocytes (Ref. 28). miR-223 levels were among the highest expressed in acute rejection. A strong positive association between intragraft levels of miRNAs (miR-142-5p, miR-155, miR-223) and mRNAs encoding T-cell CD3 and B-cell CD20 was found, which showed that intragraft levels of these mRNAs were also diagnostic of acute rejection, although with less sensitivity and specificity (Ref. 26). A further

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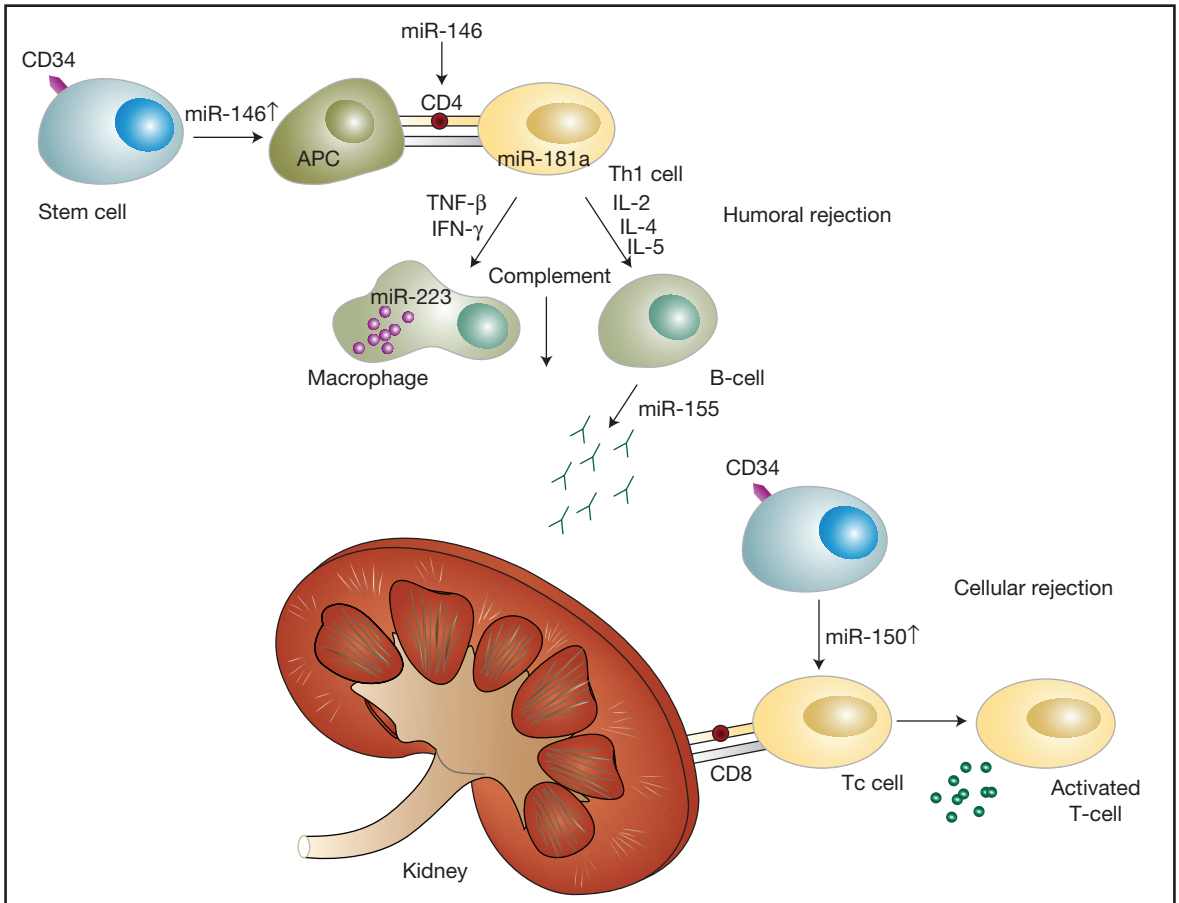

Figure 1. Schematic representation of acute rejection using the example of kidney rejection. APCs present antigens to Th cells. miR-146 is upregulated during maturation of Th1 cells and monocytes. Macrophages, neutrophils and B cells are activated, leading to complement activation, antibody production and release of cytokines. Macrophages and neutrophils express miR-223, negatively regulating the proliferation and differentiation of neutrophils. miR-223 is overexpressed in patients with acute rejection. miR-155 is important for adequate function of B cells, to produce high-affinity antibodies. T cells recognise HLA complexes on donor cells and release perforin, leading to destruction of tissue. miR-150 increases T-cell lymphopoiesis, and increased expression of miR-150 leads to the suppression of B-cell formation. miR-181a amplifies the strength and sensitivity of TCR. APC, antigen-presenting cell; HLA, human leukocyte antigen; IFN, interferon; IL, interleukin; TCR, T-cell receptor; Th, T-helper; TNF, tumour necrosis factor.

strong positive relationship between renal-tubule-specific NKCC-2 mRNA with miRNA-30-3p and miRNA-10b (which are expressed in high abundance in human renal epithelial cells) was found, indicating that the altered expression of miRNAs during acute rejection is caused partly by changing the proportions of resident renal parenchymal cells and immune cells infiltrating the graft during acute rejection. Another group compared 71 miRNAs in samples of the kidney of patients with acute rejection and normal controls by microarray analysis and subsequent quantitative real-time-RT-PCR confirmation. Twenty miRNAs with significantly different expression levels were detected. Twelve miRNAs were upregulated, for example miRNA-324-3p and miRNA-611, whereas eight were downregulated, including miRNA-125a and miRNA-381. The 20 identified miRNAs were analysed for their potential target mRNAs. A potential target of miRNA-381 included mRNA encoding patched homologue 1 Huntington,

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which is found to be involved in nonmelanoma skin cancer, a common complication of organ transplantation, which is associated with the long-term use of immunosuppressants. These findings indicate that miRNAs might have potential as diagnostic biomarkers and factors involved in the pathogenesis of acute kidney rejection (Fig. 1) (Refs 29, 30, 31).

Sixty-two differentially regulated miRNAs were identified in acute rejection samples when compared with normal controls. Among others, miR-15b, miR-28-5p, miR-106b, miR-192, miR-197, miR-200c and miR-211 were downregulated, whereas miR-125a, miR-142, miR-155 and miR-629 were upregulated (Ref. 32). Interestingly, some of these miRNAs also appear to be upregulated in acute rejection after kidney transplantation (Ref. 26). Thus, intragraft miRNAs are potentially involved in the activation of a host alloimmune response to the donor and might serve as targets for intervention and monitoring of the allograft status.

In addition to observations on acute rejection, studies also revealed changes in miRNA expression profiles in another transplant-associated organ ischaemia. After renal ischaemia reperfusion injury, an miRNA signature was described in a mouse model of kidney disease (Ref. 33). Ischaemia reperfusion injury is an important risk during organ transplantation, leading to organ dysfunction, and is associated with a significantly increased morbidity and mortality of patients. Investigators showed that after unilateral warm ischaemia, nine miRNAs (e.g. miR-21 and miR-192) were differently expressed. Thus, selected miRNA expression profiles may be used as a predictor for allograft function in the future.

Circulating miRNAs

miRNAs have been shown to be detectable in serum, plasma, urine and other body fluids, where they remain rather stable (Refs 34, 35). An explanation for this could be the inclusion of miRNAs into lipid or lipoprotein complexes, such as exosomes or microvesicles (Refs 32, 36). miRNAs are even stable in samples after formalin fixation or paraffin embedding and could be extracted and evaluated (Ref. 37). Recent studies showed that miRNAs are actively secreted to microvesicles or exosomes from different cell types (Refs 34, 36, 38). Mast cells and embryonic stem cells have been confirmed to secrete miRNAs through exosomes (Ref. 39). The occurrence of miRNAs in serum and plasma suggests that they might have a biological function outside the cell. Lawrie was the first to describe the presence of circulating miRNAs in patients with cancer (Ref. 40). The expression patterns of these circulating miRNAs correlated with several diseases, including different forms of cancer. In patients with diffuse large-B-cell lymphoma, the levels of miR-155, miR-210 and miR-21 differed from those in healthy controls. Moreover, high levels of miR-21 were negatively associated with relapse-free survival of these patients (Ref. 41). miR-208b and miR-499 are increased in the plasma of patients with acute myocardial infarction and patients with viral myocarditis, indicating myocardial damage (Ref. 42). Interestingly, in this study, inflammation per se did not seem to have an impact on miRNA release because the plasma levels of leukocyte-expressed miRNAs were not significantly increased in patients with acute myocardial infarction or viral myocarditis, despite elevated white blood cell counts. The prognostic impact of six circulating miRNAs (miR-1, miR-133a, miR-133b, miR-208a, miR-208b and miR-499) in patients with acute coronary syndrome was assessed (Ref. 43). Levels of miR-133a and miR-208b were significantly associated with the risk of death in univariate and age- and gender-adjusted analyses. Both miRNAs lost their independent association with outcome on further adjustment for hsTnT.

The existence of stable circulating miRNAs led to the hypothesis that circulating miRNAs might serve as biomarkers for different diseases. In cancer, new findings support this idea. For instance, miR-17-5p and miR-21 are increased in the serum of patients with lung cancer (Refs 44, 45). Laterza and colleagues investigated the use of liver-, muscle- and brain-specific miRNAs as circulating biomarkers of tissue injury. They used a highly sensitive quantitative PCR assay to measure miRNAs such as miR-122, miR-133a and miR-124 in plasma samples of rats that had been treated with liver and muscle toxicants or subjected to experimental stroke (Ref. 46). In line with organ damage, the group observed an increase in plasma concentration of tissue-specific miRNAs. Besides their potential as biomarkers, circulating miRNAs might also serve as paracrine mediators (Ref. 34). In

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plasma, miR-210 predicted survival in patients with acute kidney injury (Ref. 47). Whether circulating miRNAs are also useful in transplantation medicine as new biomarkers is currently under investigation. Recently, the role of urinary miRNAs as easily accessible biomarkers of acute renal allograft rejection has been evalutated (Ref. 48). Lorenzen and co-workers found that miR-210 was decreased in the urine of kidney transplant patients with acute T-cell-mediated rejection. Reduced urinary miR-210 at the time of rejection was associated with impaired kidney function 1 year after transplantation.

In conclusion, circulating miRNAs offer a potential for novel diagnostic and prognostic approaches in various disease processes.

miRNA as mediators of organ rejection, fibrosis and failure

Since their discovery, miRNAs have been widely studied and many functions have been described. miRNAs are capable of repressing target genes by post-transcriptional degradation of mRNA and translational inhibition of protein expression. They have also been shown to participate in the intercellular communication between APCs and effector cells such as T cells, B cells and dendritic cells. Profiling of exosomes revealed several miRNAs, for example miR-760, miR-632, miR-654-5p and miR-671.5p. Moreover, the analysis indicated that specific miRNAs are selectively sorted into these exosomes (Ref. 49).

The participation of miRNAs in the immune system and their possible roles as mediators in allograft function and failure are the subject of many research projects. We will therefore briefly summarise the current known role of miRNAs in various immune cells.

Monocytes

The innate immune system consists of various immune cells such as macrophages and monocytes. These cells are capable of detecting and destroying pathogens, but also present antigens to cells of the adaptive immune system (Fig. 1). miRNAs are upregulated in the monocyte–macrophage lineage in response to bacterial and viral infections (Ref. 50). The recognition of pathogens takes place by pattern-recognition receptors, such as the Toll-like receptors (TLRs). TLRs are highly expressed on macrophages and dendritic cells (Ref. 51). Human monocytes derive from haematopoietic stem cells. After maturation, they circulate in the bloodstream. On immigration into tissues, they are able to differentiate into macrophages or dendritic cells. Studies investigating umbilical cord blood $CD34^+$ progenitor cells showed that expression of miR-17-5p, miR-20a and miR-106a is involved in monocyte differentiation and maturation. Specifically, during monocytic differentiation, miR-17-5p, miR-18a and miR-106a are downregulated (Ref. 52), whereas miR-146a/b, miR-132 and miR-155 are upregulated (Ref. 53). A similar observation was made after stimulation of monocytes with lipopolysaccharide (LPS), a TLR4 ligand. Further studies of miR-146a revealed that it is regulated by NF-κB and might serve as a negative regulator of IRAK1 and TRAF6 expression (Ref. 53).

B cells

The acquired immune system is a specialised system for recognition and elimination of pathogens. By expression and production of specific antibodies, T cells and B cells are able to react quickly to invading pathogens (Fig. 1). This assumes a complex and tightly controlled maturation and differentiation process. Increasing evidence emphasises a role of miRNAs in development, function and regulation of immune response (Refs 54, 55). It has been shown that certain profiles of miRNAs are enriched in different stages of B-cell differentiation. miRNAs influence the development of naive B cells by targeting important transcription factors such as LMO2 and MYBL1 (Ref. 56). In mature B cells, miRNAs have been identified as regulators of the generation of immunoglobulin class-switched plasma cells (Ref. 57). Increased expression of miR-150 leads to the suppression of B-cell formation as a result of the block of pro-B to pre-B cell transition (Table 1). In malignancies such as chronic lymphatic leukaemia, the expression of miR-150 is decreased (Refs 65, 66). Other studies have shown that miR-155 is especially important for the adequate function of B cells, to produce high-affinity antibodies. A recent study showed that miR-155-deficient mice fail to produce a response to virulent *Salmonella typhimurium* after immunisation with a nonvirulent strain of the bacteria (Ref. 67). In malignancies, accumulation of transcripts of miRNA-155 and BIC (B-cell

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Table 1. Role of miRNAs and their target genes in various immune processes

miRNA	Target gene(s)	Function and processes regulated	Ref.
miR-21	*PTEN, PDCD4 IL12A*	Upregulated in macrophage in response to inflammation Induced by TLRs Negatively regulates macrophage activation	58
miR-17-92 (cluster)	*BIM*, *PTEN*	Regulates protransition to pretransition during development of B and T cells Promotes B- and T-cell survival	59
miR-150	*MYB*	Regulates mature B-cell production Regulates the pro- to pre-B transition Regulates T-cell activation	60
miR-155	*PU.1, MAF SHIP1*, *AID SOCS1, BACH1 CEBPB, CSFR TAB2, JARID2*	Regulates immune response to bacterial/viral infections Induced by TLR signalling Regulates TNF-α Required for normal lymphocyte functions, response of the germinal centre, class switching, plasmacyte generation Th2 and Th2 polarisation, Treg cell thymic development, granulocyte and monocyte proliferation	61
miR-181a	*DUSP5 DUSP6 SHP2 PTPN22 BCL2, CD69*	Regulates the development of B and T cells Modulates the sensitivity of T cells to antigens by regulating the expression levels of various phosphatases from TCR signalling	62
miR-223	*MEF2C*	Granulopoiesis regulation	63
miR-146	*IRAK1, TRAF6*	Th1-effector-cell specific Negative regulator of LPS signalling	64

integratin cluster), from which this miRNA is processed, leads to lymphomas such as Hodgkin and diffuse large-B-cell lymphomas (Refs 68, 69).

T cells

miR-150 increases T-cell lymphopoiesis from stem and progenitor cells, whereas it blocks development from the pro-B to pre-B cell stage (Table 1) (Ref. 70). T cells, like B cells, originate from haematopoietic stem cells in the bone marrow before populating the thymus. In 2007, a study showed that regulation of miRNA expression was characteristic for each stage of development in thymocytes (Ref. 71). Based on this discovery, many groups investigated the role of miRNAs in T-cell development. Wu and colleagues analysed expression profiles in antigen-specific naïve, effector and memory $CD8^+$ T cells (Ref. 71). They found that a total of seven miRNAs (among those miR-150, miR-16 and miR-21) accounted for more than 60% of all miRNAs. Recent data support the role of miR-181a as a modulator of T-cell maturation (Ref. 72). It was shown that miR-181a levels differ in the various stages of T-cell development. It has also been shown that this miRNA is able to augment TCR signalling, which is important for the discrimination of foreign and endogenous antigens (Ref. 73). This supports the idea that miR-181a might be promising for miRNA-based therapies for allograft dysfunction, such as GVHD or acute and chronic rejection.

Selected miRNAs and their potential use in transplantation medicine

miR-150

miR-150 is expressed in lymph nodes and its upregulation leads to maturation of functional T cells and B cells (Ref. 66). miR-150 is expressed in mature B and T cells but not in their progenitors. Likewise, the expression is lost after further differentiation of naive T cells into Th1 or

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Th2 cells. In B cells, miR-150 overexpression led to defects in B-cell development at the pro-B to pre-B cell stage, whereas it had no effect on $CD4^+$ or $CD8^+$ T cells (Refs 27, 66). miR-150 also participates in the development of B cells by downregulating cMyb. cMyb is a DNA-binding proto-oncogene expressed in haematopoietic cells, which was initially found in avian acute leukaemia viruses, AMV and E26 (Ref. 74). B cells, which are deficient in miR-150, display high expression of cMyb, whereas cells from transgenic mice overexpressing miR-150 have less cMyb (Ref. 70).

miR-155

miRNA-155 is one of the key players in the regulation of adaptive immunity and antibody-related T-cell response (Ref. 75). miRNA-155 is processed from the BIC primary transcript. After exposure to antigens, the expression of transcript and mature miR-155 is upregulated in B and T cells (Refs 76, 77, 78). miR-155 was among the first miRNAs linked to inflammation because of its upregulation in multiple immune cell lineages by TLR ligands, inflammatory cytokines and specific antigens (Refs 53, 75, 79). The B cells of mice lacking miR-155 were shown to be deficient in production of TNF-α and lymphotoxin-α (LT-α). $CD4^+$ T cells of these mice showed a Th2 profile under nonpolarising conditions (Ref. 75). The differences in miR-155 levels might influence alloimmunity effects on Th cells (Ref. 80). As discussed above, in renal allografts, miR-155 is overexpressed during acute rejection, and thus may trigger effects on T-cell polarisation. miR-155 also has a major role as a regulator of B and T cells in the adaptive immune response. miR-155-knockout mice have been shown to be immunodeficient (Ref. 67) and failed to produce an appropriate immune response towards bacteria after immunisation (Ref. 75). A further role of miRNA-155 as a mediator in inflammatory responses was also reported (Ref. 78). Researchers showed that miR-$155^{-/-}$ mice were resistant to experimental autoimmune encephalomyelitis. They also showed that these mice exhibited defective inflammatory T-cell development during encephalomyelitis. The group examined lymph nodes and splenocytes producing T cells from miR-$155^{-/-}$ mice for the presence of IL-17 (Th17) or interferon during autoimmune encephalomyelitis. Th17, a subgroup of Th cells producing IL-17, has also been shown to be involved in the pathogenesis of autoimmune diseases (Refs 81, 82). miR-155-knockout mice have a reduced number of Th17 cells in both lymph nodes and spleen tissue when compared with controls. Furthermore, miR-$155^{-/-}$ mice fail to produce Th17 cells, supporting the hypothesis that miR-155 has an important role in enhancing inappropriate chronic inflammation directed at tissue-specific antigens (Ref. 78).

miR-181a

In T cells, miR-181a is a modulator of T-cell selection and sensitivity (Ref. 73) and has also been shown to impact B- and T-cell development in haematopoietic progenitor cells (Ref. 83). In addition to expression in progenitor cells, miR-181a is also highly expressed in the thymus (Ref. 83). miR-181a positively regulates the TCR-mediated response to antigens of Th2 cells. TCRs are an important feature of the immune system in distinguishing between self and foreign antigens. T-cell response to antigen is highly influenced by the binding characteristics of its TCR to the complex between the presented peptide and the MHC (Ref. 74). Eliminating miR-181a by using an miR-181a-specific antagomir led to an increase in T cells coming out of negative selection and a decrease in the number out of positive selection (Ref. 84). miR-181a amplifies the strength and sensitivity of TCR-mediated activation. This has been shown to be due to downregulation of a variety of phosphatases known to negatively regulate the TCR signalling pathway (Ref. 73).

miR-223

Another interesting immunomodulatory miRNA is miR-223, which was among the first miRNAs suspected to regulate immune responses. This miRNA is enriched in immune cells and is confined to myeloid cells (Ref. 85). Specifically, miR-223 is expressed in neutrophils and macrophages, but not in monocytes and lymphocytes (Ref. 86). miR-223 negatively regulates the proliferation and differentiation of neutrophils through downregulation of the known promoter of myeloid progenitor proliferation, Mef2c. An miR-223-knockout model showed increased neutrophil numbers, spontaneous inflammation of the lung and tissue destruction after LPS exposure, further

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demonstrating a key role of miR-223 in inflammation (Ref. 87).

Interestingly, both miR-223 and miR-155 are overexpressed in the kidney biopsies of patients with acute rejection, as discussed above (Ref. 26), and this might explain some functional effects during organ rejection. Thus, these miRNAs might serve as interesting novel biomarkers or therapeutic targets in transplantation medicine.

Role of miRNAs in chronic rejection and organ fibrosis

Organ fibrosis is an endpoint of many diseases, including chronic rejection, diabetic nephropathy and hypertensive kidney disease. Increasingly, reports suggest that miRNAs have a role in the regulation of fibrosis processes (Refs 88, 89, 90, 91). Myofibroblasts are key players in fibrosis. Once activated by profibrotic cytokines such as transforming growth factor-beta, fibroblasts start to produce collagen and other extracellular matrix components, which if produced in excess might lead to continuous loss of organ function. Alongside resident fibroblasts, epithelial cells have also been described to mature to fibroblasts, in a process called epithelial–mesenchymal transition (Ref. 92). Recent studies extend this discovery by reporting that fibroblasts might also derive from endothelial cells by endothelial–mesenchymal transition (Refs 93, 94). In tissue remodelling, miR-21 is an interesting, yet controversial, player. miR-21 is induced at high levels after cardiac or pulmonary stress (Refs 89, 95). Treatment with an miR-21 antagonist leads to reduction of cardiac fibrosis (Ref. 89, 90), although mice with a null allele for miR-21 were normal in cardiac development and function (Ref. 96). In diabetic nephropathy, miR-21 expression is downregulated and overexpression of miR-21 inhibits the proliferation of mesangial cells, decreasing the albumin excretion rate in diabetic mice (Ref. 97). In the kidney, miR-21 knockdown halts the progression of renal fibrosis in obstructive nephropathy by a SMAD-3-dependent mechanism (Ref. 98). In kidney transplantation, miR-21 was upregulated in a model of ischaemia–reperfusion injury. In vitro studies also revealed expression of miR-21 in proliferating tubular epithelial cells (TECs). Knockdown of miR-21 leads to increased cell death, and subsequently overexpression protects TECs from cell death (Ref. 33). Thus, the impact of modulation of miR-21 in kidney or other organ transplant models remains to be investigated.

Clinical outlook and therapeutic options

In summary, miRNAs are highly expressed in immune cells and regulate particular functions. It has been shown that miRNA expression patterns are able to predict allograft function (Ref. 26), and thus tissue-specific or circulating miRNAs or combinations of both might be useful in future diagnostic or prognostic evaluation of organ rejection. Apart from their potential as biomarkers for disease and allograft function, direct mechanistic involvement of miRNAs in inflammation and rejection processes has been reported (Fig. 1). Rejection causes a progressive loss of allograft function as cells of the adaptive and innate immune system infiltrate the graft, leading to destruction of the organ. Limited knowledge on the mechanisms underlying the downregulation or silencing of the immune response prevents one from proceeding to new and effective therapeutic options. Increasing observations about the involvement of miRNAs in the modulation of immune responses have given new starting points, and new concepts might be developed from here. For instance, chemically engineered oligonucleotides targeting specific miRNAs, 'antagomirs' or 'antimiRs', are able to silence endogenous miRNAs and have been successfully used in mice (Refs 89, 98, 99). Recently, a study using an antagomir against miR-21 blocked fibrosis development in cardiovascular diseases (Ref. 89). Another group reported the successful use of an antagomir against miR-21, thereby blocking the development of lung fibrosis (Ref. 95). Others reported upregulation of miR-29 to attenuate cardiac fibrosis (Ref. 91). Altogether, these promising observations support the idea of using certain miRNA therapeutics as immunomodulating or antifibrotic drugs. Indeed, it has been shown that treatment with different miRNA antagonists can affect a wide range of organs, and thus most organs (including transplanted organs) could be targets for this approach (Refs 88, 90). A further problem to be solved is how to retain systemically injected miRNA modulators to the organ of interest without inducing side effects in

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other organs. Solutions could include coupling of miRNA modulators to cell-type-specific peptides or antibodies, or the use of viral-based miRNA modulators. Although further studies are needed, miRNA-based therapeutic strategies remain a promising new approach to treat rejection and allograft dysfunction.

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Features associated with this article

Figure
Figure 1. Schematic representation of acute rejection using the example of kidney rejection.

Table
Table 1. Role of miRNAs and their target genes in various immune processes.

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