Immuno-miRs: critical regulators of T-cell development, function and ageing

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Summary

MicroRNAs (miRNAs) are instrumental to many aspects of immunity, including various levels of T-cell immunity. Over the last decade, crucial immune functions were shown to be regulated by specific miRNAs. These ‘immuno-miRs’ regulate generic cell biological processes in T cells, such as proliferation and apoptosis, as well as a number of T-cell-specific features that are fundamental to the development, differentiation and function of T cells. In this review, we give an overview of the current literature with respect to the role of miRNAs at various stages of T-cell development, maturation, differentiation, activation and ageing. Little is known about the involvement of miRNAs in thymic T-cell development, although miR-181a and miR-150 have been implicated herein. In contrast, several broadly expressed miRNAs including miR-21, miR-155 and miR-17–92, have now been shown to regulate T-cell activation. Other miRNAs, including miR-146a, show a more T-cell-subset-specific expression pattern and are involved in the regulation of processes unique to that specific T-cell subset. Importantly, differences in the miRNA target gene repertoires of different T-cell subsets allow similar miRNAs to control different T-cell-subset-specific functions. Interestingly, several of the here described immuno-miRs have also been implicated in T-cell ageing and there are clear indications for causal involvement of miRNAs in immunosenescence. It is concluded that immuno-miRs have a dynamic regulatory role in many aspects of T-cell differentiation, activation, function and ageing. An important notion when studying miRNAs in relation to T-cell biology is that specific immuno-miRs may have quite unrelated functions in closely related T-cell subsets.

Keywords: activation; ageing; differentiation; microRNA; T cell.

MicroRNAs

MicroRNAs (miRNAs) are single stranded ~22 nucleotides (nt) long non-coding RNAs that regulate gene expression at the post-transcriptional level. MiRNAs are encoded in host genes, which can be located in introns or exons of protein-coding genes, as well as in non-coding genes.1 Biogenesis of miRNAs starts in the nucleus from a primary miRNA (pri-miR) transcript, which folds into a secondary structure containing one or more hairpin loops2 (Fig. 1). These hairpin loops are recognized and cleaved by the microprocessor complex, composed of DiGeorge syndrome critical region 8 and Drosha. This results in a stem loop precursor miRNA (pre-miR) of 60–70 nt.3 The pre-miR is subsequently transported to the cytoplasm where it undergoes a secondary processing step by Dicer resulting in a RNA duplex of 20–22 nt.2,4 The RNA duplex is incorporated into the RNA-induced silencing complex (RISC), where one of the strands undergoes degradation while the other forms the mature miRNA.5 By virtue of partial complementarity between the miRNA and its target mRNA, the miRNA guides the RISC to its target transcript.6 The miRNA : target gene binding is dependent on the degree of complementarity, which is usually high in the so-called seed sequence, i.e. the 5‘ end of the miRNA, and limited at the 3‘ part. In general, the miRNA binds to the 3‘ untranslated region (UTR) of its target mRNA transcript, but miRNAs can also bind to the 5‘ UTR and the coding region, albeit less frequently.7 Binding of RISC leads to translational inhibition or
One miRNA can regulate the expression of multiple genes, suggesting that miRNAs play a role in thymocyte selection by regulating genes involved in TCR-induced apoptosis and survival signals.

Immuno-miRs in thymic T-cell development

Committed T-cell progenitors are produced in the bone marrow and migrate to the thymus where they undergo proliferation, T-cell receptor (TCR) rearrangements and differentiation to mature naïve T cells. Based on the differential expression of the CD4 and CD8 co-receptors, progenitor T cells can be subdivided into double-negative (DN) stage 1–4, double-positive (DP) and CD4 or CD8 single-positive (SP) T cells. The development of T cells in the thymus is controlled by a complex signalling network, which ensures proper induction of genes essential for T-cell maturation and deletion of auto-reactive T cells. Transcription factors are responsible for the appropriate gene expression programmes during T-cell lineage commitment. In addition to this, it has become evident that miRNAs mediate a crucial additional level of regulation.

Dynamic regulation of miRNA expression in sequential stages of T-cell development is well documented. An overall increase in miRNA levels at early stages of T-cell maturation (DP stage) coincided with an increase in total cellular RNA content. However, individual miRNAs showed dynamic changes in distinct thymocyte subpopulations (DN, SP, DP). In both studies, an inverse correlation was observed between an increase of individual miRNAs with a depletion of predicted target genes, suggesting that miRNAs play a role in thymocyte selection by regulating genes involved in TCR-induced apoptosis and survival signals.
The biological relevance of miRNAs in T-cell development and function has been well established in vivo.\textsuperscript{29,30} Conditional deletion of Dicer at an early stage (DN to DP) of T-cell development reduced the cellularity of DP thymocytes, which was driven mainly by reduced numbers and survival of αβ T cells, whereas the number of γδ T cells was not affected.\textsuperscript{29} Remarkably, while CD8 and CD4 SP T-cell numbers were decreased in the spleen and a reduction of total CD3\(^+\) T cells was observed in the periphery, deletion of Dicer appeared to be dispensable for CD4 and CD8 commitment.\textsuperscript{29,30} Both studies clearly indicate the requirement of DICER, and therefore of miRNAs, for appropriate thymic T-cell development.

Two miRNAs were studied in more detail with respect to their function in thymic T-cell maturation.\textsuperscript{28,31,32} Inhibition of miR-181a significantly impaired both positive and negative selection of DP cells.\textsuperscript{31} Its expression is high in immature T cells (in particular at the DP stage) and low in more differentiated T helper type 1 (Th1) and Th2 cells. MiR-181a was shown to repress expression of a set of genes involved in T-cell maturation (TCR, CD69, BCL-2) and as such could regulate positive T-cell selection.\textsuperscript{25} Importantly, over-expression of miR-181a resulted in the development of low-affinity-aptitude-specific T cells by lowering the TCR signalling threshold.\textsuperscript{34} This indicates that the regulated expression of miR-181a during thymic development of immature T cells contributes to the clonal deletion of autoreactive T cells by modulating the TCR signalling threshold and survival of low-affinity peptide-specific T cells. The second miRNA studied specifically in relation to T-cell maturation is miR-150. MiRNA expression profiling in human and mouse showed that miR-150 levels are strongly up-regulated during T-cell maturation.\textsuperscript{28,33} Over-expression of miR-150 driven by a ubiquitously active promoter in miR-150 transgenic mice resulted in a block in early B-cell and thymic T-cell development. The transition from DN3 to DN4 was hampered, resulting in decreased numbers of CD4 and CD8 T cells.\textsuperscript{35} The transcription factor c-Myb was shown to be an important miR-150 target that was down-regulated in immature B and T cells upon miR-150 over-expression. Besides c-Myb, miR-150 targets NOTCH3, a main regulator of T-cell differentiation, resulting in decreased T-cell proliferation and survival.\textsuperscript{28} In conclusion, there is convincing evidence that shows the significance of miRNAs in general and miR-181a and miR-150 specifically in the process of T-cell maturation.

**Dynamic regulation of immuno-miRs during T-cell activation**

T-cell activation is initiated via TCR–MHC antigen complex interaction. The affinity of the TCR–MHC antigen complex interaction in combination with co-stimulatory signals provided by cell-cell interaction and cytokines, directs the T-cell response. Several miRNAs are regulated by transcription factors that are related to T-cell activation, such as nuclear factor (NF-κB)\textsuperscript{34–38} and PU.1\textsuperscript{39} (Fig. 2). In addition, miR-21 was shown to be under positive transcriptional control of activating protein 1 (AP1) and signal transducer and activator of transcription 3 (STAT3),\textsuperscript{40–42} while miR-155 was shown to be under positive transcriptional control of AP1\textsuperscript{43} and STAT5.\textsuperscript{44}

MiR-181a is a decisive factor in tuning the TCR signalling threshold by targeting multiple phosphatases, i.e. PTPN22, DUSP5, DUSP6 and SHP-2, involved in the regulation of kinase-mediated TCR signalling.\textsuperscript{31} Indeed, miR-181a is highly over-represented in immature thymocytes, which allows positive selection of immature thymocytes via low-affinity TCR–MHC antigen-mediated signalling (see also paragraph ‘Immuno-miRs in thymic T-cell development’).

Interleukin-2 (IL-2) is an essential autocrine cytokine for T-cell activation. Regulation of the IL-2 signalling pathway involves multiple miRNAs including miR-155, miR-181c, miR-9 and miR-31. Expression of miR-155 is markedly and quickly induced upon T-cell activation and enhances T-cell proliferation via direct targeting of the negative regulator of cytokine signalling, suppressor of cytokine signalling 1 (SOCS1)\textsuperscript{45} (Fig. 2). In addition, miR-155 targets the negative regulator of T-cell activation CTLA-4,\textsuperscript{46} indicating a second, independent route via which miR-155 promotes T-cell activation. MiR-181c has been shown to directly target IL-2 and by doing so modulates the activation state of T cells.\textsuperscript{47} MiR-9 is enhanced upon activation of T cells and results in a down-regulation of B-lymphocyte-induced maturation protein 1 (BLIMP1) and BCL6.\textsuperscript{48} Inhibition of miR-9 was shown to increase BLIMP1 and BCL6 levels and, as a consequence, to lead to decreased secretion of IL-2 and interferon-γ (IFN-γ). MiR-31 has been shown to directly repress the NF-κB-inducing kinase NIK and, as such, negatively regulates the non-canonical NF-κB signalling (Fig. 2).\textsuperscript{49} In addition, it was shown that miR-31 induces IL-2 expression in T cells via targeting of the upstream kinase suppressor KSR2.\textsuperscript{50} Hence, miR-31 is involved in the acquisition of an activated T-cell phenotype.

MiR-146a is induced upon TCR activation via activation of NF-κB signalling and has, like many miRNAs, a dual regulatory function.\textsuperscript{34} MiR-146a regulates the TCR-induced NF-κB signalling pathway via a negative feedback loop by directly regulating the levels of NF-κB signalling transducers TNF receptor-associated factor 6 (TRAF6) and Interleukin-1 receptor-associated kinase 1 (IRAK1). In addition, the delayed induction of miR-146a upon T-cell activation targets Fas-associated via death domain (FADD) and as such inhibits activation-induced T-cell death.\textsuperscript{51}

MiR-21 has been widely studied in relation to its anti-apoptotic function, especially in cancer.\textsuperscript{52} MiR-21 expression is induced upon TCR triggering in the presence
of co-stimulation and regulates several processes that are relevant for T-cell activation, such as apoptosis, proliferation and migration. Importantly, the regulatory function of miR-21 seems to depend on the differentiation status of the T cell. Whereas miR-21 in activated memory T cells functions as an anti-apoptotic factor, in
recently activated naive T cells miR-21 may be involved in regulating homing properties via targeting CCR7.55

The miR-17–92 cluster is strongly induced in T cells upon T-cell activation.36,57 Activation induced miR-17–92 promoted cell cycle progression of effector cells via targeting of phosphatase and tensin homolog (PTEN). This resulted in enhanced mTOR signalling and mediated skewing towards short-lived terminal effector cells. Failure to down-regulate miR-17–92 resulted in a gradual loss of memory cells and defective central memory T-cell development.56

It is evident that TCR stimulation induces a marked change in miRNA pattern, and regulation of IL-2 seems to be an essential role of the immuno-miRs involved in T-cell activation.

The role of immuno-miRs in peripheral T-cell subsets

Differentiation of naive CD4+ and CD8+ T cells into memory, effector or regulatory T (Treg) cells is a dynamic process that depends on the presence of many environmental determinants. This differentiation results in a marked change in the transcriptional programme and acquisition of lineage-specific functions.58,59 Several studies have shown that miRNAs are dynamically regulated during T-cell maturation and specific miRNA signatures were observed for specialized T-cell subsets.60–62 It should be noted that, although the overall miRNome of T lymphocytes of mice and human is relatively well conserved there is a poor concordance between specific T-cell subset miRNA signatures of the two species.63

CD4+ T cells

Upon interaction with cognate antigen presented by antigen-presenting cells, such as dendritic cells, CD4+ T cells can differentiate into a variety of effector subsets. Specific CD4+ T-cell subsets secrete cytokines to activate macrophages, cytotoxic T cells and natural killer cells (Th1), stimulate B cells and inflammation (Th2), enhance mucosal barrier functions (Th17), or suppress the immune response (Treg), while others remain in lymphoid organs and help B cells to differentiate into antibody secreting cells [follicular helper T (Thf)].

Th1 and Th2 cells

The Th1 and Th2 cells develop from naive CD4+ T cells in response to cognate antigen and polarizing cytokines in the initiating phase of immune responses.63 Th1 cells are defined as IFN-γ-producing cells and Th2 cells produce IL-4 and IL-5 but not IFN-γ.

Deletion of Dicer in CD4+ T cells results in a dramatic decrease in peripheral T cells. T cells derived from Dicer-deficient mice show impaired expansion and dysregulated cytokine expression upon Th1 and Th2 differentiation.60 In particular, repression of IFN-γ expression was impaired resulting in a Th1-skewed immune response.30

One of the critical miRNAs that regulate differentiation towards Th cells is miR-125b, which is preferentially expressed in naive CD4+ T cells compared with memory CD4+ T cells. MiR-125b enforces the naive T-cell state by regulating targets such as IFN-γ, IL-10RA, IL-2RB and BLIMP1, which all have a role in T-cell differentiation.60

The miR-17–92 cluster enhanced polarization towards a Th1 phenotype.64 MiR-19b, one of the six cluster members, was indispensable for IFN-γ production. Together with miR-17, another member of the cluster, miR-19b was critical in promoting Th1 responses and preventing differentiation into inducible Treg cells by targeting PTEN (miR-19b), transforming growth factor, beta receptor II (TGFBRII) and cAMP responsive element binding protein 1 (CREB1) (both miR-17).64

Over-expression of miR-155 in activated CD4+ T cells also promoted Th1 cell differentiation possibly by targeting the IFN-γ receptor x-chain (IFNGR1).65 MiR-155-deficient CD4+ T cells on the other hand, were more prone to polarize towards Th2 cells.65,66 This was at least in part due to enhanced levels of the miR-155 target c-Maf, which is a potent transactivator of the Th2-specific cytokine IL-4.66

An miRNA that can limit Th1 cell differentiation is miR-29. The miR-29 seed family members can rescue the aberrant IFN-γ expression of miRNA-deficient CD4+ T cells by targeting the IFN-γ-inducing transcription factors T-box expressed in T cells (T-bet) and eomesodermin (EOMES).67 Importantly, it was shown that miR-29 can also directly target IFN-γ and miR-29b expression levels are enhanced by IFN-γ, resulting in a negative feedback loop.68

Th17 cells

The development of Th17 cells is stimulated by pro-inflammatory cytokines produced in response to bacteria and fungi. Several miRNAs have been shown to promote Th17 differentiation. Mice deficient for miR-155 are characterized both by reduced numbers of Th1 and Th17 cells.69 Besides miR-155, miR-301 is also required for Th17 differentiation. Th17 cells are characterized by relatively high miR-301 expression levels in comparison to other T-cell subsets and in vitro differentiated T cells.70 Inhibition of miR-301 results in an impaired in vitro Th17 differentiation through a decreased signalling in the Th17 critical IL-6/23-induced STAT3 pathway. Protein inhibitor of activated STAT3 (PIAS3), an inhibitor of STAT3 signalling was shown to be a target of miR-301 and, in line with this, PIAS3 inhibition could phenocopy the effect of miR-301 up-regulation.
The miR-132/212 cluster enhances Th17 differentiation when induced via the Aryl hydrocarbon receptor under Th17 polarizing conditions.\(^7\) Inhibition of the miR-132/212 cluster effectively repressed Th17 differentiation via lack of effective down-regulation of the miR-132/212 target gene BCL6, which is a negative regulator of Th17 differentiation.

Two miRNAs of the miR-17–92 cluster, i.e. miR-19b and miR-17, are critically involved in Th17 differentiation.\(^7\) MiR-19 was shown to target PTEN resulting in enhanced phosphoinositide 3-kinase (PI3K) signalling and miR-17 inhibited IKZF4, a zinc finger transcription factor shown to negatively regulate Th17 differentiation.

Compared with Th cells and inducible Treg cells, Th17 showed the highest miR-326 expression and Th17 differentiation was promoted by increasing the levels of miR-326.\(^7\) Ets1, a negative regulator of Th17 differentiation was identified as a functional target of miR-326 by showing that an ‘miR-326-resistant’ Ets1 variant showed normal Th17 differentiation.

Hypoxia-inducible factor 1α (HIF-1α) a key transcriptional regulator of Th17 cell differentiation, regulates expression of miR-210.\(^7\) HIF-1α was in turn shown to be regulated by miR-210, resulting in a negative feedback loop. In line with these findings, T-cell-specific deletion of miR-210 resulted in an increase in Th17 differentiation.\(^7\)

**Treg cells**

Regulatory T cells play an essential role in controlling the immune response and preventing autoimmunity.\(^7\) Treg cells from both mice and human were shown to express a set of miRNAs that is distinct from conventional T cells.\(^6\) Specifically, miR-146a and miR-21 are consistently more highly expressed in Treg cells whereas the expression level of miR-31 is lower in Treg cells. Of note, in a detailed comprehensive analysis of miRNA expression profiles in naïve and memory regulatory and conventional T cells, high miR-21 expression was found to be restricted to the memory T-cell compartment.\(^6\) Hence, the widely reported high expression of miR-21 by Treg cells seems attributable to the predominant memory phenotype of Treg cells.

Using a Foxp3-induced conditional Dicer knockout (KO), it was shown that miRNAs are essential for Treg-mediated tolerance. These Foxp3-specific Dicer KO mice developed a spontaneous systemic autoimmune disease similar to Foxp3 KO mice.\(^5\) One of the miRNAs that has been implicated in the development of Treg cells is miR-155, an miRNA that is induced by Foxp3.\(^6\) MiR-155 KO mice have reduced Treg cell numbers without an apparent reduction in their suppressive function. The reduction in Treg cell numbers was explained by a reduced sensitivity to IL-2 signalling due to lack of suppression of the miR-155 target gene SOCS1.\(^7\) Besides miR-155, a Foxp3-induced deficiency of the miR-17–92 cluster also resulted in reduced numbers of Treg cells due to decreased proliferation and increased apoptosis.\(^8\) The involvement of miR-17–92 in Treg cells, however, extends beyond promoting accumulation of antigen-specific Treg cells. In later phases of the immune response, miR-17–92 controls IL-10 production and, possibly, migration.\(^9\)

In contrast to miR-155 or miR-17–92 KO, miR-146a KO resulted in increased numbers of Treg cells.\(^8\) However, in vivo analysis of miR-146a-deficient Treg cells in an miR-146a-sufficient environment showed that these cells have a strongly impaired suppressive activity resulting in severe immune-mediated pathology. This phenotype was induced via the targeting of STAT1 by miR-146a.

MiR-142-3p is negatively regulated by Foxp3 and the lower miR-142-3p levels in Treg cells results in higher levels of cyclic adenosine monophosphate (cAMP) by targeting the cAMP-producing enzyme adenyl cyclase 9.\(^8\) Transfer of cAMP through gap junctions from Treg cells into conventional T cells is a known suppressive mechanism employed by Treg cells.

MiR-126 was shown to be a regulator of the suppressive activity of Treg cells.\(^8\) Inhibition of miR-126 leads to reduced Foxp3 expression by targeting PIK3R2, a regulatory component of PI3K.

Several other miRNAs, i.e. miR-24, miR-210 and miR-31, were shown to regulate Foxp3 by direct binding to the 3’ UTR, indicating that a network of miRNAs is involved in regulating the levels of Foxp3 expression.\(^7\)\(^8\)

**T follicular helper cells**

Th cells that migrate towards sites of activated B cells may be triggered to differentiate into Tfh cells. Tfh cells are antigen-experienced CD4+ CXCR5+ BCL6+ PD1+ T cells that have a critical role in germinal centre formation and germinal centre B-cell maturation.\(^6\) So far only a few studies have addressed the role of miRNAs in the differentiation and/or function of Tfh cells.\(^7\)\(^8\)

The miR-17–92 cluster is an important regulator of Tfh cell biology. Deletion of the miR-17–92 cluster in CD4+ T cells resulted in decreased numbers of Tfh and germinal centre B cells upon viral infection while transgenic expression of this miRNA cluster in CD4+ T cells resulted in increased numbers of both Tfh and germinal centre B cells.\(^7\)\(^8\) The underlying mechanism included the miR-17–92 target genes PTEN, nuclear receptor RORα and phosphatase PHLPP2. Deletion of one *pten* allele partially rescued the miR-17–92 KO phenotype, and deletion of one *rora* allele restored expression of several Tfh subset inappropriate genes.\(^7\) Inhibition of PHLPP2 in the KO background rescued Tfh differentiation while over-expression in wild-type CD4+ T cells impaired their ability to migrate to B-cell follicles but not...
to differentiate into Tfh cells.\textsuperscript{88} A second miRNA linked to Tfh cell differentiation is miR-10a. MiR-10a targets BCL-6, which is essential for Tfh cell differentiation and maintenance. Inhibition of miR-10a enhanced the conversion of inducible Treg to Tfh cells and over-expression could inhibit this process.\textsuperscript{89}

Our current knowledge on how miRNAs affect the phenotype of Tfh cells is still limited. It may be anticipated that miRNAs targeting PD-1 and CXCR5, which are crucial for Tfh cells, will be important herein.

**CD8\(^+\) T cells**

Differeniation of naive CD8\(^+\) T cells into effector or memory cytotoxic T cells follows upon activation by interacting with antigen-presenting cells. Deletion of Dicer at the CD8 SP stage resulted in a lack of CD8 interacting with antigen-presenting cells. Deletion of memory cytotoxic T cells follows upon activation by higher in effector memory CD8\(^+\) T cells as compared to regulated upon CD8\(^+\) T-cell activation.\textsuperscript{90} Others showed CD69 by the miR-130/301 family, which is strongly up-regulated in rapidly proliferating activated CD8\(^+\) T cells and a prolonged expression of CD69 expression which was associated with a prolonged retention of the cells in lymph nodes.

The increased CD69 expression in Dicer-deficient T cells could mechanistically be explained by targeting of CD69 by the miR-130/301 family, which is strongly upregulated upon CD8\(^+\) T-cell activation.\textsuperscript{90} Others showed that activated murine CD8\(^+\) T cells that lack Dicer have, next to the increase in CD69 expression, strongly enhanced expression of Perforin.\textsuperscript{91} MiR-139 was shown to regulate the expression of Perforin both directly and indirectly via targeting of its transcriptional activator EO-MES.\textsuperscript{91}

To further identify miRNAs that can explain the phenotype of Dicer-deficient CD8\(^+\) T cells, miRNA profiles were generated of antigen-stimulated CD8\(^+\) subsets. This revealed a strong up-regulation of the miR-17–92 cluster in rapidly proliferating activated CD8\(^+\) T cells and a down-regulation during the differentiation from effector to memory cells.\textsuperscript{56} Indeed, expression of the miR-17–92 cluster is critically involved in the expansion of CD8\(^+\) T cells as mice deficient for this cluster specifically in TCR-activated mature T cells have an impaired CD8 expansion upon viral infection. Over-expression of miR-17–92 promoted effector CD8\(^+\) T-cell differentiation and resulted in decreased numbers of memory T cells. The over-expression of miR-17–92 resulted in a decreased expression of PTEN and other negative regulators of PI3K-Akt-mTOR signalling.\textsuperscript{56,92} As it has been shown that reduced mTOR signalling favours generation of memory cells, it is likely that down-regulation of negative regulators of mTOR signalling by miR-17–92 contributes to this effect.\textsuperscript{93}

Similar to miR-17–92, miR-155 levels are up-regulated during activation of naive CD8\(^+\) T cells and levels are higher in effector memory CD8\(^+\) T cells as compared to naive and central memory CD8\(^+\) T cells.\textsuperscript{45,94,95} Absence of miR-155 impaired antiviral CD8\(^+\) T-cell responses and conversely, miR-155 over-expression enhanced the antiviral responses.\textsuperscript{45,95,96} Inhibition of STAT1 and IRF7, two type I IFN signalling-associated transcription factors partially rescued the impaired response of miR-155-deficient CD8\(^+\) T cells in vivo.\textsuperscript{95} Over-expression of SOCS1, a known miR-155 target, resulted in a similar phenotype as observed for the miR-155-deficient CD8\(^+\) T cells suggesting an important role for this molecule.\textsuperscript{45} However, inhibition of SOCS1 in miR-155-deficient CD8\(^+\) T cells could not rescue their response to virus infection in vivo, indicating that miR-155 exerts its effects via another pathway or additionally targets genes downstream of SOCS1.\textsuperscript{95}

**Immuno-miRs in T-cell ageing**

Ageing-related alteration of immune function, termed immunosenescence, involves a number of characteristic changes in the T-cell compartment.\textsuperscript{97,98} First, as a result of thymic involution and continuous antigenic stimulation, a gradual shift is observed from T cells with a naive phenotype to T cells with a memory phenotype and terminally differentiated T cells.\textsuperscript{99,100} Second, characteristic changes are observed in the expression of defined cell surface markers including down-regulation of co-stimulatory receptor CD28 and up-regulation of NK-cell-related receptors.\textsuperscript{98} At present, little is known about the mechanisms underlying the observed age-related changes in T-cell composition and function. However, a number of studies indicate that miRNAs are involved.\textsuperscript{101–106}

The miR-17–92 cluster, which regulates cell cycle control and apoptosis, is down-regulated in old compared with young CD8\(^+\) CD28\(^+\) T cells.\textsuperscript{102,105} Expression of the miR-17–92 cluster negatively correlates with p21 protein levels, suggesting regulation of replicative exhaustion of aged T cells.\textsuperscript{106} One of the members of the miR-17–92 cluster, miR-92, was found to positively correlate with the percentage of naive CD8\(^+\) T cells (CD8\(^+\) CD27\(^-\) and CD3\(^+\) CD8\(^+\) CD62L). This indicates that the expression of miR-92a in CD8\(^+\) T cells is mainly derived from naive cells, and that miR-92a expression in CD8\(^+\) T cells declines progressively with age. Indeed, age-related attrition of naive T cells is linked to a reduction of miR-92a in human T lymphocytes.\textsuperscript{103} MiR-181a originally described in relation to its role in tuning the threshold for TCR signalling,\textsuperscript{31} was under-represented in aged CD4\(^+\) T cells.\textsuperscript{106} This correlated with increased expression of DUSP6, which impaired TCR sensitivity of aged CD4\(^+\) T cells. Consequently, dual specificity phosphatase 6 (DUSP6) was coined as a potential target for restoring T-cell responses in the elderly, to augment the effectiveness of vaccination.\textsuperscript{106}

An miRNA cluster over-represented in CD8\(^+\) CD28\(^+\) T cells is the miR-23–27 cluster.\textsuperscript{104} This miRNA cluster targets multiple members of the DNA damage repair (DDR) pathway.\textsuperscript{107} Consistent with this, it was reported that a
decreased activity of the DDR pathway is involved in cellular senescence and T-cell differentiation. Specifically, decreased expression of the histone H2A family member X (H2AX), a validated target of miR-24 (part of miR-23~27 cluster), has been reported in CD8⁺ CD28⁻ T cells. Experimentally induced DNA damage resulted in decreased expression of H2AX as well as a decreased potential to activate the DDR response, especially in CD8⁺ CD28⁻ T cells. MiR-24 could modulate expression of H2AX as well as a decreased potential to activate the DDR response, especially in CD8⁺ CD28⁻ T cells. MiR-24 could modulate expression of H2AX when over-expressed in Jurkat T cells. However, the direct functional involvement of miR-24 on DDR signalling and apoptosis in CD8⁺ CD28⁻ T cells was not clearly established and might involve differential IL-15 expression between CD28⁺ and CD28⁻ cells.

The concept of immunosenescence and the involvement of ageing T cells herein is just beginning to be understood. As a consequence, comprehensive concepts on how miRNAs are involved in this process are still lacking. Available literature shows that aged T cells are characterized by defined changes in miRNA expression levels. The miRNAs involved are mechanistically linked with various aspects of T-cell ageing and possibly play causal roles in immunosenescence.

Conclusions

The involvement of miRNAs in the process of T-cell differentiation, activation and function is irrefutable. Often, the exact molecular impact of these immuno-miRNAs on the T-cell phenotype is not easily defined experimentally and therefore qualified in terms of fine-tuning. However, fine-tuning of the T-cell phenotype is the foundation of well-balanced T-cell immunity and, as such, should not be judged trivial. Interestingly and completely in line with the current dogma of miRNA biology, similar miRNAs have been described in quite different and unrelated aspects of T-cell biology.

A number of miRNAs, like miR-21, miR-17~92 and miR-155, play crucial roles in T cells (Fig. 2 and Table 1). Their function may be generic and independent of a specific T-cell subset, i.e. sustaining proliferation and repression of apoptosis, but also subset specific, i.e. regulating CCR7 (miR-21), RORα (miR-17~92) or CTLA-4 (miR-155). The generic versus specific functions are probably explained by differences in the miRNA targetome at certain stages of T-cell development or differentiation. This clearly exemplifies the importance to consider the function of defined miRNAs in the relevant T-cell subset. The interplay between miRNAs and their targetome, both of which are expressed independently in time and by differentiation stage, defines signalling circuits and allows regulation of a spatio-temporal T-cell response.

Importantly, given the T-cell-specific miRNA expression patterns, assessment of differential miRNA expression in unfractionated or undefined T-cell samples is of limited relevance. Specifically, differential miRNA expression patterns assessed in unfractionated or undefined T-cell samples may simply reflect slight differences in the composition of classically defined T-cell subsets. However, when studied in carefully defined T-cell subsets,
immuno-miRs may help to expand our knowledge of T-cell phenotypes and the molecular circuits that direct T-cell responses in health and disease.61

Disclosures
The authors declare no competing interests.

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