

Mini-review

The interplay between critical transcription factors and microRNAs in the control of normal and malignant myelopoiesis

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ABSTRACT

Myelopoiesis is a complex process driven by essential transcription factors, including C/EBP α , PU.1, RUNX1, KLF4 and IRF8. Together, these factors are critical for the control of myeloid progenitor cell expansion and lineage determination in the development of granulocytes and monocytes/macrophages. MicroRNAs (miRNAs) are expressed in a cell type and lineage specific manner. There is increasing evidence that miRNAs fine-tune the expression of hematopoietic lineage-specific transcription factors and drive the lineage decisions of hematopoietic progenitor cells. In this review, we discuss recently discovered self-activating and feed-back mechanisms in which transcription factors and miRNAs interact during myeloid cell development. Furthermore, we delineate how some of these mechanisms are affected in acute myeloid leukemia (AML) and how disrupted transcription factor-miRNA interplays contribute to leukemogenesis.

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1. Introduction

Hematopoiesis is a complex of stepwise processes controlled by transcription factors that are expressed in a cell type- and developmental stage-dependent fashion. Delicate regulation of expression of transcription factors, such as CCAAT/enhancer-binding protein alpha (C/EBP α), spleen focus forming virus proviral integration oncogene SPI1 (PU.1), runt related transcription factor 1 (RUNX1), kruppel-like factor 4 (KLF4) and interferon regulatory factor 8 (IRF8) is crucial for myelopoiesis [1–5]. It has become increasingly clear that the expression of miRNAs is essential for normal hematopoiesis. For instance, expression of *Dicer*, the critical RNaseIII enzyme for miRNA biogenesis [6], in hematopoietic stem cells (HSCs) is indispensable for hematopoietic cell differentiation in mice [7]. Conditional deletion of *Dicer* in HSCs, causing a full depletion of miRNAs, results in complete loss of HSCs due to induction of apoptosis and thereby loss of all hematopoietic cell lineages [7]. MiRNAs control gene expression by recruitment of the

RNA-induced Silencing Complex (RISC) to 3'-UTRs of protein-coding transcripts in a sequence-dependent fashion [6]. In addition, recent data show strong evidence for miRNA activities in the nucleus e.g. mediating epigenetic regulation and targeting long non-coding RNAs [8,9]. Like transcription factors, the expression of miRNAs in myeloid cells is highly cell type and developmental stage-dependent. In this review, we will focus on the interplay between the major myeloid transcription factors C/EBP α , PU.1, RUNX1, KLF4 and IRF8 and miRNAs in the course of myeloid cell development (Fig. 1). These interactions include essential regulation of these transcription factors by miRNAs as well as the induction of critical miRNAs by these myeloid transcription factors.

2. The interplay of C/EBP α with miRNAs in normal and malignant myelopoiesis

The transcription factor C/EBP α is a master regulator of myeloid differentiation [10]. *C/EBP α* is a single exon gene and encodes an mRNA with two translation initiation sites, resulting in either a 42 kD (p42) or a 30 kD (p30) protein. C/EBP α -p42, but not C/EBP α -p30, contains two N-terminal transactivation domains involved in the repression of E2F transcription factors. Down-regulation of E2F activity is an essential requirement for normal granulopoiesis [11]. In agreement, murine conditional *C/ebp α* null hematopoietic progenitor cells are unable to develop into mature granulocytes and are blocked in their differentiation at the common myeloid

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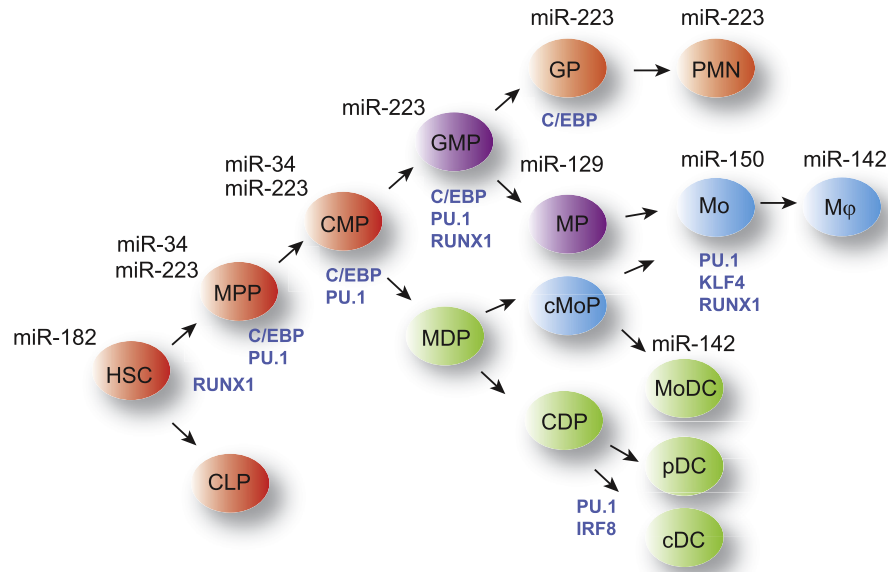


Fig. 1. Schematic overview of myeloid cell development. The hematopoietic stem cells (HSCs) and the early myeloid progenitor cells (multi-potent progenitor (MPP), common lymphoid progenitor (CLP), common myeloid progenitor (CMP)) are indicated in red, the granulocyte-macrophage progenitor (GMP) and the macrophage progenitor are indicated in purple. DC populations are indicated in green (monocyte and dendritic cell progenitor (MDP), common dendritic cell progenitor (CDP), monocyte derived DC (MoDC), plasmacytoid DC (pDC), conventional DC (cDC)). The macrophage/monocyte populations (monocytes (Mo), macrophages (Mφ)) are indicated in blue and the granulocytes are indicated in orange (granulocyte progenitor (GP), polymorphonuclear neutrophil (PMN)). Transcription factors and examples of miRNAs regulating the different stages of myeloid differentiation are indicated by the blue and black text respectively below the cell types. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

progenitor (CMP) stage [5,12]. Aberrant down-regulation of *C/EBPα* expression by hyper methylation of its promoter, post-translational modifications or frame shift mutations at the 5'-end and point mutations at the 3'-end of the gene encoding the basic region-leucine zipper domain lead to aberrant *C/EBPα* proteins, disrupt the normal activities of *C/EBPα* and are all associated with AML [10,13]. The AML cases with *C/EBPα* gene mutations are characterized by a unique miRNA expression profile [14], strongly suggesting that *C/EBPα* controls the expression of a specific set of miRNAs.

MiRNAs are essential for HSC survival, as indicated above. In striking contrast to the findings in HSCs, miRNA expression is not essential for survival of *C/ebpα*-positive hematopoietic progenitor cells, as CRE-mediated deletion of *Dicer* in *C/EBPα* positive cells in mice results in normal frequencies of myeloid progenitor cells in the bone marrow [15]. However, these mice have a high number of dysplastic myeloid cells in BM and blood and lack monocytes and macrophages [15]. Thus, *Dicer* expression is essential for normal differentiation of *C/EBPα* positive hematopoietic stem and progenitors (HSPCs) into granulocytes and monocytes/macrophages. Therefore, it's not surprising that *C/EBPα* directly or indirectly regulates the expression of miRNAs that are essential for myeloid cell development. A set of at least 20 miRNAs is found to be highly active in *C/ebpα*-positive HSPCs, including Let-7, miR-17 and miR-223, which are critical for the repression of genes involved in stem cell biology and erythropoiesis [15]. In agreement, AML cases with inactivating mutations in *C/EBPα* are characterized by low level expression of a set of at least 28 miRNAs compared to other AML samples, including miR-9 and Let-7 [14].

Recent data show that the expression level of miR-223 is tightly regulated during myelopoiesis, indicating that activity of miR-223 in hematopoietic cells is highly dynamic. *C/EBPα* is lowly expressed in HSCs. E2F1, a known antagonist of *C/EBPα* at this stage, is a direct transcriptional repressor of *MIR223* in HSCs (Fig. 2A). *C/EBPα* is upregulated in early myeloid progenitor cells and directly

enhance the expression of miR-223 [9]. *C/EBPα* competes at the *MIR223* promoter with the transcription factor nuclear factor (NF)I-A, an inhibitor of miR-223 expression, and upregulates miR-223 expression (Fig. 2A) [16]. Also, after passing a certain threshold, miR-223 mediates repression of E2F1 expression thereby enhancing its own expression, indicating a self-amplifying mechanism. Interestingly, *C/EBPα*-upregulated miR-223 is not only loaded into RISC, but is also associated with the polycomb group proteins (PcGs)-RNA-interfering complex. PcG complexes localize into the nucleus and silence target gene expression [9]. For instance, the PcG-miR-223 complex targets the promoter of NFI-A at a region that contains miR-223 complementary sequences and causes transcriptional repression [9]. The regulation of NFI-A levels in myeloid progenitor cells is crucial for myeloid lineage decision, because NFI-A induction drives erythropoiesis, whereas its down-regulation enables granulopoiesis [16,17]. Because miR-223 strongly inhibits the expression of NFI-A, this *C/EBPα*-stimulated process results in an activating circuitry that drives retinoic acid-induced granulocytic differentiation (Fig. 2B) [16].

There is strong evidence for different mechanisms that fine-tune the levels and activities of miR-223 in hematopoietic precursor cells. For instance, transcriptional variants of primary miR-223 are differentially expressed during hematopoiesis [18]. The longest primary miR-223 transcript is expressed at the highest level in CD34⁺ progenitors undergoing granulocytic differentiation, while cells that undergo monocytic differentiation express low levels. The expression of this variant is high in erythroid progenitors but declines strongly during erythroid differentiation. In addition to the long miR-223 variant, a shorter variant of primary miR-223 is induced only during granulopoiesis, but not upon monocytic or erythroid differentiation. Both primary miR-223 transcripts are processed and contribute to the enhancement of mature miR-223 levels in granulocytic progenitor cells. These transcripts originate from two different transcriptional start sites and are controlled by

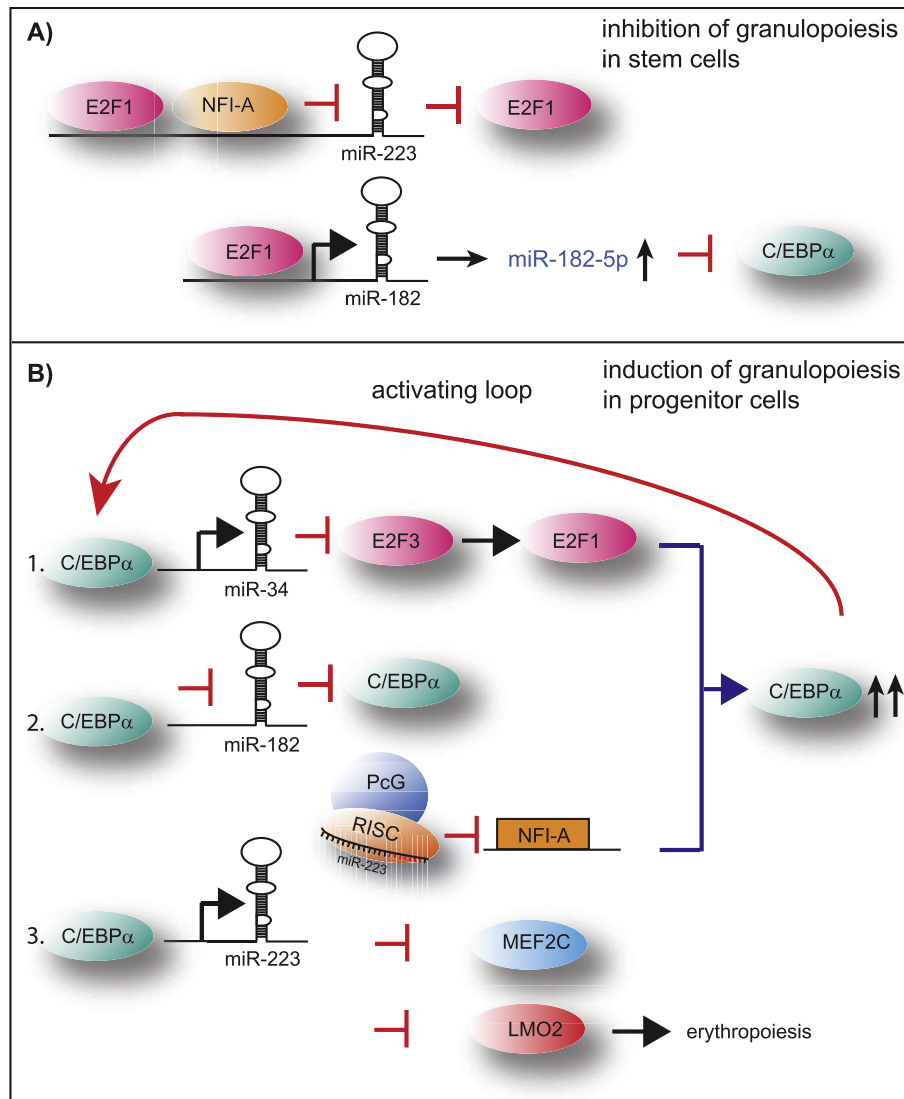


Fig. 2. The interplay between C/EBP α and miRNAs. Arrows indicate stimulatory activity, while inhibitory activities are indicated by block-signs (–). Notably, the signs are used as such that they represent the functional link between the indicated factor before the sign and the next step. This implies that stimulation and inhibition are indicated per step and not as the result of the whole represented cascade. **A)** E2F1 and NFI-A control the inhibition of granulopoiesis in stem cells. E2F1 and NFI-A inhibit transcription of miR-223. E2F1 is highly expressed and activates miR-182, which is an inhibitor of C/EBP α . **B)** C/EBP α -induced self-activating loop during granulopoiesis. C/EBP α induction in myeloid progenitors causes down-regulation of miR-182 and upregulation of miR-34 and miR-223. These three events represses the indicated inhibitors of C/EBP α such as E2F1, NFI-A and miR-182, resulting in a further upregulation of C/EBP α . Furthermore, miR-223 represses MEF2C and LMO2, thereby inhibiting erythropoiesis and stimulating granulopoiesis.

different transcription factors [18]. During monocytic differentiation, PU.1 and C/EBP β are recruited to the distal *MIR223* regulatory region. The enrichment of these transcription factors in early monocyte development explains the initial expression of miR-223 in monocytic cells. However, PU.1 and C/EBP β are released from the promoter at late stages of monocytic differentiation, resulting in a decreased expression of miR-223 in mature macrophages. During erythroid differentiation, erythroid transcription factors including gata-binding protein 1 (GATA-1), transcription factor 3 (TCF3, E2A), lim domain only 2 (LMO2) and T-cell acute lymphocytic leukemia 1 (TAL1), interact with the proximal transcriptional start site of the *MIR223* promoter and silence primary miR-223 transcription, thereby maintaining a low miR-223 expression. Forced miR-223 expression suppresses erythroid differentiation of K562 cells [18]. Vice versa, downregulation of miR-223 expression in human CD34⁺ cord blood cells results in the induction of LMO2, which is a confirmed target of miR-223, and expansion and

differentiation of erythroid progenitor cells (Fig. 2B) [20]. Together, these results strongly suggest that high miR-223 expression is critical for normal granulopoiesis. Surprisingly, *Mir223* KO mice show enhanced progenitor cell proliferation, granulocyte differentiation and activation [19]. This phenotype may at least in part be explained by overexpression of the transcription factor mads box transcription enhancer factor 2, polypeptide C (MEF2C) and E2F1, which are direct targets of miR-223 [19,20].

The expression of C/EBP α itself is directly controlled by miR-182-5p in HSCs via interaction with the 3'-UTR of *C/EBP α* mRNA (Fig. 2A) [21]. E2F1 is a strong activator of *MIR182* and overrules the inhibitory effects of C/EBP α on well-conserved interaction sites within the *MIR182* promoter [21]. When C/EBP α is induced, an auto-regulatory loop involving miR-182 is activated that results in further C/EBP α induction, which is critical for granulopoiesis as described above (Fig. 2B). C/EBP α -mediated upregulation of miR-34 expression strongly represses E2F3 expression, which is an

activator of E2F1 (Fig. 2B) [22]. Decreased E2F1 levels allow for the competing C/EBP α binding at the *MIR182* promoter, resulting in transcriptional silencing of *MIR182* [21]. In this way, enhanced C/EBP α expression inhibits its negative regulators E2F1 and miR-182 via transcriptional activation of *MIR223* and *MIR34*. Thus, these miRNA-mediated mechanisms may largely explain the stable myeloid cell determination of progenitor cells that is initiated by C/EBP α .

More complexity to the network involving C/EBP α regulation of granulopoiesis is added by the notion that other miRNAs than miR-182 also control C/EBP α expression. An important role is ascribed to miR-124, which targets *C/EBPA* transcripts [23]. The 3'-UTR of *C/EBPA* is 1.4 kb in length and contains two well-conserved 7mer-m8 sites for miR-124-3p. There are three copies of the *MIR124a* gene and one copy of the *MIR124b* gene in the human genome. The genes encoding *MIR124a-1* and *MIR124a-3*, but not *MIR124a-2*, are located within CpG islands. These CpG islands are aberrantly epigenetically silenced in leukemia cells, strongly suggesting that *MIR124* inactivation is an acquired event in leukemogenesis [23,24]. Downregulation of miR-124a occurs most-frequently in AML cases with t(15:17) by an unknown mechanism [24]. In addition, in cell lines and isolated AML cells, *MIR124* is silenced by ecotropic viral integration site 1 (EV11) [25]. Treatment of leukemia cells with DNA-demethylating agent 5-aza-2'-deoxycytidine (DAC) and histone deacetylase inhibitor trichostatin A (TSA) causes strong downregulation of C/EBP α expression as a result of *MIR124a* reactivation [23]. As suggested by the investigators, the epigenetic status of *MIR124a* in AML cells may be an important predictor for the efficiency of epigenetic therapy [23]. Interestingly, overexpression of miR-124 indirectly inhibits the expression of PU.1 by the downregulation of C/EBP α , thereby affecting monocyte/macrophage development and activation [26]. The functions of miR-124 as a negative regulator of C/EBP α -induced myelopoiesis still needs *in vivo* validation.

Loss of C/EBP α collaborates with oncogenic miRNAs in AML. For instance, enhanced expression of miR-125b accelerates leukemogenesis in mice that carry a C-terminal mutant of *C/ebpa* (*C/ebpa*-C^m) [27]. Furthermore, we have recently shown that forced expression of miR-199-3p, a DNA-damage-induced miRNA that is frequently upregulated in AML, in *C/ebpa* null progenitors causes AML in mice [28]. Notably, overexpression of miR-106, a miRNA that induces progenitor cell expansion but no oncogenic transformation in this model, did not result in AML [28]. Collaboration of C/EBP α loss and enhanced miR-155 levels in leukemogenesis has been strongly suggested in subsets of AML characterized by activating mutations in FMS-related tyrosine kinase 3 (*FLT3*) [29]. The most common mutations are in-frame internal tandem duplications (ITDs) in the juxtamembrane domain of the receptor *FLT3* and these occur in 30% of AML cases. *FLT3*-ITD receptors cause upregulation of miR-155 by constitutive activation of nuclear factor (NF)- κ B (p65), and signal transducer and activator of transcription 5 (STAT5). In addition *FLT3*-ITD causes reduced expression and activity of C/EBP α by constitutive activation of the extracellular signal-regulated kinase (ERK) 1/2 pathway and cyclin-dependent kinase-1 (CDK1)-mediated phosphorylation of C/EBP α serine 21 [30,31]. We have recently shown that enhanced miR-155 expression in *C/ebpa* null myeloid progenitor cells causes AML independently of additional signaling aberrations associated with *FLT3*-ITD in mice [29].

Gene expression analysis showed that the transcription factor PU.1, which is a well-studied target of miR-155, was downregulated in *FLT3*-AML [29]. The main evidence for miR-155-mediated regulation of PU.1 levels came from a study published by Lu et al. in which the interaction of miR-155 and *Pu.1* was well-studied *in vivo* by mutating the miR-155 binding site in the 3'-UTR of *Pu.1* in mice

(*Pu.1*^{155-/155-} mice) [32]. *Pu.1* homozygous mutant mice were viable and born at normal frequencies and without any clear aberrancies in the numbers and proportions of lymphoid and myeloid cell lineages. However, the *Pu.1*^{155-/155-} mutation resulted in a 2-fold increased PU.1 protein level and caused a significantly impaired class switch recombination and a reduced plasma cell differentiation of B-cells due to enhanced paired box gene 5 (PAX5) levels [32]. The effects of disrupted miR-155-mediated PU.1 regulation on the functions of myeloid cell types still needs to be determined.

3. PU.1 miRNA interactions

The hematopoietic-specific transcription factor PU.1 binds to GAGGAA sequences at regulatory elements of lineage-specific genes [33]. PU.1 directly or indirectly regulates the expression of several miRNAs involved in the control of macrophage development including the upregulated miR-146a, miR-342, miR-338 and miR-155 and downregulated miRNAs such as members of the *MIR17~92* cluster miR-19, miR-20 and miR-92. The interplay between PU.1 and these miRNAs have been recently reviewed by Alemdehy et al. [34].

More recent work show that PU.1 controls the transcriptional activation of miR-22 during monocyte/macrophage differentiation [35]. Upregulated miR-22 promotes differentiation by targeting *EV11* transcripts and increasing the c-JUN expression and c-JUN-PU.1 interactions. MiR-22 is significantly downregulated in AML samples compared to normal CD34⁺ bone marrow cells and peripheral mono nuclear cells (PMNCs) and may be an interesting diagnostic marker. Forced expression of miR-22 in human leukemia cell lines and in CD34⁺ cells of AML patients induced differentiation, suggesting a potential value in AML therapy. Moreover, miR-22 controls IRF8 and CSF1R (M-CSFR) expression, involved in differentiation of dendritic cells (DC) (see below).

Downregulation of PU.1 collaborates with loss of P53 and causes aggressive AML in mice [36]. The expression of the transcription factor MYB is silenced by P53 in hematopoietic cells. The authors discovered that MYB is an upstream regulator of *Mir-155*. Loss of P53 in AML causes upregulation of MYB and miR-155 expression. As a result, PU.1 expression is strongly decreased. This event significantly accelerates the pathogenesis of AML [36].

A study of Grassilli et al. showed that a network of ATRA-induced PU.1 in collaboration with vav guanine exchange factor 1 (VAV1) upregulates miR-142 expression, which in turn supports the differentiation of acute promyelocytic leukemia (APL) cells [37]. The positive correlation of PU.1 expression and miR-142 upregulation was also found in a human induced pluripotent stem cell (iPSC) model during myeloid progenitor cell differentiation induced by SCF, Flt3-L, IL-6 TPO and IL-3 [38]. A more recent study by Solomon et al., showed a different mechanism of PU.1-induced miRNAs in myeloid cell differentiation [39]. This study provides evidence for a mechanism in which PU.1-upregulated miRNAs, such as miR-342, miR-141 and miR-200c, downregulate genes involved in lipid anabolism, including ATP citrate lyase (ACLY). In their model, PU.1 controls the cell cycle by reducing the expression of E2F1 through direct activation of miR-223 transcription. The increased miR-223 level is sufficient to downregulate *E2f1*, which is a direct target of miR-223 (Fig. 2A, see above). These data suggest that PU.1 controls a set of miRNAs involved in different aspects of myeloid differentiation.

An intriguing finding concerning PU.1-controlled monocyte/macrophage differentiation is recently described [40]. In this study the authors show that PU.1 upregulates the expression of the long non-coding monocytic RNA (Lnc-MC) during monocyte/macrophage differentiation. Lnc-MC binds and inhibits the activities of miR-199a-2-5p, thereby preventing the repression of target

mRNAs. MiR-199a-2-5p is a potent repressor of activating A receptor type 1B (ACVR1B), an important factor driving monocyte/macrophage differentiation [41]. These data show that PU.1–upregulated Lnc-MC stimulate differentiation by inhibiting miR-199a-2-5p and thereby releasing ACVR1B expression.

4. The interplay between miRNAs and the transcription factor RUNX1

The RUNX family of transcription factors consists of 3 members (RUNX1, RUNX2 and RUNX3), which are highly conserved between species and control the expression of genes that are cell lineage-specific and important for normal mouse development [3]. Genetic deletion of the RUNX1-encoding gene in mice causes lethality at embryonic day E12.5, because of central nervous system (CNS) defects [42]. RUNX1, also known as AML1, is a transcription factor that is critical for normal hematopoiesis (Fig. 1). RUNX1 is the alpha component of the core binding factor (CBF) and binds to the non-DNA-binding partner CBF β , which is critical for regulation of RUNX1 target genes. The role of RUNX1 in normal hematopoiesis and leukemia has been reviewed recently in two publications [3,43]. RUNX1 is frequently deregulated in AML due to chromosomal translocation, mostly t(8;21)(q22;q23), which generates a fusion with eight twenty one (ETO, or RUNX1T1), in AML. RUNX1 has been shown to positively or negatively regulate the expression of numerous miRNAs including miR-223 and the miR-222–221 cluster. Vice versa, several miRNAs target RUNX1 and regulate its expression at different stages of hematopoiesis. Important insights into RUNX1 activities in this miRNA network have been reviewed by Rossetti et al. who describe the role of miRNA-mediated RUNX1 deregulation in human leukemia [44]. Here, we will discuss new insights concerning the interplay of miRNAs with RUNX1 (Fig. 3).

Studies with knockout mice show that the deletion of the *Mir23a~Mir27a~Mir24-2* cluster directs the lineage commitment of Multi-Potential Progenitor (MPPs) populations to differentiation into Common Lymphoid Progenitor CLPs, while the total number of MPPs is unchanged [45]. This finding has been confirmed in transplantation experiments with *Mir23^{-/-}* CD34⁺ lineage-negative, Sca1-positive, c-Kit-positive cells (LSK) transplantation into sub-lethally irradiated mice, where an increase in lymphoid cell populations was observed 20 days post transplantation [45]. The change in differentiation capacity of stem cells is explained by an increased lymphoid stem cell gene expression program and enhanced expression of miR-23a targets including transcription factors *Runx1*, special AT-rich sequence-binding protein1 (*Satb1*) and *Btb* and *cnc* homologue 1 (*Bach1*) [45]. Together, these data suggest that miR-23a and its target *Runx1* play a pivotal role in the myeloid-lymphoid fate decisions in hematopoiesis.

The miR-23a-miR-27a-miR-24-2 cluster of miRNAs controls RUNX1 expression levels in human and murine hematopoietic cells during megakaryopoiesis (Fig. 3A) [46]. RUNX1 transcripts may have different isoforms containing 3'-UTRs of different sizes dependent on the usage of alternative polyadenylation signals [46]. However, miR-27a interaction sites are close to the translational stop and therefore miR-27a is capable to potentially regulate all RUNX1 isoforms [46]. Strikingly, RUNX1 itself induces the expression of miR-27a by direct binding to the promoter region of *MIR23a~MIR27a~MIR24-2* gene (Fig. 3A) [46]. The RUNX1 transcripts containing the long 3'-UTR sequence are less sensitive to miR-27a-mediated repression compared to the short 3'-UTR-containing transcript, most-likely due to structural changes of the RNA or by protection of RNA-binding proteins [46]. This allows for simultaneous upregulation of RUNX1 and miR-23a, miR-27a, miR-24-2 targeting other transcripts that need to be repressed during megakaryopoiesis. In addition to this cluster, it has now become

clear that RUNX1 controls the expression the *MIR144~451* cluster (Fig. 3B). This cluster is repressed by RUNX1 by its binding to the promoter and to lesser extent the enhancer region of this gene [47]. This regulation by RUNX1 has been confirmed in primary human CD34⁺ cells [47]. Besides a role in immature progenitors, the RUNX1-mediated repression of the *MIR144/451* cluster occurs during megakaryopoiesis [47]. The binding of RUNX1 to the *MIR144/451* promoter is accompanied by a decrease in histone activation marks, H3K9ac and H3K4me3 and an increase in repressive chromatin H3R2me2 mark. Silencing of *MIR144/451* in progenitor cells inhibits the miR-451-mediated erythropoiesis, thereby allowing megakaryopoiesis (Fig. 3B).

The expression of RUNX1 needs to be tightly regulated during myelopoiesis, which is at least in part controlled by RNA-binding proteins [48]. When CD34⁺ progenitor cells differentiate into monocytes and granulocytes upon *in vitro* stimulation with colony-stimulating factor (CSF) 1 or CSF3, several RNA-binding proteins are differentially expressed. From these, KH-type splicing regulatory protein (KSRP) is the most downregulated RNA-binding protein during monocytopoiesis and most upregulated during granulopoiesis [48]. KSRP interacts with the miRNA biogenesis components DGCR8 and DROSHA, thereby promoting the processing of miR-129 (Fig. 3C) [48]. When KSRP is induced, miR-129 is processed, targets RUNX1 and downregulates its translation. RUNX1 inhibits CSF3-receptor (CSF3R) expression. Therefore, miR-129-mediated downregulation of RUNX1 causes an upregulation of CSF3R and thereby promotes granulocytic differentiation [48]. This is different in monocytopoiesis, where there is no induction of KSRP and therefore no miR-129-mediated inhibition of RUNX1. RUNX1 stimulates the expression of the CSF1R, thereby promoting monocyte development [48]. Therefore, miR-129 is involved in monocytic and granulocytic lineage determination by RUNX1 through regulation by KSRP (Fig. 3C).

Studies in zebrafish show a very different relation between miR-142 and *runx1* in HSCs [49]. By using *runx1* and *cmyb* levels as a measure for HSC development, the authors show that a decrease in miR-142 expression correlates with a decreased *runx1* expression [49]. This downregulation of *runx1* is caused by interferon regulatory factor 7 (*irf7*), which is a direct target of miR-142 (Fig. 3D). The mechanism of RUNX1 regulation via miR-142-controlled *Irf7* was verified in mice [49]. Inhibiting miR-142 causes enhanced IRF7 levels, a decreased RUNX1 expression and a diminished colony forming capacity of murine c-Kit⁺CD34⁺ cells and splenocytes, while normal miR-142 levels are associated with normal RUNX1 expression and normal HSC numbers [46]. The binding of C/EBP β , PU.1 and RUNX1 to the *Mir142* promoter has been shown in macrophages (Fig. 3E) and an increase of miR-142 expression was observed compared to fibroblasts where only C/EBP β and RUNX1 bind to the promoter [50]. The expression of miR-142 was even higher in DCs where RUNX1 partner CBF β , in addition to the other transcription factors, was found to be enriched on the *Mir142* promoter (Fig. 3F) [50]. The transcription factor PU.1 appears to be critical for miR-142 expression and synergizes with RUNX1, C/EBP β , and CBF β [50]. Together, these findings suggest that miR-142 controls the expression of RUNX1 in HSCs. In macrophages and DC's RUNX1 fine-tunes the expression of miR-142.

In leukemia RUNX1-fusions are involved in silencing of miRNA expression. Translocation t(8;21)(q22;q22) involving RUNX1 and the ETO gene generates the RUNX1-ETO fusion oncoprotein. The RUNX1-ETO oncoprotein directly inhibits the expression of miR-9-1 (Fig. 3G), which is in concordance with the lack of miR-9-1 expression in clinical t(8;21)(q22;q22) AML [51]. This inhibition is mediated by five putative RUNX1 binding sites that are present in the regulatory region located 2 kb upstream of the *MIR9-1* gene (Fig. 3G). Luciferase reporter assays show that the RUNX1 binding

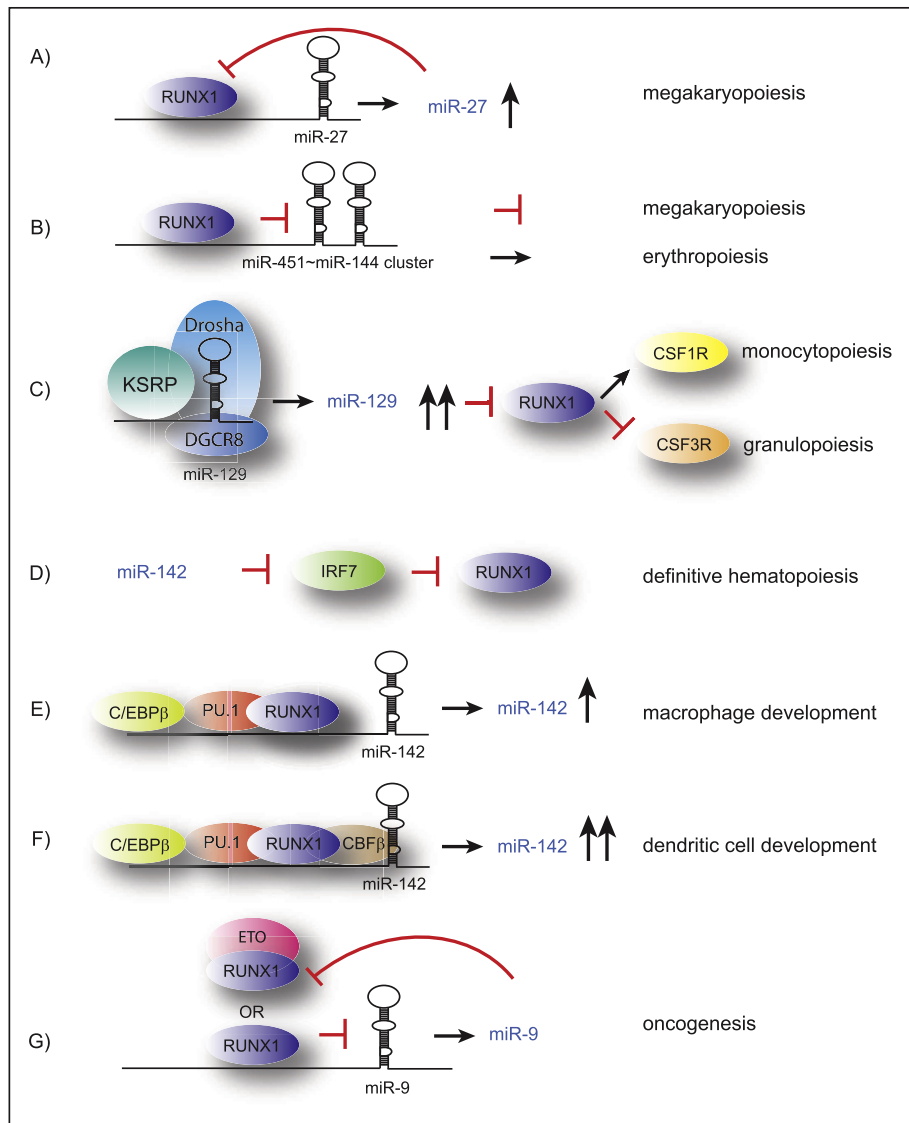


Fig. 3. RUNX1-controlled miRNA activities in different aspects of hematopoiesis. A) RUNX1 mediates the activation of miR-27. When miR-27 expression passes a certain threshold, this results in specific downregulation of short RUNX1 transcripts during megakaryopoiesis. B) RUNX1 inhibits the expression of miR-451~miR-144, thereby driving the switch between erythropoiesis towards megakaryopoiesis. C) The RNA-binding protein KSRP activate the processing of pre-miR-129. Enhanced miR-129 expression results in the downregulation of RUNX1 and the switch of monocytopoiesis towards granulopoiesis. D) Upregulation of miR-142 causes a down-regulation of IRF7, thereby inducing RUNX1 expression, which is critical for definitive hematopoiesis. E) C/EBPβ, PU.1, RUNX1-mediated upregulation of miR-142 is important for macrophage development. F) CBFβ enhance the C/EBPβ, PU.1, RUNX1-mediated expression of miR-142 and drives dendritic cell development. G) RUNX1 and the oncogenic fusion RUNX1-ETO are potent inhibitors of miR-9 expression, which is in turn an inhibitor of RUNX1-ETO. Inhibition of miR-9 is an oncogenic event in AML.

site in the region most proximal to the transcriptional start site is sufficient to silence the expression of miR-9-1 by recruiting epigenetic modulators such as histone deacetylase 1 (HDAC1) and DNA methyltransferase 1 (DNMT1), DNMT3b in t(8;21)(q22; q22) positive cells [51]. Interestingly, miR-9-1 directly targets *RUNX1*, *ETO*, *RUNX1-ETO* and other downstream targets that are overexpressed in t(8;21)(q22;q22) AML (Fig. 3G) [51]. It is clear that RUNX1-fusions repress the expression of their inhibitor miR-9. Therefore, miR-9 may be interesting as a potential drug in treatment of t(8;21)(q22;q22) AML.

5. KLF4 and miRNA regulation in myeloid cells

KLF4 plays an important role in myeloid differentiation at different stages: differentiation of monocytic cells from myeloid precursors [52,53] and polarization and activation of mature

mononuclear phagocytes into alternatively activated M2-like cells [54,55]. Related to KLF4 function and miRNA regulation in early precursor differentiation (Fig. 4A), most information stems from studies in leukemic cells. Profiling of miRNAs that are differentially expressed between M1 (AML with minimal differentiation) and M5 (acute monocytic leukemia) identified 12 miRNAs that delineated these two AML types [56]. Among these, miR-130a and miR-135b were shown to target KLF4. These miRNAs are down-regulated upon induction of differentiation in AML cell lines with vitamin D and phorbol-12-myristate-13-acetate (PMA), with a concomitant up-regulation of KLF4 expression. Interestingly, miR-130a also targets *IRF8*, encoding another transcription factor involved with myelomonocytic differentiation (see below). Mutations of the nucleolar protein NPM1 occur frequently in AML. Comparison of nucleophosmin 1 (NPM1)-mutated with NPM1-wt AML showed overexpression of miRNAs, including miR-10a in the former [57].

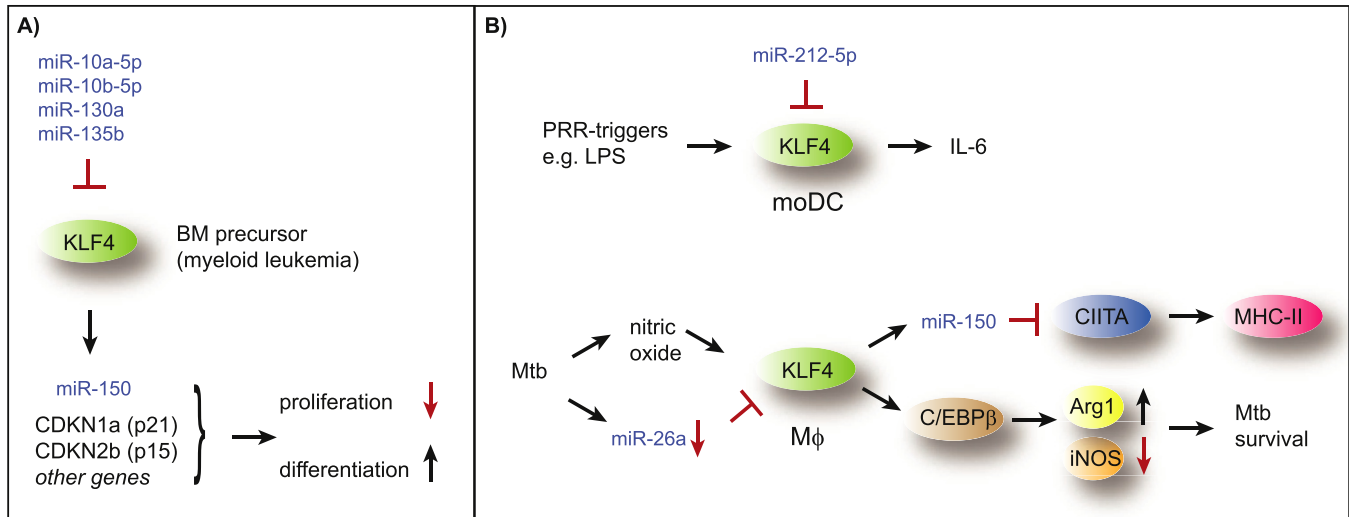


Fig. 4. MiRNAs involved with regulation of monocytic differentiation from precursors induced by KLF4 (A) and activation of mature monocyte-derived cells (B). The meaning of the signs are described in the legend of Fig. 2. A) In myeloid leukemia, miRNA-mediated inhibition of KLF4 enhances proliferation and represses differentiation of myeloid precursor cells. B) Activation of mature monocyte derived cells is inhibited by indicated miRNAs.

MiR-10a targets *KLF4*, and inhibition of miR-10a causes increased AML cell death. Similarly, miR-10b overexpression is also commonly observed in NPM1-mutated AML, causing KLF4 down-regulation and limiting myeloid differentiation [58].

Functional involvement of KLF4 with precursor cell differentiation includes induction of miR-150 expression (Fig. 4A) [59]. This stimulates myeloid differentiation and inhibits proliferation of AML cells [59]. Accordingly, the *MIR150* promoter contains KLF-family transcription factor binding sites. However, additional genes other than only *MIR150* are necessary to explain the KLF4 effects on myeloid differentiation as manipulating expression of miR-150 and the KLF4-target cyclin-dependent kinase inhibitor 1a (CDKN1a, p21) did not fully recapitulate the KLF4 effects. Besides inducing expression of CDKN1a, KLF4 also stimulates CDKN2b (p15) expression [2,60]. Up-regulation of these CDKNs upon KLF4 induction is a likely mechanism for effective cell cycle inhibition.

In mature monocyte-related cells, KLF4 drives polarization and activation into alternatively activated M2-like cells [55]. Interestingly, Kapoor et al. [54] showed that KLF4 promotes M2 polarization of macrophages by inducing MCP-1–induced protein (MCPiP). The transcriptional activator MCPiP, in turn, induces expression of C/EBPβ and peroxisome proliferator-activated receptor-γ (PPARγ), which are both transcription factors important for M2 polarization (recently reviewed in Ref. [61]). Fig. 4B shows the involvement of miRNAs in the regulation of KLF4 activity in mature cells. MiR-212-5p was found upregulated upon fungal infection in monocyte-derived DC and shown to inhibit KLF4 expression [62]. The same group showed before that KLF4 is important for IL-6 induction in fungal responses, and accordingly fungus-stimulated DC express significantly lower levels of IL-6 than DC stimulated by, for instance, lipopolysaccharide (LPS) [63].

Infection of macrophages with *Mycobacterium tuberculosis* causes down-regulation of miR-26a, which targets KLF4 [64]. This allows increased KLF4 expression and causes a shift of polarization from M1-to M2-like phenotype, as indicated by increased arginase 1 (ARG1) and decreased nitric oxide synthase 2A (iNOS) expression. This route of M2 polarization is further favored by simultaneously increased C/EBPβ signaling, probably mediated by KLF4 activity, as indicated above. *M. tuberculosis* infection also hampers major histocompatibility complex class II (MHCII) expression by macrophages. Ghorpade et al. showed that KLF4 expression is

induced in these cells by a nitric oxide-dependent mechanism [65]. Comparable to the situation in precursors, KLF4 induces expression of miR-150, which inhibits the MHCII transcriptional activator CIITA.

Several additional studies show KLF4 regulation by miRNAs in other mesenchymal cell types, such as vascular endothelial cells and smooth muscle cells [66–69]. Of these, miR-92a, miR-103, miR-143 and miR-145, amongst others, have been reported to be expressed also by myeloid cells, and therefore it is likely that these miRNAs have similar impact on KLF4 in myeloid function as well. Notably, miR-143 and miR-145 appear to be associated with M2 polarization [70], and thus possibly ameliorates KLF4 protein expression and function as negative feedback, but this remains to be shown.

6. IRF8 and miRNA regulation in myeloid cells

In addition to KLF4, IRF8 operates in myeloid differentiation at multiple stages [1]. It stimulates differentiation of monocytes from myeloid progenitors [53] and differentiation of subsets of dendritic cells [71], while inhibiting development of osteoclasts from myeloid precursors [72] and suppressing neutrophil production [73]. Furthermore, in line with its induction by interferon-gamma (IFN-γ), IRF8 is important in shaping macrophage and DC inflammatory phenotype and function [74,75]. IRF8 may heterodimerize with other IRF family members to bind interferon-stimulated response elements. In addition, IRF8 interaction with PU.1 appears to be essential for the production of IFN-β by monocytes [76].

At several levels, miRNA regulation of IRF8 has been demonstrated (Fig. 5). As described above, profiling of M1 and M5 subtypes of leukemia identified 12 differentially expressed miRNAs [56]. Among these, miR-130a and miR-181 were found to target IRF8. These miRNAs were down-regulated upon induction of differentiation in AML cell lines with vitamin D and PMA, with a concomitant up-regulation of IRF8 expression. Interestingly, miR-130a also targets KLF4, another transcription factor involved with myelomonocytic differentiation at this stage (see above).

Studying the effect of polymorphisms in miRNA-binding sites on leukemia risk, Dzikiewicz-Krawczyk et al. observed an increased Chronic Myeloid Leukemia (CML) risk associated with the IRF8rs_10514611 allelic variant [77]. The T allele of

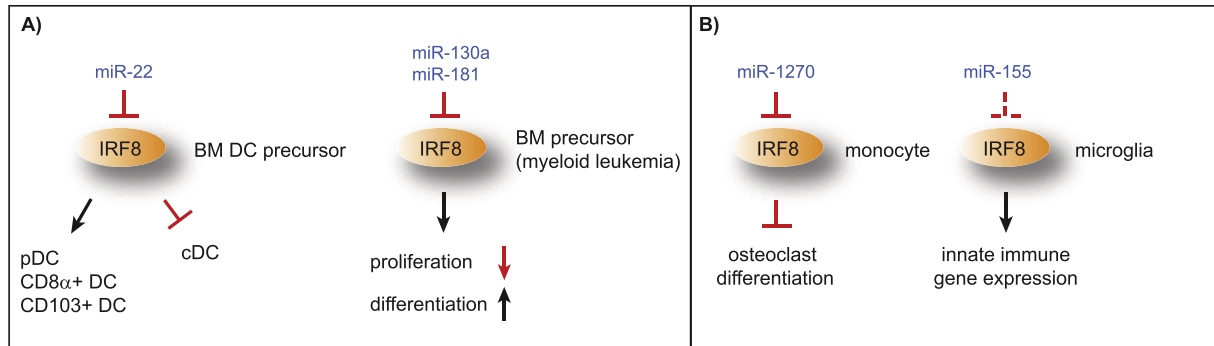


Fig. 5. MiRNAs involved with regulation of IRF8 in differentiation of (A) BM precursor cells and (B) mature osteoclasts and microglia. The meaning of the signs are described in the legend of Fig. 2. Dashed block signs means indirect repression. A) MiR-22 controls the expression of IRF8, which determines the differentiation of DCs. In myeloid leukemia, miR-130a and miR-181 aberrantly represses IRF8 in BM precursor cells resulting in an enhanced proliferation and reduced differentiation. B) In monocytes, miR-1270-mediated IRF8 mediates osteoclast differentiation. In microglia, miR-155-mediated repression IRF8 represses innate immune gene expression.

IRF8rs_10514611C>T was predicted to increase binding of miR-330-3p but luciferase experiments failed to confirm the presumed functional interaction.

Watowich and colleagues identified miR-22 as an important regulator of IRF8 protein expression, determining differentiation of mouse DC precursors into different subsets [78]. Overexpression of miR-22 mediated an enhanced generation of conventional DC (cDC) at the expense of IRF8-dependent plasmacytoid (pDC) development, while miR-22 knock-down caused the opposite effect. Not only does miR-22 influence differentiation of DC precursor cells into different subsets, we observed it also is induced in the final maturation step of monocyte-derived DC, where it targets CSF1R [79]. Down-regulation of this homeostatic growth factor receptor is required for these DC to achieve a phenotype associated with antigen-presenting capacity.

Comparing miRNA expression profiles in circulating monocytes of women with or without osteoporosis, Jimenez-Ortega et al. found miR-1270 over-expressed in these osteoclast precursor cells in osteoporosis [80]. MiR-1270 down-regulates expression of IRF8, which is known to inhibit osteoclastogenesis [72]. Therefore, increased expression of miR-1270 probably contributes to increased generation of osteoclasts. Furthermore, miR-155 was shown to decrease IRF8 expression in a human viral infection model of microglial cells [81]. Bioinformatic analysis predicts *IRF8* as one of the potential targets for miR-155, but testing of the 3'-UTR of *IRF8* in the luciferase reporter system for miRNA target validation did not confirm a direct interaction.

7. Concluding remarks

The regulatory role of miRNAs has been widely studied in hematopoiesis in the past decade. It has become evident that the interplay between lineage-specific transcription factors and miRNAs is critical for normal differentiation and function of myeloid cells. In this review we presented multiple examples of how miRNAs control cell lineage determination. In these examples miRNAs downregulate molecular programs that are active at different cellular states or in different cell types, implying their regulatory contribution at multiple developmental levels. Deregulations of miRNA activities are frequently seen in different types of leukemia, underscoring the importance of proper miRNA regulation for normal hematopoietic cell behavior. Although the molecular networks presented in this review are just a tip of the iceberg of the complex mechanisms of myeloid differentiation and function, it is clear that myeloid lineage-specific transcription factors fully rely on miRNA activities to drive normal myeloid cell development.

The networks controlled by miRNAs at the different stages of myeloid differentiation are still largely elusive and the identification of essential miRNA target genes is technically challenging. Although conventional miRNA function relies on the reverse complementary interaction of miRNAs in the seed region with 3'-UTRs of mRNAs, increasing data show that the non-seed region plays a role in the non-canonical functions of miRNAs [82–84]. Several experimental observations discussed in this review indicate that mature miRNAs are also localized and active in the nucleus and may change the epigenetic status of myeloid progenitor cells. In addition, the RNA binding proteins controlling canonical miRNA activities and non-canonical nuclear localization and epigenetic functions are mostly unidentified. Furthermore, it is becoming more evident that long-non-coding RNAs are important mediators of miRNA activity and cell type-specific gene expression. These observations add even a new level of complexity to the regulatory functions of transcription factors and miRNAs during myeloid cell development. Understanding the complicated networks involved in myeloid differentiation may open new avenues for therapeutic intervention for the treatment of AML.

Conflicts of interest

The authors declare no conflict of interests.

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References

- [1] A. Yanez, H.S. Goodridge, Interferon regulatory factor 8 and the regulation of neutrophil, monocyte, and dendritic cell production, *Curr. Opin. Hematol.* 23 (2016) 11–17.
- [2] A.M. Ghaleb, V.W. Yang, Kruppel-like factor 4 (KLF4): what we currently know, *Gene* 611 (2017) 27–37.
- [3] M. de Bruijn, E. Dzierzak, Runx transcription factors in the development and function of the definitive hematopoietic system, *Blood* 129 (2017) 2061–2069.
- [4] A.D. Friedman, Transcriptional regulation of granulocyte and monocyte development, *Oncogene* 21 (2002) 3377–3390.
- [5] D.E. Zhang, P. Zhang, N.D. Wang, C.J. Hetherington, G.J. Darlington, D.G. Tenen, Absence of granulocyte colony-stimulating factor signaling and neutrophil development in CCAAT enhancer binding protein alpha-deficient mice, *Proc. Natl. Acad. Sci. U. S. A.* 94 (1997) 569–574.
- [6] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, *Cell* 116 (2004) 281–297.
- [7] S. Guo, J. Lu, R. Schlanger, H. Zhang, J.Y. Wang, M.C. Fox, L.E. Purton, H.H. Fleming, B. Cobb, M. Merckenschlager, T.R. Golub, D.T. Scadden, MicroRNA miR-125a controls hematopoietic stem cell number, *Proc. Natl. Acad. Sci. U. S. A.*

- A. 107 (2010) 14229–14234.
- [8] E. Leucci, F. Patella, J. Waage, K. Holmstrom, M. Lindow, B. Porse, S. Kauppinen, A.H. Lund, microRNA-9 targets the long non-coding RNA MALAT1 for degradation in the nucleus, *Sci. Rep.* 3 (2013) 2535.
 - [9] G. Zardo, A. Ciolfi, L. Vian, L.M. Starnes, M. Billi, S. Racanicchi, C. Maresca, F. Fazi, L. Travaglini, N. Noguera, M. Mancini, M. Nanni, G. Cimino, F. Lo-Coco, F. Grignani, C. Nervi, Polycombs and microRNA-223 regulate human granulopoiesis by transcriptional control of target gene expression, *Blood* 119 (2012) 4034–4046.
 - [10] A.D. Friedman, C/EBPalpha in normal and malignant myelopoiesis, *Int. J. Hematol.* 101 (2015) 330–341.
 - [11] B.T. Porse, T.A. Pedersen, X. Xu, B. Lindberg, U.M. Wewer, L. Friis-Hansen, C. Nerlov, E2F repression by C/EBPalpha is required for adipogenesis and granulopoiesis in vivo, *Cell* 107 (2001) 247–258.
 - [12] P. Zhang, J. Iwasaki-Arai, H. Iwasaki, M.L. Fenys, T. Dayaram, B.M. Owens, H. Shigematsu, E. Levantini, C.S. Huettner, J.A. Lektrom-Himes, K. Akashi, D.G. Tenen, Enhancement of hematopoietic stem cell repopulating capacity and self-renewal in the absence of the transcription factor C/EBP alpha, *Immunity* 21 (2004) 853–863.
 - [13] S. Koschmieder, B. Halmos, E. Levantini, D.G. Tenen, Dysregulation of the C/EBPalpha differentiation pathway in human cancer, *J. Clin. Oncol.* 27 (2009) 619–628.
 - [14] M. Jongen-Lavrencic, S.M. Sun, M.K. Dijkstra, P.J. Valk, B. Lowenberg, MicroRNA expression profiling in relation to the genetic heterogeneity of acute myeloid leukemia, *Blood* 111 (2008) 5078–5085.
 - [15] M.F. Alemdehy, N.G. van Bortel, H.W. de Looper, I.J. van den Berge, M.A. Sanders, T. Cupedo, I.P. Touw, S.J. Erkeland, Dicer1 deletion in myeloid-committed progenitors causes neutrophil dysplasia and blocks macrophage/dendritic cell development in mice, *Blood* 119 (2012) 4723–4730.
 - [16] F. Fazi, A. Rosa, A. Fatica, V. Gelmetti, M.L. De Marchis, C. Nervi, I. Bozzoni, A minicircuitry comprised of microRNA-223 and transcription factors NF1-A and C/EBPalpha regulates human granulopoiesis, *Cell* 123 (2005) 819–831.
 - [17] L.M. Starnes, A. Sorrentino, E. Pelosi, M. Ballarino, O. Morsilli, M. Biffoni, S. Santoro, N. Felli, G. Castelli, M.L. De Marchis, G. Mastroberardino, M. Gabbianelli, A. Fatica, I. Bozzoni, C. Nervi, C. Peschle, NF1-A directs the fate of hematopoietic progenitors to the erythroid or granulocytic lineage and controls beta-globin and G-CSF receptor expression, *Blood* 114 (2009) 1753–1763.
 - [18] L. Vian, M. Di Carlo, E. Pelosi, F. Fazi, S. Santoro, A.M. Cerio, A. Boe, V. Rotilio, M. Billi, S. Racanicchi, U. Testa, F. Grignani, C. Nervi, Transcriptional fine-tuning of microRNA-223 levels directs lineage choice of human hematopoietic progenitors, *Cell Death Differ.* 21 (2014) 290–301.
 - [19] J.B. Johnnidis, M.H. Harris, R.T. Wheeler, S. Stehling-Sun, M.H. Lam, O. Kirak, T.R. Brummelkamp, M.D. Fleming, F.D. Camargo, Regulation of progenitor cell proliferation and granulocyte function by microRNA-223, *Nature* 451 (2008) 1125–1129.
 - [20] J.A. Pulikkan, V. Dengler, P.S. Peramangalam, A.A. Peer Zada, C. Muller-Tidow, S.K. Bohlander, D.G. Tenen, G. Behre, Cell-cycle regulator E2F1 and microRNA-223 comprise an autoregulatory negative feedback loop in acute myeloid leukemia, *Blood* 115 (2010) 1768–1778.
 - [21] A.A. Wurm, P. Zjablovskaja, M. Kardosova, D. Gerloff, D. Brauer-Hartmann, C. Katzerke, J.U. Hartmann, T. Benoukraf, S. Fricke, N. Hilger, A.M. Muller, M. Billi, S. Schwind, D.G. Tenen, D. Niederwieser, M. Alberich-Jorda, G. Behre, Disruption of the C/EBPalpha-miR-182 balance impairs granulocytic differentiation, *Nat. Commun.* 8 (2017) 46.
 - [22] J.A. Pulikkan, P.S. Peramangalam, V. Dengler, P.A. Ho, C. Preudhomme, S. Meshinchi, M. Christopheit, O. Nibourel, S.K. Bohlander, D.G. Tenen, G. Behre, C/EBPalpha regulated microRNA-34a targets E2F3 during granulopoiesis and is down-regulated in AML with CEBPA mutations, *Blood* 116 (2010) 5638–5649.
 - [23] B. Hackanson, K.L. Bennett, R.M. Brena, J. Jiang, R. Claus, S.S. Chen, N. Blagitko-Dorfs, K. Maharry, S.P. Whitman, T.D. Schmittgen, M. Lubbert, G. Marcucci, C.D. Bloomfield, C. Plass, Epigenetic modification of CCAAT/enhancer binding protein alpha expression in acute myeloid leukemia, *Canc. Res.* 68 (2008) 3142–3151.
 - [24] X.X. Chen, J. Lin, J. Qian, W. Qian, J. Yang, J.C. Ma, Z.Q. Deng, D. Xie, C. An, C.Y. Tang, Z. Qian, Dysregulation of miR-124-1 predicts favorable prognosis in acute myeloid leukemia, *Clin. Biochem.* 47 (2014) 63–66.
 - [25] I. Vazquez, M. Maicas, N. Marcotegui, A. Conchillo, E. Guruceaga, J. Roman-Gomez, M.J. Calasanz, X. Agirre, F. Prosper, M.D. Otero, Silencing of hsa-miR-124 by EVI1 in cell lines and patients with acute myeloid leukemia, *Proc. Natl. Acad. Sci. U. S. A.* 107 (2010) E167–E168 author reply E169–E170.
 - [26] E.D. Ponomarev, T. Veremeyko, N. Barteneva, A.M. Krichevsky, H.L. Weiner, MicroRNA-124 promotes microglia quiescence and suppresses EAE by deactivating macrophages via the C/EBP-alpha-PU.1 pathway, *Nat. Med.* 17 (2011) 64–70.
 - [27] Y. Enomoto, J. Kitaura, K. Hatakeyama, Y. Watanuki, T. Akasaka, N. Kato, M. Shimanuki, K. Nishimura, M. Takahashi, M. Taniwaki, C. Haferlach, R. Siebert, M.J. Dyer, N. Asou, H. Aburatani, H. Nakakuma, T. Kitamura, T. Sonoki, Emu/miR-125b transgenic mice develop lethal B-cell malignancies, *Leukemia* 25 (2011) 1849–1856.
 - [28] M.F. Alemdehy, J.R. Haanstra, H.W. de Looper, P.M. van Strien, J. Verhagen-Oldenampsen, Y. Caljouw, M.A. Sanders, R. Hoogenboezem, A.H. de Ru, G.M. Janssen, S.E. Smetsers, M.B. Bierings, P.A. van Veelen, M. von Lindern, I.P. Touw, S.J. Erkeland, ICL-induced miR139-3p and miR199a-3p have opposite roles in hematopoietic cell expansion and leukemic transformation, *Blood* 125 (2015) 3937–3948.
 - [29] M.F. Alemdehy, H.W. de Looper, F.G. Kavelaars, M.A. Sanders, R. Hoogenboezem, B. Lowenberg, P.J. Valk, I.P. Touw, S.J. Erkeland, MicroRNA-155 induces AML in combination with the loss of C/EBPA in mice, *Leukemia* 30 (2016) 2238–2241.
 - [30] H.S. Radomska, D.S. Basseres, R. Zheng, P. Zhang, T. Dayaram, Y. Yamamoto, D.W. Sternberg, N. Lokker, N.A. Giese, S.K. Bohlander, S. Schnittger, M.H. Delmotte, R.J. Davis, D. Small, W. Hiddemann, D.G. Gilliland, D.G. Tenen, Block of C/EBP alpha function by phosphorylation in acute myeloid leukemia with FLT3 activating mutations, *J. Exp. Med.* 203 (2006) 371–381.
 - [31] R. Zheng, A.D. Friedman, M. Levis, L. Li, E.G. Weir, D. Small, Internal tandem duplication mutation of FLT3 blocks myeloid differentiation through suppression of C/EBPalpha expression, *Blood* 103 (2004) 1883–1890.
 - [32] D. Lu, R. Nakagawa, S. Lazzaro, P. Staudacher, C. Abreu-Goodger, T. Henley, S. Boiani, R. Leyland, A. Galloway, S. Andrews, G. Butcher, S.L. Nutt, M. Turner, E. Vigorito, The miR-155-PU.1 axis acts on Pax5 to enable efficient terminal B cell differentiation, *J. Exp. Med.* 211 (2014) 2183–2198.
 - [33] M.J. Klemm, S.R. McKercher, A. Celada, C. Van Beveren, R.A. Maki, The macrophage and B cell-specific transcription factor PU.1 is related to the ets oncogene, *Cell* 61 (1990) 113–124.
 - [34] M.F. Alemdehy, S.J. Erkeland, MicroRNAs: key players of normal and malignant myelopoiesis, *Curr. Opin. Hematol.* 19 (2012) 261–267.
 - [35] C. Shen, M.T. Chen, X.H. Zhang, X.L. Yin, H.M. Ning, R. Su, H.S. Lin, L. Song, F. Wang, Y.N. Ma, H.L. Zhao, J. Yu, J.W. Zhang, The PU.1-modulated MicroRNA-22 is a regulator of monocyte/macrophage differentiation and acute myeloid leukemia, *PLoS Genet.* 12 (2016), e1006259.
 - [36] P. Basova, V. Pospisil, F. Savvulidi, P. Burda, K. Vargova, L. Stanek, M. Dluhosova, E. Kuzmova, A. Jonasova, U. Steidl, P. Laslo, T. Stopka, Aggressive acute myeloid leukemia in PU.1/p53 double-mutant mice, *Oncogene* 33 (2014) 4735–4745.
 - [37] S. Grassilli, E. Nika, E. Lambertini, F. Brugnoli, R. Piva, S. Capitani, V. Bertagnolo, A network including PU.1, Vav1 and miR-142-3p sustains ATRA-induced differentiation of acute promyelocytic leukemia cells - a short report, *Cell. Oncol.* 39 (2016) 483–489.
 - [38] C.L. Sweeney, R. Teng, H. Wang, R.K. Merling, J. Lee, U. Choi, S. Koontz, D.G. Wright, H.L. Malech, Molecular analysis of neutrophil differentiation from human induced pluripotent stem cells delineates the kinetics of key regulators of hematopoiesis, *Stem Cell.* 34 (2016) 1513–1526.
 - [39] L.A. Solomon, S. Podder, J. He, N.L. Jackson-Choronenki, K. Gibson, R.G. Ziliotto, J. Rhee, R.P. DeKoter, Coordination of myeloid differentiation with reduced cell cycle progression by PU.1 induction of MicroRNAs targeting cell cycle regulators and lipid anabolism, *Mol. Cell Biol.* 37 (2017).
 - [40] M.T. Chen, H.S. Lin, C. Shen, Y.N. Ma, F. Wang, H.L. Zhao, J. Yu, J.W. Zhang, PU.1-Regulated long noncoding RNA Inc-MC controls human monocyte/macrophage differentiation through interaction with MicroRNA 199a-5p, *Mol. Cell Biol.* 35 (2015) 3212–3224.
 - [41] H.S. Lin, J.N. Gong, R. Su, M.T. Chen, L. Song, C. Shen, F. Wang, Y.N. Ma, H.L. Zhao, J. Yu, W.W. Li, L.X. Huang, X.H. Xu, J.W. Zhang, miR-199a-5p inhibits monocyte/macrophage differentiation by targeting the activin A type 1B receptor gene and finally reducing C/EBPalpha expression, *J. Leukoc. Biol.* 96 (2014) 1023–1035.
 - [42] Q. Wang, T. Stacy, M. Binder, M. Marin-Padilla, A.H. Sharpe, N.A. Speck, Disruption of the Cbfa2 gene causes necrosis and hemorrhaging in the central nervous system and blocks definitive hematopoiesis, *Proc. Natl. Acad. Sci. U. S. A.* 93 (1996) 3444–3449.
 - [43] R. Sood, Y. Kamikubo, P. Liu, Role of RUNX1 in hematological malignancies, *Blood* 129 (2017) 2070–2082.
 - [44] S. Rossetti, N. Sacchi, RUNX1: a microRNA hub in normal and malignant hematopoiesis, *Int. J. Mol. Sci.* 14 (2013) 1566–1588.
 - [45] J.L. Kurkewich, J. Hansen, N. Klopfenstein, H. Zhang, C. Wood, A. Boucher, J. Hickman, D.E. Muench, H.L. Grimes, R. Dahl, The miR-23a-27a-24-2 microRNA cluster buffers transcription and signaling pathways during hematopoiesis, *PLoS Genet.* 13 (2017), e1006887.
 - [46] O. Ben-Ami, N. Pencovich, J. Lotem, D. Levanon, Y. Groner, A regulatory interplay between miR-27a and Runx1 during megakaryopoiesis, *Proc. Natl. Acad. Sci. U. S. A.* 106 (2009) 238–243.
 - [47] N. Kohrs, S. Kolodziej, O.N. Kuvardina, J. Herglotz, J. Yillah, S. Herkt, A. Piechatek, G. Salinas Riester, T. Lingner, C. Wichmann, H. Bonig, E. Seifried, U. Platzbecker, H. Medyouf, M. Grez, J. Lausen, MiR144/451 expression is repressed by RUNX1 during megakaryopoiesis and disturbed by RUNX1/ETO, *PLoS Genet.* 12 (2016), e1005946.
 - [48] H. Zhao, X. Wang, P. Yi, Y. Si, P. Tan, J. He, S. Yu, Y. Ren, Y. Ma, J. Zhang, D. Wang, F. Wang, J. Yu, KSRP specifies monocytic and granulocytic differentiation through regulating miR-129 biogenesis and RUNX1 expression, *Nat. Commun.* 8 (2017) 1428.
 - [49] X. Lu, X. Li, Q. He, J. Gao, Y. Gao, B. Liu, F. Liu, miR-142-3p regulates the formation and differentiation of hematopoietic stem cells in vertebrates, *Cell Res.* 23 (2013) 1356–1368.
 - [50] Y. Sun, J. Sun, T. Tomomi, E. Nieves, N. Mathewson, H. Tamaki, R. Evers, P. Reddy, PU.1-dependent transcriptional regulation of miR-142 contributes to its hematopoietic cell-specific expression and modulation of IL-6, *J. Immunol.* 190 (2013) 4005–4013.
 - [51] L. Fu, J. Shi, A. Liu, L. Zhou, M. Jiang, H. Fu, K. Xu, D. Li, A. Deng, Q. Zhang, Y. Pang, Y. Guo, K. Hu, J. Zhou, Y. Wang, W. Huang, Y. Jing, L. Dou, L. Wang,

- K. Xu, X. Ke, C. Nervi, Y. Li, L. Yu, A minicircuitry of microRNA-9-1 and RUNX1-RUNX1T1 contributes to leukemogenesis in t(8;21) acute myeloid leukemia, *Int. J. Canc.* 140 (2017) 653–661.
- [52] M.W. Feinberg, A.K. Wara, Z. Cao, M.A. Lebedeva, F. Rosenbauer, H. Iwasaki, H. Hirai, J.P. Katz, R.L. Haspel, S. Gray, K. Akashi, J. Segre, K.H. Kaestner, D.G. Tenen, M.K. Jain, The Kruppel-like factor KLF4 is a critical regulator of monocyte differentiation, *EMBO J.* 26 (2007) 4138–4148.
- [53] O. Ma, S. Hong, H. Guo, G. Ghiaur, A.D. Friedman, Granulopoiesis requires increased C/EBPalpha compared to monopoiesis, correlated with elevated Cebpa in immature G-CSF receptor versus M-CSF receptor expressing cells, *PLoS One* 9 (2014), e95784.
- [54] N. Kapoor, J. Niu, Y. Saad, S. Kumar, T. Sirakova, E. Becerra, X. Li, P.E. Kolattukudy, Transcription factors STAT6 and KLF4 implement macrophage polarization via the dual catalytic powers of MCP1P, *J. Immunol.* 194 (2015) 6011–6023.
- [55] X. Liao, N. Sharma, F. Kapadia, G. Zhou, Y. Lu, H. Hong, K. Paruchuri, G.H. Mahabeshwar, E. Dalmás, N. Venteclef, C.A. Flask, J. Kim, B.W. Doreian, K.Q. Lu, K.H. Kaestner, A. Hamik, K. Clement, M.K. Jain, Kruppel-like factor 4 regulates macrophage polarization, *J. Clin. Invest.* 121 (2011) 2736–2749.
- [56] M. Lutherborrow, A. Bryant, V. Jayaswal, D. Agapiou, C. Palma, Y.H. Yang, D.D. Ma, Expression profiling of cytogenetically normal acute myeloid leukemia identifies microRNAs that target genes involved in monocytic differentiation, *Am. J. Hematol.* 86 (2011) 2–11.
- [57] A. Bryant, C.A. Palma, V. Jayaswal, Y.W. Yang, M. Lutherborrow, D.D. Ma, miR-10a is aberrantly overexpressed in Nucleophosmin1 mutated acute myeloid leukaemia and its suppression induces cell death, *Mol. Canc.* 11 (2012) 8.
- [58] Q. Zou, S. Tan, Z. Yang, J. Wang, J. Xian, S. Zhang, H. Jin, L. Yang, L. Wang, L. Zhang, The human nucleophosmin 1 mutation A inhibits myeloid differentiation of leukemia cells by modulating miR-10b, *Oncotarget* 7 (2016) 71477–71490.
- [59] V.A. Morris, C.L. Cummings, B. Korb, S. Boaglio, V.G. Oehler, Deregulated KLF4 expression in myeloid leukemias alters cell proliferation and differentiation through MicroRNA and gene targets, *Mol. Cell Biol.* 36 (2015) 559–573.
- [60] G.A. Bjerke, C. Hyman-Walsh, D. Wotton, Cooperative transcriptional activation by Klf4, Meis2, and Pbx1, *Mol. Cell Biol.* 31 (2011) 3723–3733.
- [61] C. Li, M.M. Xu, K. Wang, A.J. Adler, A.T. Vella, B. Zhou, Macrophage polarization and meta-inflammation, *Transl. Res.* 191 (2018) 29–44.
- [62] A. Dix, K. Czakai, I. Leonhardt, K. Schaferhoff, M. Bonin, R. Guthke, H. Einsele, O. Kurzai, J. Löffler, J. Linde, Specific and novel microRNAs are regulated as response to fungal infection in human dendritic cells, *Front. Microbiol.* 8 (2017) 270.
- [63] K. Czakai, I. Leonhardt, A. Dix, M. Bonin, J. Linde, H. Einsele, O. Kurzai, J. Loeffler, Kruppel-like Factor 4 modulates interleukin-6 release in human dendritic cells after in vitro stimulation with *Aspergillus fumigatus* and *Candida albicans*, *Sci. Rep.* 6 (2016) 27990.
- [64] S.K. Sahu, M. Kumar, S. Chakraborty, S.K. Banerjee, R. Kumar, P. Gupta, K. Jana, U.D. Gupta, Z. Ghosh, M. Kundu, J. Basu, MicroRNA 26a (miR-26a)/KLF4 and CREB-C/EBPbeta regulate innate immune signaling, the polarization of macrophages and the trafficking of *Mycobacterium tuberculosis* to lysosomes during infection, *PLoS Pathog.* 13 (2017), e1006410.
- [65] D.S. Ghorpade, S. Holla, A.Y. Sinha, S.K. Alagesan, K.N. Balaji, Nitric oxide and KLF4 protein epigenetically modify class II transactivator to repress major histocompatibility complex II expression during *Mycobacterium bovis* bacillus Calmette-Guerin infection, *J. Biol. Chem.* 288 (2013) 20592–20606.
- [66] Y. Fang, P.F. Davies, Site-specific microRNA-92a regulation of Kruppel-like factors 4 and 2 in athero susceptible endothelium, *Arterioscler. Thromb. Vasc. Biol.* 32 (2012) 979–987.
- [67] B.N. Davis-Dusenbery, M.C. Chan, K.E. Reno, A.S. Weisman, M.D. Layne, G. Lagna, A. Hata, Down-regulation of Kruppel-like factor-4 (KLF4) by microRNA-143/145 is critical for modulation of vascular smooth muscle cell phenotype by transforming growth factor-beta and bone morphogenetic protein 4, *J. Biol. Chem.* 286 (2011) 28097–28110.
- [68] P. Hartmann, Z. Zhou, L. Ntarelli, Y. Wei, M. Nazari-Jahantigh, M. Zhu, J. Grommes, S. Steffens, C. Weber, A. Schober, Endothelial Dicer promotes atherosclerosis and vascular inflammation by miRNA-103-mediated suppression of KLF4, *Nat. Commun.* 7 (2016) 10521.
- [69] F. Lovren, Y. Pan, A. Quan, K.K. Singh, P.C. Shukla, N. Gupta, B.M. Steer, A.J. Ingram, M. Gupta, M. Al-Omran, H. Teoh, P.A. Marsden, S. Verma, MicroRNA-145 targeted therapy reduces atherosclerosis, *Circulation* 126 (2012) S81–S90.
- [70] Y. Zhang, M. Zhang, M. Zhong, Q. Suo, K. Lv, Expression profiles of miRNAs in polarized macrophages, *Int. J. Mol. Med.* 31 (2013) 797–802.
- [71] F. Ginhoux, K. Liu, J. Helft, M. Bogunovic, M. Greter, D. Hashimoto, J. Price, N. Yin, J. Bromberg, S.A. Lira, E.R. Stanley, M. Nussenzweig, M. Merad, The origin and development of nonlymphoid tissue CD103+ DCs, *J. Exp. Med.* 206 (2009) 3115–3130.
- [72] B. Zhao, M. Takami, A. Yamada, X. Wang, T. Koga, X. Hu, T. Tamura, K. Ozato, Y. Choi, L.B. Ivashkiv, H. Takayanagi, R. Kamijo, Interferon regulatory factor-8 regulates bone metabolism by suppressing osteoclastogenesis, *Nat. Med.* 15 (2009) 1066–1071.
- [73] H. Tsujimura, T. Nagamura-Inoue, T. Tamura, K. Ozato, IFN consensus sequence binding protein/IFN regulatory factor-8 guides bone marrow progenitor cells toward the macrophage lineage, *J. Immunol.* 169 (2002) 1261–1269.
- [74] P. Tailor, T. Tamura, H.J. Kong, T. Kubota, M. Kubota, P. Borghi, L. Gabriele, K. Ozato, The feedback phase of type I interferon induction in dendritic cells requires interferon regulatory factor 8, *Immunity* 27 (2007) 228–239.
- [75] H. Xu, J. Zhu, S. Smith, J. Foldi, B. Zhao, A.Y. Chung, H. Outtz, J. Kitajewski, C. Shi, S. Weber, P. Saftig, Y. Li, K. Ozato, C.P. Blobel, L.B. Ivashkiv, X. Hu, Notch-RBP-J signaling regulates the transcription factor IRF8 to promote inflammatory macrophage polarization, *Nat. Immunol.* 13 (2012) 642–650.
- [76] P. Li, J.J. Wong, C. Sum, W.X. Sin, K.Q. Ng, M.B. Koh, K.C. Chin, IRF8 and IRF3 cooperatively regulate rapid interferon-beta induction in human blood monocytes, *Blood* 117 (2011) 2847–2854.
- [77] A. Dzikiewicz-Krawczyk, A. Maciejka, E. Maly, D. Januszkiewicz-Lewandowska, M. Mosor, M. Fichna, E. Strauss, J. Nowak, Polymorphisms in microRNA target sites modulate risk of lymphoblastic and myeloid leukemias and affect microRNA binding, *J. Hematol. Oncol.* 7 (2014) 43.
- [78] H.S. Li, N. Greeley, N. Sugimoto, Y.J. Liu, S.S. Watowich, miR-22 controls Irf8 mRNA abundance and murine dendritic cell development, *PLoS One* 7 (2012), e52341.
- [79] J. Riepsaame, A. van Oudenaren, B.J. den Broeder, W.F. van Ijcken, J. Pothof, P.J. Leenen, MicroRNA-mediated down-regulation of m-CSF receptor contributes to maturation of mouse monocyte-derived dendritic cells, *Front. Immunol.* 4 (2013) 353.
- [80] R.F. Jimenez-Ortega, E.G. Ramirez-Salazar, A.Y. Parra-Torres, S.A. Munoz-Montero, C. Rangel-Escareno, I. Salido-Guadarrama, M. Rodriguez-Dorantes, M. Quiterio, J. Salmeron, R. Velazquez-Cruz, Identification of microRNAs in human circulating monocytes of postmenopausal osteoporotic Mexican-Mestizo women: a pilot study, *Exp Ther Med* 14 (2017) 5464–5472.
- [81] S. Pareek, S. Roy, B. Kumari, P. Jain, A. Banerjee, S. Vrat, MiR-155 induction in microglial cells suppresses Japanese encephalitis virus replication and negatively modulates innate immune responses, *J. Neuroinflammation* 11 (2014) 97.
- [82] N. Cloonan, Re-thinking miRNA-mRNA interactions: intertwining issues confound target discovery, *Bioessays* 37 (2015) 379–388.
- [83] L. Miao, H. Yao, C. Li, M. Pu, X. Yao, H. Yang, X. Qi, J. Ren, Y. Wang, A dual inhibition: microRNA-552 suppresses both transcription and translation of cytochrome P450 2E1, *Biochim. Biophys. Acta* 1859 (2016) 650–662.
- [84] M. Pu, C. Li, X. Qi, J. Chen, Y. Wang, L. Gao, L. Miao, J. Ren, MiR-1254 suppresses HO-1 expression through seed region-dependent silencing and non-seed interaction with TFAP2A transcript to attenuate NSCLC growth, *PLoS Genet.* 13 (2017), e1006896.