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REVIEW

MicroRNAs in erythropoiesis and red blood cell disorders

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Abstract **MicroRNAs (miRNAs) are 19-24 nucleotide non-coding ribonucleic acids binding DNA or RNA and controlling gene expression via mRNA degradation or its transcription inhibition. Erythropoies is a multi step differentiation process of erythroid progenitors to nucleate red blood cells. Maturation, proliferation and differentiation of red blood cells is affected by erythroid factors, signaling pathways in niche of hematopoietic cells, transcription factors as well as miRNAs. Expression of different types of miRNAs during erythroid development provides a background for the study of these molecules to control erythroid differentiation and maturation as well as their use as diagnostic and prognostic markers to treat erythroid disorders like thalassemia, sickle cell disease and erythrocyte enzyme deficiencies. In this paper, with reference to biosynthesis of miRNAs, their function in normal and anemic erythropoiesis has been investigated. The target molecule of each of these miRNAs has been cited in an attempt to elucidate their role in erythropoiesis.**

Keywords miRNA, erythropoiesis, red blood cell

Introduction

Erythropoiesis is the differentiation process of hematopoietic stem cells (HSCs) to erythroid lineage, including several stages such as burst forming unit-erythroid (BFU-E), colony forming unit-erythroid (CFU-E) and erythroid precursor cells, which is regulated and controlled by several intrinsic and extrinsic signals (Cantor and Orkin, 2002; Kim and Bresnick, 2007). At different stages of erythropoiesis, several factors participate, including erythropoietin (EPO), EPO receptor (EpoR), GATA-1, STAT-5, BCL-xl, Bnip3L (also called Nix), c-Myb and microRNAs (miRNAs) (Leonard et al.,1993; Silva et al., 1999; Aerbajinai et al., 2003; Bruchova et al., 2009).

miRNAs are a group of small, 19–24 nucleotide non-coding RNAs that regulate gene expression by degradation or translation inhibition of target mRNAs (Valencia-Sanchez et al., 2006; Bracht et al., 2010). These molecules usually affect the 3′untranslated region (UTR) of target mRNA, and induce decreased or suppressed mRNA expression (Fig. 1) (Fabbri et al., 2008; Yang and Lai, 2011; Kandhavelu and Kandhavelu, 2012). In addition, several miRNAs are involved in such processes as development, apoptosis, metabolism of fats and sugars, cell differentiation and proliferation, hematopoiesis, chromatin structure and remodeling, tumorigenesis, cancer and viral resistance (Aalto and Pasquinelli, 2012; Guerau-de-Arellano et al., 2012; Karius et al., 2012; Saki et al., 2015). Several miRNAs participate in regulation of different stages of erythropoiesis including proliferation, differentiation and maturation. A set of miRNAs have been shown to affect erythroid cultures of cord blood *in vitro*, including miR-32, miR-136 and miR-137 that affect early erythroid commitment as well as miR-22, miR-28 and miR-185, which have an impact on maturation (Choong et al., 2007). Increased expression of miR-16 and miR-451 as well as reduced

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expression of miR-150, miR-155, miR-221 and miR-222 is associated with late stages of erythroid differentiation, high levels of miR-339 and miR-378 is related with the intermediate stage of erythropoiesis and miR-451 may be considered as an erythroid specific miRNA (Bruchova et al., 2007; Hattangadi et al., 2011). During normal erythropoiesis, expression of miR-150, miR-155, miR-221 and miR-222 is decreased and that of miR-451 (which is erythroid specific) and miR-16 (in reticulocytes) increased, while miR-339 and miR-378 have a biphasic pattern of expression (Vasilatou et al., 2010). It has been observed that miRNAs modify erythropoiesis by affecting transcription factors or surface antigens required for erythropoiesis (Felli et al., 2005). Also these agents regulate hemoglobin biosynthesis by increasing and decreasing globin chains, and result in iron hemostasis, increased resistance to oxidative stress and antioxidants in red cells via expression of several genes (Chen et al., 2008). In this review, the important role of miRNAs in normal erythropoiesis and erythroid disorders has been discussed.

Role of miRNAs in erythropoiesis

Erythropoiesis in humans is a multistep process in which multipotent HSCs are committed to differentiate into red cell lineage. During this process, erythroid progenitors including BFU-E and CFU-E are generated followed by other cells in this lineage such as normoblasts, erythroblasts, reticulocytes and finally mature erythrocytes (Moritz et al., 1997). Various factors such as EPO, testosterone, estrogen, interleukin-3, granulocyte-macrophage colony-stimulating factor and interleukin-9 as well as cell-cell interactions and other external factors binding cell surface receptors and triggering several signaling pathways are able to regulate this process (Shiozaki et al., 1992; Palis, 2008).

Maturation, proliferation and differentiation of red blood cells (RBCs) is affected by key regulators of erythropoiesis (iron, hypoxia, stress, growth factors), bone marrow (BM) niche signaling pathways regulating homeostasis (Wnt, Hox, Notch, SCF/C-kit) and erythroid differentiation (EPO-R,

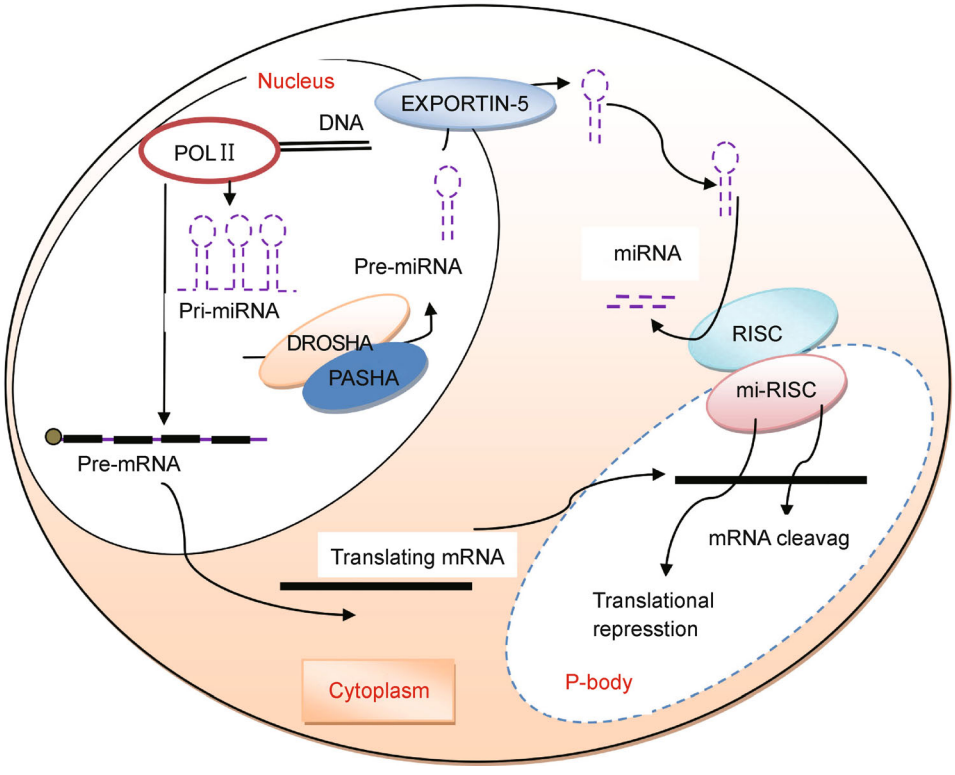

Figure 1 Schematic diagram of microRNA biosyn thesis and function in human cells. miRNAs are first transcribed in the nucleus by RNA polymerase II as a primary transcript known as pri-miRNA (200–300 nucleotides). Specific ribonuclease of Drosha in association with Pasha (DGCR8) cuts pri-miRNA and pre-miRNA, which are transported from nucleus to cytoplasm by a molecule called Exportin/Ran GTP. Dicer-I cuts and processes Pre-miRNA in the cytoplasm. Then, the leading or lagging strand bind a multi-protein complex known as RISC and is converted to active miRNA or miRISC. In P-body of the cell, it acts as a complementary sequence to bind its target sequence in mRNA. In case of fully compatible binding of it to target mRNA, the latter will be degraded and cut but in case of non-compliant relative binding, it reduces stability and structurally prevents the ribosome binding, translation initiation or elongation. Abbreviations: Pol II: RNA polymerase II; miRNA: micro RNA; pre-miRNA: precursor miRNA; pri-miRNA: primary miRNA; pre-mRNA: precursor mRNA; RISC: RNA-induced silencing complex.

HIF) as well as various transcription factors (such as GATA, FOG, TAL-1, LMO2, SCL, E2A, EKLF, BCL11A) (Wu et al., 1995; García and Frampton, 2008; Bruchova et al., 2009). In addition, studies show that miRNAs are involved in the development of RBCs and play their part by affecting the genes or transcription factors involved in erythropoiesis (Zhang et al., 2012) (Table 1). In this section of the paper, we discuss the importance of a number of miRNAs in normal erythropoiesis.

miR-24: Activin is a member of transforming growth factor-β (TGF-β) family with an important role in proliferation and differentiation of erythroid progenitors, and stimulates hemoglobin synthesis in CFU-E together with erythropoietin, increasing the formation of BFU-E (Shiozaki et al., 1992; Moritz et al., 1997). Activin binding to type II activin receptor leads to phosphorylation and activation of type I activin receptor (ALK4, also known as ActRIB) as well as intracellular signal transduction through Smad signaling pathway, which increases the expression of target genes (Xu et al., 2008). Chen et al. showed that miR-24 binding to 3′-UTR of hALK4 mRNA decreases the expression of AKT4 and reduces activin signaling (Chen et al., 2008). They also showed that erythroid differentiation of K562 cells, erythroid colony formation and maturation of human $CD34^+$ hematopoietic progenitor cells is inhibited by miR-24. Overexpression of miR-24, an AKT4 antagonist in K562 cells, resulted in accumulation of hemoglobin in these cells, reduced the formation of CFU-E as well as BFU-E and decreased erythroid differentiation of $CD34^+$ cells (Chen et al., 2008). These results indicate the role of miR-24 in erythroid differentiation; however, the effect of miR-24 on other genes involved in erythropoiesis as well as the impact of other miRNAs on AKT4 cannot be ignored.

miR-126: MiR-126 is considered as a regulator of primitive erythroid lineage with an inhibitory effect on erythropoietic development (Sturgeon et al., 2012). Protein tyrosine phosphatase non-receptor type 9 (PTPN9) is a target of this miRNA, which is necessary for growth and expansion of normal erythroid cells (Huang et al., 2011). The expression of PTPN9 has been shown to increase in erythroid progenitor cells of polycythemia vera (PV) patients, and reduced miR-126 expression may account for this phenomenon (Xu et al., 2003). Some studies have presented PTPN9 as the main target of miR-126 (Huang et al., 2011). There is a high expression of miR-126 in hematopoietic precursors, while the expression of it is reduced in the process of differentiation into erythroid cells *in vivo* (Byon and Papayannopoulou, 2012). In addition to PTPN9, expression suppression of other downstream target

Table 1 MiRNAs involved in regulating gene expression during erythropoiesis.

miRNA	Expression	Target gene	Function	References
miR-15a, miR-16-1	Down	*c-Myb*	Differentiation toward the erythroid lineage from CFU-EM	Hattangadi et al., 2011; Azzouzi et al., 2012
miR-24	Bi-phasic	*hALK4*	Modulates negatively erythropoiesis	Wang et al., 2008; Havelange and Garzon, 2010
miR122	Down	*Hfe Hjv*	Encode activation of hepcidin expression	
miR126	Down	*PTPN9*	Growth and expansion of normal erythroid cell	Huang et al., 2011
miR-144/451	Up	*FOXO3 GATA-2 KLFD*	Regulates oxidative stress, positive regulation of terminal erythroid differentiation and in zebrafish hemoglobin synthesis (miR-144)	Fu et al., 2009; Pase et al., 2009; Havelange and Garzon, 2010
miR-155	Down	*PU-1/ETS-1 CEBPβ/SHIP1*	Inhibits erythropoiesis	Masaki et al., 2007; O'Connell et al., 2008; Havelange and Garzon, 2010
miR-191	Down	*Riok-3 Mxi-1*	Erythroblast chromatin condensation and enucleation	Zhang et al., 2011; Bianchi et al., 2012
miR-210	Up	*HIF Scf EPO*	Induced erythroid differentiation from erythroid progenitor	Kosaka et al., 2008; Byon and Papayannopoulou, 2012
miR-221/222	Down	*c-Kit*	Inhibits early erythroid proliferation Controls perinatal Hb switching	Felli et al., 2005; Bianchi et al., 2012
miR-223	Down	*LMO2*	Inhibits erythropoiesis	Felli et al., 2009; Havelange and Garzon, 2010
miR-320	Down	*CD71(TFR-1)*	Central mediator of iron-loaded transferrin uptake	Chen et al., 2008; Lawrie, 2010
miR-376a	Down	*CDK-2 AGO*	Differentiation toward the committed erythroid/ megakaryocyte lineage from HSC	Wang et al., 2011
Let-7d	Down	*DMT-IRE*	Regulation Hemin-induced erythroid maturation and iron homeostasis	Andolfo et al., 2010; Listowski et al., 2013

Abbreviations: hALK4: human activin A receptor, type 1B; HFE: hemochromatosis gene; Hjv: hemojuvelin gene; PTPN9: protein tyrosine phosphatase non-receptor type 9; FOXO3: Forkhead box protein O3; KLFD: Krüppel-like factor D; HIF: hypoxia inducible factor; Scf: stem cell factor; EPO: erythropoietin; LMO2: LIM domain only protein 2; TFR-1: transferrin receptor-1; CDK-2: Cyclin dependent kinase-2; AGO: Argonaute proteins; DMT: divalent metal transporter; IRE: Iron responsive element; CFU-EM: Colony forming unit-myeloid/erythroid; Hb: hemoglobin; HSC: hematopoietic stem cell.

(s) by miR-126 may be involved in erythropoiesis inhibition, and further studies are needed to identify them (Huang et al., 2011; Byon and Papayannopoulou, 2012).

miR-191: During the development of RBCs, nuclear chromatin is fully compressed until the reticulocytes lose their nucleus. This process is influenced by genes like Riok3 and Mxi1, the expression level of which is increased with maturity of erythroblasts as well as decreased expression of Gcn5, which results in histone deacetylation, chromatin assembly for chromatin maturation and erythroblastic enucleation (Zhang et al., 2011). Studies on murine erythroid precursors indicate that miR-191 is reduced during maturation of erythroid cells. This miRNA targets Riok3 and Mxi1, inhibiting their expression and thus preventing erythroblast enucleation, which results in maturity of erythroblasts (Zhang et al., 2011; Listowski et al., 2013).

miR-210: MiR-210 expression is observed during erythroid maturation, and is associated with HbF production induction, regulation of O2 homeostasis as well as inhibition of apoptosis in erythrocytes (Kosaka et al., 2008). The expression of this miRNA in hypoxic conditions is associated with changing type of hemoglobins by erythroid cells as well as increased HbF in stress erythropoiesis conditions. Hypoxia-associated miR-210 may be associated with increased expression of γ-globin genes in differentiating erythroid cells (Bianchi et al., 2009; Sarakul et al., 2013). However, further studies like targeted deletion in vivo are required on the function of miR-210 to provide more details in order to reveal the physiologic function of this miRNA.

miR-221/222: MiR-221/222 is able to regulate erythropoiesis and angiogenesis via c-kit targeting (Zhan et al., 2007). c-kit is a tyrosine kinase receptor for stem cell factor (SCF) and is an essential factor to control the proliferation of primitive hematopoietic and erythroid cells (Williams et al., 1990). c-Kit and SCF signaling pathway play an important role in erythroid cell development; however, despite the importance of this pathway in erythropoiesis, the mechanisms regulating cellular responses by this pathway are unknown (Munugalavadla and Kapur, 2005). SCF and c-Kit are involved in survival, proliferation and differentiation of erythroid precursor cells in association with EPO and EPO-R, and their presence is essential for differentiation of hematopoietic cells to committed erythroid precursors (van de Loosdrecht and Vellenga, 2000). In addition, SCF activates EPO and EPO-R signaling pathway through phosphorylation of tyrosine, followed by increased expression of BCL2 in erythroblasts, increased BCL-xl expression in CFU-E and in more mature erythroid lineages as well as inhibition of apoptosis in erythroblasts (van de Loosdrecht and Vellenga, 2000; Bakker et al., 2004). Research on proliferating erythroid cells showed that c-Kit expression is inversely related to expression of miR-221/222, and its expression inhibits normal erythropoiesis and erythroleukemic cell growth, while has no impact on Kit^- hematopoietic cell lines. Therefore, miR-221/222 may have target genes other than *Kit*, and other miRNAs may target the *Kit* gene, which requires further practical studies (Felli et al., 2005). It has been shown that human perinatal hemoglobin switching is controlled by Kit-receptor and miR-221/222, and increased expression of these miRNAs in progenitor cells of umbilical cord blood (UCB) causes reduced erythroblastic proliferation and decreased fetal hemoglobin content (Havelange and Garzon, 2010).

miR-223: Several studies have indicated reduced expression of miR-223 during maturation and differentiation of monocytic, erythroid and mast-cell series. However, few practical studies have been performed to assess the role of miR-223 during the differentiation of the mentioned lineages (Baltimore et al., 2008). Felli et al. showed that miR-223 expression is considerably reduced during erythropoiesis, which is essential for erythrocyte proliferation and differentiation. This study also suggests that LMO2 protein may be a critical target of miR-223 (Felli et al., 2009). LMO2 (LIM-only protein 2, RBNT2) is a family member of LIM-only class of transcriptional co-regulators with an important role in regulation of HSCs development and erythropoiesis, and mice lacking this gene show defects in hematopoiesis and formation of fetal erythrocytes (Warren et al., 1994). LMO2 participates in the regulation of genes involved in erythroid differentiation by forming a DNA binding complex together with basic helix–loop–helix proteins of SCL, E2A, LIM domain binding protein 1 (Ldb-1) and GATA1 (Wadman et al., 1997). It has also been shown that LMO2 expression in multipotent progenitor cells increases erythroid differentiation by increasing the transcription of erythroid genes (Hansson et al., 2007).

miR-144/451: GATA-1 is a member of GATA family, and was first identified as a protein that specifically binds to β-globin 3′ enhancer (Wall et al., 1988). GATA1 expression is observed in erythroid cells, megakaryocytes, eosinophils and mast cells, and is essential for normal erythropoiesis (Ferreira et al., 2005). Without GATA1, lineage-committed erythroid precursors are formed but undergo differentiation arrest and apoptosis (Welch et al., 2004). Several erythroid genes are regulated by GATA-1, including miR 144/451, which is directly induced by GATA1 (Dore et al., 2008; Rasmussen et al., 2010). Dore et al. showed that miR-144/451 locus is a major downstream effector of GATA-1 in erythroid cells, and altered expression of this miRNA as a result of genetic changes or pharmacologic manipulation can affect RBC production in different diseases (Dore et al., 2008). Other studies have shown that miR-155 and miR-451 are two key factors in normal erythroid differentiation, and decreased expression of miR-155 and overexpression of miR-451 may lead to progression of erythroid differentiation (Masaki et al., 2007; Merkerova et al., 2008; Svasti et al., 2010). In addition to miR-144/451, miR-23a is activated by GATA-1 during erythroid differentiation and can act as a positive erythroid regulator (Bruchova-Votavova et al., 2010; Zhang et al., 2012).

Ago2 protein from Argonaute superfamily, a component of

RISC (RNA-induced silencing complex), is another factor involved in expression regulation of miR-451 (O'Carroll et al., 2007; Yang and Lai, 2011; Aalto and Pasquinelli 2012). In mice lacking Ago2 Gene, defective B cells and erythroid maturation as well as increased basophilic erythroblasts together with significant reduction in mature orthochromatic erythroblasts is observed (Cheloufi et al., 2010). It is not clear how the reduction or absence of Ago2 impairs erythropoiesis, and its impact on expression of miRNAs is a suggested mechanism because the lack of Ago2 protein reduces the expression level of miRNAs, including miR-451 (O'Carroll et al., 2007; Byon and Papayannopoulou, 2012). It has also been shown that the expression level of miRNA-376a is high in early precursor cells and is reduced during cell differentiation. This reduction is associated with increased Ago2 and CDK-2 levels, and plays an effective role in differentiation of hematopoietic progenitor cells toward committed erythroid/megakaryocyte progenitors (Wang et al., 2011).

RAB14 is another target gene of miR-144/451, is a member of the RAS oncogene family of small GTPase proteins and may play a crucial role in regulation of erythroid cell proliferation and differentiation (Stenmark, 2009) . Recently, it was shown that RAB14 is a potential target for miR-144 and/or miR-451, and decreased RAB14 expression during erythropoiesis is correlated with increased miR-144 and miR-451 expression (Kim et al., 2014). However, the role of these miRNAs and their target genes in erythroid differentiation is still not well understood, and further studies are needed to understand their function in the cell (Wang et al., 2013; Zhu et al., 2013).

Other miRNAs involved in erythropoiesis: In addition to the mentioned miRNAs, studies have indicated the over-expression of miR-20, miR-106a, Let-7a and Let-7d during erythroid differentiation versus decreased expression of them during megakaryocytic differentiation (Garzon et al., 2006; Choong et al., 2007). Furthermore, it has been shown that reduced expression of miR-150 is essential for erythroid differentiation. This miRNA directly interferes with *Myb* mRNA and induces differentiation of myeloid-erythroid progenitor cells (MEP) to erythrocytes or megakaryocytes (Listowski et al., 2013).

Several studies have reported that miR-155 expression is reduced during erythropoiesis in primary cells, and over-expression of it is associated with a reduction in the number of erythroid precursors and circulating red cells (Bruchova et al., 2007; O'Connell et al., 2008). A negative autoregulative feedback loop between miR-15a and c-Myb has also been reported, whereas c-Myb binds miR-15a promoter and regulates its expression. MiR-15a also binds 3′ UTR of c-Myb and inhibits its translation. Reverse expression of these two factors plays a role in $CD34^{+}$ cells undergoing erythroid differentiation. Increased expression of miR-15a in normal marrow mononuclear cells inhibits erythroid and myeloid colony formation in vitro (Zhao et al., 2009).

Recently, Wang et al. reported that the expression of miR-486-5p during erythroid differentiation can enhance PI3K/AKT signaling activity via reduction of PTEN and FOXO1 transcription factors levels, which plays an important role in erythropoietin signal transduction and erythroid phenotype (Wang et al., 2014). In addition, Zhai et al. demonstrated that miR-146b is able to promote the erythroid and megakaryocytic development of K562 cells, and decreased miR-146b levels can inhibit these processes (Zhai et al., 2014). In addition, they showed that GATA-1 is linked to chromatin sites of *miR-146b* promoter and promotes its transcriptional activation during erythroid and megakaryocytic differentiation.

Therefore, according to the above, it can be concluded that the gain/loss-of-function of miRNAs can affect erythropoiesis, and impaired expression of them causes erythropoiesis defects like congenital and acquired red cell disorders, which requires comprehensive and practical studies to take advantage of miRNAs to treat diseases (Choong et al., 2007).

miRNA expression/function in RBC Disorders

Impaired expression of miRNAs may be important in development or aggravation of a number of erythroid disorders, including impaired hemoglobin synthesis that causes genetic diseases like thalassemia in humans (Byon and Papayannopoulou, 2012). The human α-globin cluster has three functional α-globin genes including αI, α II and ζ, which are differently expressed during embryonic and adult erythropoiesis, respectively (Fig. 2) and β-globin cluster consists of five ϵ genes in the yolk sac, Gγ and Aγ in fetal liver as well as δ and β genes that are expressed in the BM (Fig. 2) (Tsiftsoglou et al., 2009; Buccheri et al., 2013). Impaired structure or function of globins results in disorders such as α- and β-thalassemia (Maniatis et al., 1980; Hilliard and Berkow, 1996; Higgs et al., 2005; Fu et al., 2009). In this type of disorder, globin chains are often structurally normal and are mainly produced due to lack of or decreased synthesis of globin chains. Synthesis of α and β chains is respectively decreased in α and β thalassemia, thereby producing hypochromic cells due to lack of β-globin production and precipitation of excess globin chains in red cell precursors, which is associated with hemolysis in α thalassemia and ineffective erythropoiesis in β thalassemia (Papayannopoulou et al., 1979; Yuan et al., 1993; Hilliard and Berkow, 1996; Centis et al., 2000; Mathias et al., 2000; Pekarsky et al., 2006). It has been shown that miR-15b, miR-16, miR-22 and miR-185 play an important role in the development of erythroid surface antigens and hemoglobin synthesis (Lulli et al., 2013).

A study has shown that miR-144, which is a type of erythroid lineage-specific microRNA gene, is expressed in specific stages of embryogenesis and can cause negative regulation of embryonic α-globin but has no effect on

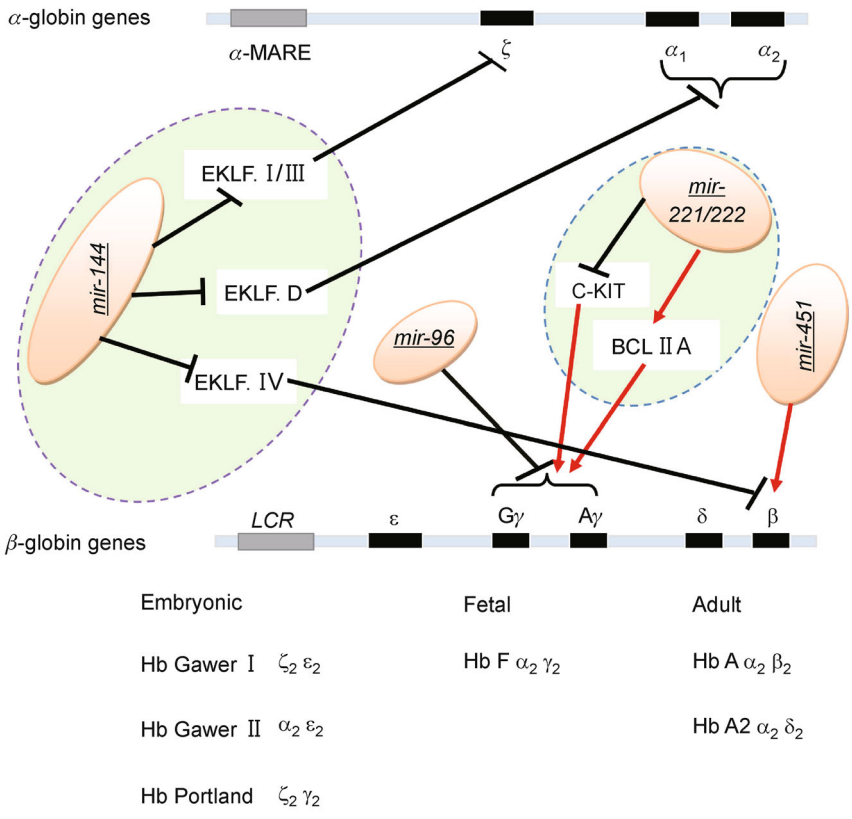

Figure 2 The role of miRNAs in regulation of hemoglobin synthesis. Globin chains are expressed on different chromosomes: ζ and ε on chromosome 16 and β, γ and ε chains on chromosome 11. Combination of different chains during development results in different hemoglobins like fetal hemoglobins of Gower I (ζ2 ε2), Gower II (α2 ε2) and Portland (ζ2 γ2), embryonic hemoglobins of F(α2γ2) as well as adult hemoglobins of HB A (α2 β2) and A2 (α 2δ2). miR-96 and miR-451 directly affect mRNA of γ and β chains and inhibit their expression. miR-144 inhibits the transcription of α, ε and β genes through expression inhibition of EKLF (Basu et al., 2007; Byon and Papayannopoulou, 2012). miR-221/222 reduces the synthesis of c-Kit and increases BCL11A synthesis, increasing the expression of γ -globin chain (Bianchi et al., 2010; Zhou et al., 2010). Abbreviations: Hb: hemoglobin; miR: micro-RNA; EKLF: erythroid Krupple-like factor; BCL11A: B cell CLL/lymphoma 11A (zinc finger protein).

embryonic β-globin gene expression. Thus, miR-144 may regulate embryonic α-globin expression in primitive erythropoiesis (Fu et al., 2009). The miR-144 mechanism of action on embryonic α-globin appears to be mediated by targeting the erythroid Krupple-like factor (EKLF) (Fig. 2) (Basu et al., 2007). This factor is essential for transcription activation of adult β-globin gene, and mice that are deficient in this gene experience severe β-thalassemia (Stamatoyannopoulos, 2005; Bank, 2006). This transcription factor binds the promoter region of miR-144 and induces its expression. Thus, miR-144 and EKLF constitute a negative feedback loop that results in reduced level of EKLF (Fig. 2) (Byon and Papayannopoulou, 2012).

It has been shown that overexpression of human let-7 miRNA is associated with fetal-to-adult hemoglobin switching. Increased expression of this miRNA may be involved in regulation of changes during erythropoiesis or patterns of hemoglobin expression, which demands further studies (Noh et al., 2009).

A number of studies have indicated that miR-15a, which may be involved in cell cycle control as well as miR-16-1 are associated with increased expression of γ-globin and HbF mediated by down-modulation of Myb as an inhibitor of γ-globin gene transcription in hematopoietic progenitors in adults (Guglielmelli et al., 2011; Lulli et al., 2013; Shivdasani, 2006). Therefore, treatment strategies regulating the expression of these miRNAs and increasing HbF level may be effective upon improvement of symptoms in patients with β-thalassemia or sickle-cell disease (SCD) (Sankaran, 2011).

SCD is a qualitative abnormality in globin chain due to a single amino acid change in β-globin gene, resulting in abnormal hemoglobin polymerization and hemolysis (Fard et al., 2013). Patients with homozygous mutation for SCD (HbSS) show the most severe form of disease. Chen et al. studied the miRNA expression profile in reticulocytes and mature erythrocytes of SCD patients and found that the expression of miR-29a, miR-140, miR-144 and miR-451 was

increased and that of miR-320, miR-let-7, miR-181 and miR-141 was decreased in SSD erythrocytes (Chen et al., 2008). Decreased expression of miR-320 in reticulocytes of patients with HbSS is associated with a high expression of CD71 marker. MiR-320 directly regulates the expression of CD71, and low expression level of this miR in HbSS cells can be associated with a high level of CD71 expression during terminal differentiation, maturation disruption and increase in reticulocytes in patients with SCD (Chen et al., 2008; Lawrie, 2010).

MiR-144 is another miRNA undergoing increased expression in HbSS reticulocytes, which is associated with severe disease and hemolysis (Sangokoya et al., 2010). NRF2 is a basic leucine zipper transcription factor and a main target of miR-144, which plays an important role in the regulation of antioxidant response (Ishii et al., 2000; Lee and Johnson, 2004; Motohashi and Yamamoto, 2004). Overexpression of miR-144 in patients with SCD reduces NRF2 and results in reduction of intracellular glutathione levels and increased sensitivity to oxidative stress (Sangokoya et al., 2010; Walker et al., 2011).

In addition to miR-144, miR-451 has been shown to play an important role in regulating responses to cellular stress, undergoing increased expression in HbSS erythrocytes (Chen et al., 2008). GATA-1 transcription factor directly activates bicistronic locus encoding miR-144 and miR-451, the expression of which is important during erythroid maturation. MiR-451 has also been detected in mature circulating RBCs. This miRNA directly targets Ywhaz mRNA that encodes phospho-serine/threonine binding protein 14-3-3ζ (Patrick et al., 2010; Listowski et al., 2013), and loss of miR-451 causes protein 14-3-3ζ accumulation in erythroid precursors, activation of AKT and impaired transcription activity of FoxO3, a key positive expression regulator of anti-oxidant genes (Fig. 3) (Yu et al., 2010). AKT is responsible for several intracellular signals that can be activated in miR-144/451$^{-/-}$ erythroblasts through other 14-3-3-independent mechanisms. In early-stage erythroid precursors, the signals generated by Kit receptor and/or EpoR activate AKT followed by FoxO3 phosphorylation, which may enable its binding to 14-3-3 proteins and inhibit transcription activity of FoxO3 (Bakker et al., 2007; Yu et al., 2010). Reduced Kit and EpoR signals during normal erythroid maturation causes entry of FoxO3 into the nucleus and its anti-oxidant activity. MiR-451 increases the activity of FOX3 in terminal erythropoiesis via inhibition of 14-3-3ζ proteins (Listowski et al., 2013). Therefore, reduction of this miRNA in HbSS patients who are more sensitive to oxidative stress than normal subjects can increase the severity of disease, and its overexpression may cause relief of symptoms, which requires further comprehensive studies.

PV is a myeloproliferative disorder characterized by hyperactive erythropoiesis, resulting in the accumulation of normal RBCs (Bruchova et al., 2009). EPO-independent erythrocytosis is a feature of these patients, and approximately 90%–95% of them bear JAK2 V617F mutation (James, 2008; Levine and Gilliland, 2008). MiRNA profiling in these patients indicates that expression regulation of miR-150, miR-451, miR-155, miR-222 and miR-378 in cultured cells is impaired in these patients (Bruchova et al., 2007). In granulocytes of PV patients, the expression of miR-182 and let-7a is increased and decreased, respectively. In platelets of these patients, miR-26b is increased, and miR-30b, miR-30c and miR-150 are decreased in their reticulocytes (Bruchova et al., 2008; Byon and Papayannopoulou, 2012). The role of these miRNAs in pathogenesis of this disease is still not well established, and requires further investigations.

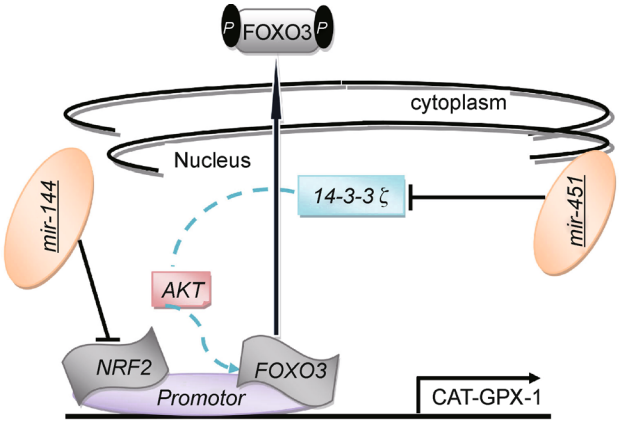

Figure 3 The role of miRNAs in responses to cellular oxidative stress. The expression of CAT and GPX-1 genes results in production of antioxidants and increased resistance of RBCs to oxidative stress. FOXO3 and NRF2 affect the promoter of these genes, leading to increased expression of them. MiR-144 directly affects the mRNA gene of NRF2 and restricts its expression (Ishii et al., 2000; Lee and Johnson, 2004; Motohashi and Yamamoto, 2004). On the other hand, 14-3-3ζ gene activates AKT, phosphorylates FOXO3 and causes its departure from the nucleus. Hence, suppressed FOXO3 and NRF2 activity reduces the expression of CAT and GPX-1 genes. MiR-451 suppresses the expression of 14-3-3ζ that prevents FOXO3 phosphorylation and acts as a stimulant to increase antioxidant agents in RBCs (Yu et al., 2010). Abbreviations: RBCs: red blood cells; FOXO3: Forkhead box protein O3; AKT: serine/threonine protein kinase Akt; NRF2: NF-E2-related factor 2; CAT: catalase gene; GPX-1: glutathione peroxidase 1.

Impaired absorption of iron and its use in hemoglobin synthesis can also result in erythroid disorders. Divalent metal transporter (DMT1) and iron-responsive element (IRE) are important factors in enterocyte iron absorption and erythroid iron utilization, and are subject to increased expression in CD34^{+} cells during erythroid differentiation (Andolfo et al., 2010). Several mechanisms including miRNAs expression can regulate DMT-IRE expression. Let-7d is one of the most important regulators of DMT-IRE expression, and increased expression of it reduces DMT-IRE mRNA and protein levels in human erythroid cell lines by binding DMT-IRE 3′UTR

(Davis and Clarke, 2013). Increased miR-Let-7d in K562 cells is associated with defects in erythroid differentiation by accumulation of iron in endosomes (Andolfo et al., 2010). Increased Let-7d expression is associated with reduced ferritin heavy chain (FH1), increased expression of transferrin receptor C (TfRC) and thus reduced erythroid maturation and disrupted iron hemostasis. This changing pattern of expression is seen in iron-deficient states (Byon and Papayannopoulou, 2012). Yet, there are still many uncertainties about the role of this miRNA in disorders of iron homeostasis, and further studies are required to elucidate its role.

Conclusions and future directions

Erythropoiesis is a complex developmental process during which erythroid progenitors like BFU-E and CFU-E as well as other cells in this series including normoblasts, erythroblasts, reticulocytes and finally mature erythrocytes are produced (Palis, 2014). Several factors including miRNAs are involved in the regulation of this process (Tsiftsoglou et al., 2009). MiRNAs are small non-coding RNAs acting post-transcriptionally and regulating protein expression by inhibiting translation or destabilizing the target mRNA (Bianchi et al., 2009). In erythroid-committed precursors, the expression of miR-126, miR-15a, miR-221, miR-222 and miR-24 is reduced, resulting in the expression of c-Myb, c-kit and ALK4 transcription factors (Azzouzi et al., 2012). c-Myb is an essential transcription factor for normal erythropoiesis, and activates GATA-1, c-kit, LMO2 and KLF1 (Melotti and Calabretta, 1996; Bianchi et al., 2010). After activation by c-Myb and derepression of ALK4 receptor that is necessary to stimulate β-globin synthesis, KLF1 activates BCL11A transcription factor and increases β-globin synthesis, inhibits γ-globin synthesis and increases Hb A synthesis (Bianchi et al., 2010; Zhou et al., 2010). c-Myb-activated GATA-1 activates the expression of miR-451 and miR-144 by interfering with LMO2 (Dore et al., 2008). MiR-451 inhibits GATA-2 and 14-3-3ζ transcription factors, promoting cell maturation and inhibiting apoptosis (Pase et al., 2009). C-kit is another essential factor for erythropoiesis, which activates EPOR and causes autophosphorylation of JAK2 as well as activation of STAT5 and PI3K signaling pathways, and thus promotes cell survival, proliferation and differentiation (Grebien et al., 2008). Finally, PTPN9 expression induces erythroid expansion, and miR-126 can inhibit the growth and expansion of normal erythroid cells via inhibition of this factor (Huang et al., 2011). In maturation stage, decreased expression of miR-191 is essential for erythroid enucleation by Riok3 and Mxi1 factors (Zhang et al., 2011). Impaired expression of each of the mentioned miRNAs and their target genes can cause red cell disorders, especially disrupted red cell maturation and hemoglobin expression. Moreover, due to importance of a number of miRNAs including miR-144/451 in regulating responses to cellular stress, impaired expression of them can increase destruction of erythroid cells when faced with oxidative agents (Chen et al., 2008).

Therefore, given the importance of miRNAs during erythropoiesis and their role in development or intensification of a number of disorders related to this lineage, better knowledge of them and their target genes can help us in effective treatment of patients, which demands further practical studies.

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Compliance with ethics guidelines

Javad Mohammdai-asl, Abolfazl Ramezani, Fatemeh Norozi, Amal Saki Malehi, Ali Amin Asnafi, Mohammad Ali Jalali Far, Seyed Hadi Mousavi, Najmaldin Saki declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Aalto A P, Pasquinelli A E (2012). Small non-coding RNAs mount a silent revolution in gene expression. Curr Opin Cell Biol, 24(3): 333–340

Aerbajinai W, Giattina M, Lee Y T, Raffeld M, Miller J L (2003). The proapoptotic factor Nix is coexpressed with Bcl-xL during terminal erythroid differentiation. Blood, 102(2): 712–717

Andolfo I, De Falco L, Asci R, Russo R, Colucci S, Gorrese M, Zollo M, Iolascon A(2010). Regulation of divalent metal transporter 1 (DMT1) non-IRE isoform by the microRNA Let-7d in erythroid cells. Haematologica, 95(8): 1244–1252

Azzouzi I, Schmugge M, Speer O (2012). MicroRNAs as components of regulatory networks controlling erythropoiesis. Eur J Haematol, 89 (1): 1–9

Bakker W J, Blázquez-Domingo M, Kolbus A, Besooyen J, Steinlein P, Beug H, Coffer P J, Löwenberg B, von Lindern M, van Dijk T B (2004). FoxO3a regulates erythroid differentiation and induces BTG1, an activator of protein arginine methyl transferase 1. J Cell Biol, 164(2): 175–184

Bakker W J, van Dijk T B, Parren-van Amelsvoort M, Kolbus A, Yamamoto K, Steinlein P, Verhaak R G, Mak T W, Beug H, Löwenberg B, von Lindern M (2007). Differential regulation of Foxo3a target genes in erythropoiesis. Mol Cell Biol, 27(10): 3839–3854

Baltimore D, Boldin M P, O'Connell R M, Rao D S, Taganov K D (2008). MicroRNAs: new regulators of immune cell development and function. Nat Immunol, 9(8): 839–845

Bank A (2006). Regulation of human fetal hemoglobin: new players, new complexities. Blood, 107(2): 435–443

Basu P, Lung T K, Lemsaddek W, Sargent T G, Williams D C Jr, Basu M, Redmond L C, Lingrel J B, Haar J L, Lloyd J A (2007). EKLF and KLF2 have compensatory roles in embryonic β-globin gene expression and primitive erythropoiesis. Blood, 110(9): 3417–3425

Bianchi E, Zini R, Salati S, Tenedini E, Norfo R, Tagliafico E, Manfredini R, Ferrari S (2010). c-myb supports erythropoiesis through the transactivation of KLF1 and LMO2 expression. Blood, 116(22): e99–e110

Bianchi N, Zuccato C, Finotti A, Lampronti I, Borgatti M, Gambari R (2012). Involvement of miRNA in erythroid differentiation. Epigenomics, 4(1): 51–65

Bianchi N, Zuccato C, Lampronti I, Borgatti M, Gambari R (2009). Expression of miR-210 during erythroid differentiation and induction of gamma-globin gene expression. BMB Rep, 42(8): 493–499

Bracht J R, Van Wynsberghe P M, Mondol V, Pasquinelli A E (2010). Regulation of lin-4 miRNA expression, organismal growth and development by a conserved RNA binding protein in *C. elegans*. Dev Biol, 348(2): 210–221

Bruchova H, Merkerova M, Prchal J T (2008). Aberrant expression of microRNA in polycythemia vera. Haematologica. 93(7): 1009–1016

Bruchova H, Yoon D, Agarwal A M, Mendell J, Prchal J T (2007). Regulated expression of microRNAs in normal and polycythemia vera erythropoiesis. Exp Hematol, 35(11): 1657–1667

Bruchova H, Yoon D, Agarwal A M, Swierczek S, Prchal J T (2009). Erythropoiesis in polycythemia vera is hyper-proliferative and has accelerated maturation. Blood Cells Mol Dis, 43(1): 81–87

Bruchova-Votavova H, Yoon D, Prchal J T (2010). miR-451 enhances erythroid differentiation in K562 cells. Leuk Lymphoma, 51(4): 686–693

Buccheri M A, Spina S, Ruberto C, Lombardo T, Labie D, Ragusa A A (2013). Annotated definition of BCL11A and HMIP-2 haplotypes through the analysis of sicilian β-thalassemia patients with high levels of fetal hemoglobin. Hemoglobin, 37(5): 423–434

Byon J C, Papayannopoulou T (2012). MicroRNAs: Allies or foes in erythropoiesis? J Cell Physiol, 227(1): 7–13

Cantor A B, Orkin S H (2002). Transcriptional regulation of erythropoiesis: an affair involving multiple partners. Oncogene, 21 (21): 3368–3376

Centis F, Tabellini L, Lucarelli G, Buffi O, Tonucci P, Persini B, Annibali M, Emiliani R, Iliescu A, Rapa S, Rossi R, Ma L, Angelucci E, Schrier S L (2000). The importance of erythroid expansion in determining the extent of apoptosis in erythroid precursors in patients with β-thalassemia major. Blood, 96(10): 3624–3629

Cheloufi S, Dos Santos C O, Chong M M, Hannon G J (2010). A dicer-independent miRNA biogenesis pathway that requires Ago catalysis. Nature, 465(7298): 584–589

Chen S Y, Wang Y, Telen M J, Chi J T (2008). The genomic analysis of erythrocyte microRNA expression in sickle cell diseases. PLoS ONE, 3(6): e2360

Choong M L, Yang H H, McNiece I (2007). MicroRNA expression profiling during human cord blood-derived CD34 cell erythropoiesis. Exp Hematol, 35(4): 551–564

Davis M, Clarke S (2013). Influence of microRNA on the maintenance of human iron metabolism. Nutrients, 5(7): 2611–2628

Dore L C, Amigo J D, Dos Santos C O, Zhang Z, Gai X, Tobias J W, Yu D, Klein A M, Dorman C, Wu W, Hardison R C, Paw B H, Weiss M J (2008). A GATA-1-regulated microRNA locus essential for erythropoiesis. Proc Natl Acad Sci USA, 105(9): 3333–3338

Fabbri M, Garzon R, Andreeff M, Kantarjian H M, Garcia-Manero G, Calin G A (2008). MicroRNAs and noncoding RNAs in hematological malignancies: molecular, clinical and therapeutic implications. Leukemia, 22(6): 1095–1105

Fard A D, Hosseini S A, Shahjahani M, Salari F, Jaseb K (2013). Evaluation of novel fetal hemoglobin inducer drugs in treatment of β-hemoglobinopathy disorders. Int J Hematol Oncol Stem Cell Res, 7 (3): 47–54

Felli N, Fontana L, Pelosi E, Botta R, Bonci D, Facchiano F, Liuzzi F, Lulli V, Morsilli O, Santoro S, Valtieri M, Calin G A, Liu C G, Sorrentino A, Croce C M, Peschle C (2005). MicroRNAs 221 and 222 inhibit normal erythropoiesis and erythroleukemic cell growth via kit receptor down-modulation. Proc Natl Acad Sci USA, 102(50): 18081–18086

Felli N, Pedini F, Romania P, Biffoni M, Morsilli O, Castelli G, Santoro S, Chicarella S, Sorrentino A, Peschle C, Marziali G (2009). MicroRNA 223-dependent expression of LMO2 regulates normal erythropoiesis. haematologica. Haematologica, 94(4): 479–86

Ferreira R, Ohneda K, Yamamoto M, Philipsen S (2005). GATA1 function, a paradigm for transcription factors in hematopoiesis. Mol Cell Biol, 25(4): 1215–1227

Fu Y F, Du T T, Dong M, Zhu K Y, Jing C B, Zhang Y, Wang L, Fan H B, Chen Y, Jin Y, Yue G P, Chen S J, Chen Z, Huang Q H, Jing Q, Deng M, Liu T X (2009). Mir-144 selectively regulates embryonic α-hemoglobin synthesis during primitive erythropoiesis. Blood, 113(6): 1340–1349

García P, Frampton J (2008). Hematopoietic lineage commitment: miRNAs add specificity to a widely expressed transcription factor. Dev Cell, 14(6): 815–816

Garzon R, Pichiorri F, Palumbo T, Iuliano R, Cimmino A, Aqeilan R, Volinia S, Bhatt D, Alder H, Marcucci G, Calin G A, Liu C G, Bloomfield C D, Andreeff M, Croce C M (2006). MicroRNA fingerprints during human megakaryocytopoiesis. Proc Natl Acad Sci USA, 103(13): 5078–5083

Grebien F, Kerenyi M A, Kovacic B, Kolbe T, Becker V, Dolznig H, Pfeffer K, Klingmüller U, Müller M, Beug H, Müllner E W, Moriggl R (2008). Stat5 activation enables erythropoiesis in the absence of EpoR and Jak2. Blood, 111(9): 4511–4522

Guerau-de-Arellano M, Alder H, Ozer H G, Lovett-Racke A, Racke M K (2012). miRNA profiling for biomarker discovery in multiple sclerosis: from microarray to deep sequencing. J Neuroimmunol, 248(1-2): 32–39

Guglielmelli P, Tozzi L, Bogani C, Iacobucci I, Ponziani V, Martinelli G, Bosi A, Vannucchi A M, and the AGIMM (AIRC-Gruppo Italiano Malattie Mieloproliferative) Investigators (2011). Overexpression of microRNA-16-2 contributes to the abnormal erythropoiesis in polycythemia vera. Blood, 117(25): 6923–6927

Hansson A, Zetterblad J, van Duren C, Axelson H, Jönsson J I (2007). The Lim-only protein LMO2 acts as a positive regulator of erythroid differentiation. Biochem Biophys Res Commun, 364(3): 675–681

Hattangadi S M, Wong P, Zhang L, Flygare J, Lodish H F (2011). From stem cell to red cell: regulation of erythropoiesis at multiple levels by multiple proteins, RNAs, and chromatin modifications. Blood, 118 (24): 6258–6268

Havelange V, Garzon R (2010). MicroRNAs: emerging key regulators of hematopoiesis. Am J Hematol, 85(12): 935–942

Higgs D R, Garrick D, Anguita E, De Gobbi M, Hughes J, Muers M, Vernimmen D, Lower K, Law M, Argentaro A, Deville M A, Gibbons R (2005). Understanding α-globin gene regulation: Aiming to improve the management of thalassemia. Ann N Y Acad Sci, 1054 (1): 92–102

Hilliard L M, Berkow R L (1996). The thalassemia syndromes. Prim Care Update Ob Gyns, 3(5): 157–162

Huang X, Gschweng E, Van Handel B, Cheng D, Mikkola H K, Witte O N (2011). Regulated expression of microRNAs-126/126* inhibits erythropoiesis from human embryonic stem cells. Blood, 117(7): 2157–2165

Ishii T, Itoh K, Takahashi S, Sato H, Yanagawa T, Katoh Y, Bannai S, Yamamoto M (2000). Transcription factor Nrf2 coordinately regulates a group of oxidative stress-inducible genes in macrophages. J Biol Chem, 275(21): 16023–16029

James C (2008). The JAK2V617F mutation in polycythemia vera and other myeloproliferative disorders: one mutation for three diseases? Hematology (Am Soc Hematol Educ Program), 2008(1): 69–75

Kandhavelu M, Kandhavelu J (2012). pre-piRNA biogenesis mimics the pathway of miRNA. Biochem Syst Ecol, 43: 200–204

Karius T, Schnekenburger M, Dicato M, Diederich M (2012). MicroRNAs in cancer management and their modulation by dietary agents. Biochem Pharmacol, 83(12): 1591–1601

Kim M, Tan Y S, Cheng W C, Kingsbury T J, Heimfeld S, Civin C I (2014). MIR144 and MIR451 regulate human erythropoiesis via RAB14. Br J Haematol, 168(4): 583–597

Kim S I, Bresnick E H (2007). Transcriptional control of erythropoiesis: emerging mechanisms and principles. Oncogene, 26(47): 6777–6794

Kosaka N, Sugiura K, Yamamoto Y, Yoshioka Y, Miyazaki H, Komatsu N, Ochiya T, Kato T (2008). Identification of erythropoietin-induced microRNAs in haematopoietic cells during erythroid differentiation. Br J Haematol, 142(2): 293–300

Lawrie C H (2010). microRNA expression in erythropoiesis and erythroid disorders. Br J Haematol, 150(2): 144–151

Lee J M, Johnson J A (2004). An important role of Nrf2-ARE pathway in the cellular defense mechanism. J Biochem Mol Biol, 37(2): 139–143

Leonard M, Brice M, Engel J D, Papayannopoulou T (1993). Dynamics of GATA transcription factor expression during erythroid differentiation. Blood, 82(4): 1071–1079

Levine R L, Gilliland D G (2008). Myeloproliferative disorders. Blood, 112(6): 2190–2198

Listowski M A, Heger E, Bogusławska D M, Machnicka B, Kuliczkowski K, Leluk J, Sikorski A F (2013). microRNAs: fine tuning of erythropoiesis. Cell Mol Biol Lett, 18(1): 34–46

Lulli V, Romania P, Morsilli O, Cianciulli P, Gabbianelli M, Testa U, Giuliani A, Marziali G (2013). MicroRNA-486-3p regulates γ-globin expression in human erythroid cells by directly modulating BCL11A. PLoS ONE, 8(4): e60436

Maniatis T, Fritsch E F, Lauer J, Lawn R M (1980). The molecular genetics of human hemoglobins. Annu Rev Genet, 14(1): 145–178

Masaki S, Ohtsuka R, Abe Y, Muta K, Umemura T (2007). Expression patterns of microRNAs 155 and 451 during normal human erythropoiesis. Biochem Biophys Res Commun, 364(3): 509–514

Mathias L A, Fisher T C, Zeng L, Meiselman H J, Weinberg K I, Hiti A L, Malik P (2000). Ineffective erythropoiesis in β-thalassemia major is due to apoptosis at the polychromatophilic normoblast stage. Exp Hematol, 28(12): 1343–1353

Melotti P, Calabretta B (1996). Induction of hematopoietic commitment and erythromyeloid differentiation in embryonal stem cells constitutively expressing c-myb. Blood, 87(6): 2221–2234

Merkerova M, Belickova M, Bruchova H (2008). Differential expression of microRNAs in hematopoietic cell lineages. Eur J Haematol, 81(4): 304–310

Moritz K M, Lim G B, Wintour E M (1997). Developmental regulation of erythropoietin and erythropoiesis. Am J Physiol, 273(6 Pt 2): R1829–R1844

Motohashi H, Yamamoto M (2004). Nrf2-Keap1 defines a physiologically important stress response mechanism. Trends Mol Med, 10(11): 549–557

Munugalavadla V, Kapur R (2005). Role of c-Kit and erythropoietin receptor in erythropoiesis. Crit Rev Oncol Hematol, 54(1): 63–75

Noh S J, Miller S H, Lee Y T, Goh S H, Marincola F M, Stroncek D F, Reed C, Wang E, Miller J L (2009). Let-7 microRNAs are developmentally regulated in circulating human erythroid cells. J Transl Med, 7(1): 98

O'Carroll D, Mecklenbrauker I, Das P P, Santana A, Koenig U, Enright A J, Miska E A, Tarakhovsky A (2007). A Slicer-independent role for Argonaute 2 in hematopoiesis and the microRNA pathway. Genes Dev, 21(16): 1999–2004

O'Connell R M, Rao D S, Chaudhuri A A, Boldin M P, Taganov K D, Nicoll J, Paquette R L, Baltimore D (2008). Sustained expression of microRNA-155 in hematopoietic stem cells causes a myeloproliferative disorder. J Exp Med, 205(3): 585–594

Palis J (2008). Ontogeny of erythropoiesis. Curr Opin Hematol, 15(3): 155–161

Palis J (2014). Primitive and definitive erythropoiesis in mammals. Front Physiol, 5: 3

Papayannopoulou T, Kalmantis T, Stamatoyannopoulos G (1979). Cellular regulation of hemoglobin switching: evidence for inverse relationship between fetal hemoglobin synthesis and degree of maturity of human erythroid cells. Proc Natl Acad Sci USA, 76(12): 6420–6424

Pase L, Layton J E, Kloosterman W P, Carradice D, Waterhouse P M, Lieschke G J (2009). miR-451 regulates zebrafish erythroid maturation *in vivo* via its target gata2. Blood, 113(8): 1794–1804

Patrick D M, Zhang C C, Tao Y, Yao H, Qi X, Schwartz R J, Jun-Shen Huang L, Olson E N (2010). Defective erythroid differentiation in miR-451 mutant mice mediated by 14-3-3ζ. Genes Dev, 24(15): 1614–1619

Pekarsky Y, Santanam U, Cimmino A, Palamarchuk A, Efanov A, Maximov V, Volinia S, Alder H, Liu C G, Rassenti L, Calin G A, Hagan J P, Kipps T, Croce C M (2006). Tcl1 expression in chronic lymphocytic leukemia is regulated by miR-29 and miR-181. Cancer Res, 66(24): 11590–11593

Rasmussen K D, Simmini S, Abreu-Goodger C, Bartonicek N, Di Giacomo M, Bilbao-Cortes D, Horos R, Von Lindern M, Enright A J, O'Carroll D (2010). The miR-144/451 locus is required for erythroid homeostasis. J Exp Med, 207(7): 1351–1358

Saki N, Abroun S, Soleimani M, Hajizamani S, Shahjahani M, Kast R E, Mortazavi Y (2015). Involvement of microRNA in t-cell differentia-

tion and malignancy. Int J Hematol Oncol Stem Cell Res, 9(1): 33–49

Sangokoya C, Telen M J, Chi J T (2010). microRNA miR-144 modulates oxidative stress tolerance and associates with anemia severity in sickle cell disease. Blood, 116(20): 4338–4348

Sankaran V G (2011). Targeted therapeutic strategies for fetal hemoglobin induction. Hematology (Am Soc Hematol Educ Program), 2011(1): 459–465

Sarakul O, Vattanaviboon P, Tanaka Y, Fucharoen S, Abe Y, Svasti S, Umemura T (2013). Enhanced erythroid cell differentiation in hypoxic condition is in part contributed by miR-210. Blood Cells Mol Dis, 51(2): 98–103

Shiozaki M, Sakai R, Tabuchi M, Nakamura T, Sugino K, Sugino H, Eto Y (1992). Evidence for the participation of endogenous activin A/erythroid differentiation factor in the regulation of erythropoiesis. Proc Natl Acad Sci USA, 89(5): 1553–1556

Shivdasani R A (2006). MicroRNAs: regulators of gene expression and cell differentiation. Blood, 108(12): 3646–3653

Silva M, Benito A, Sanz C, Prosper F, Ekhterae D, Nuñez G, Fernandez-Luna J L (1999). Erythropoietin can induce the expression of bcl-x(L) through Stat5 in erythropoietin-dependent progenitor cell lines. J Biol Chem, 274(32): 22165–22169

Stamatoyannopoulos G (2005). Control of globin gene expression during development and erythroid differentiation. Exp Hematol, 33 (3): 259–271

Stenmark H (2009). Rab GTPases as coordinators of vesicle traffic. Nat Rev Mol Cell Biol, 10(8): 513–525

Sturgeon C M, Chicha L, Ditadi A, Zhou Q, McGrath K E, Palis J, Hammond S M, Wang S, Olson E N, Keller G (2012). Primitive erythropoiesis is regulated by miR-126 via nonhematopoietic Vcam-1 + cells. Dev Cell, 23(1): 45–57

Svasti S, Masaki S, Penglong T, Abe Y, Winichagoon P, Fucharoen S, Umemura T (2010). Expression of microRNA-451 in normal and thalassemic erythropoiesis. Ann Hematol, 89(10): 953–958

Tsiftsoglou A S, Vizirianakis I S, Strouboulis J (2009). Erythropoiesis: model systems, molecular regulators, and developmental programs. IUBMB Life, 61(8): 800–830

Valencia-Sanchez M A, Liu J, Hannon G J, Parker R (2006). Control of translation and mRNA degradation by miRNAs and siRNAs. Genes Dev, 20(5): 515–524

van de Loosdrecht A A, Vellenga E (2000). Myelodysplasia and apoptosis: new insights into ineffective erythropoiesis. Med Oncol, 17(1): 16–21

Vasilatou D, Papageorgiou S, Pappa V, Papageorgiou E, Dervenoulas J (2010). The role of microRNAs in normal and malignant hematopoiesis. Eur J Haematol, 84(1): 1–16

Wadman I A, Osada H, Grütz G G, Agulnick A D, Westphal H, Forster A, Rabbitts T H (1997). The LIM-only protein Lmo2 is a bridging molecule assembling an erythroid, DNA-binding complex which includes the TAL1, E47, GATA-1 and Ldb1/NLI proteins. EMBO J, 16(11): 3145–3157

Walker A L, Steward S, Howard T A, Mortier N, Smeltzer M, Wang Y D, Ware R E (2011). Epigenetic and molecular profiles of erythroid cells after hydroxyurea treatment in sickle cell anemia. Blood, 118 (20): 5664–5670

Wall L, deBoer E, Grosveld F (1988). The human beta-globin gene 3′ enhancer contains multiple binding sites for an erythroid-specific protein. Genes Dev, 2(9): 1089–1100

Wang F, Yu J, Yang G H, Wang X S, Zhang J W (2011). Regulation of erythroid differentiation by miR-376a and its targets. Cell Res, 21(8): 1196–1209

Wang F, Zhu Y, Guo L, Dong L, Liu H, Yin H, Zhang Z, Li Y, Liu C, Ma Y, Song W, He A, Wang Q, Wang L, Zhang J, Li J, Yu J (2013). A regulatory circuit comprising GATA1/2 switch and microRNA-27a/24 promotes erythropoiesis. Nucleic Acids Res, 42(1): 442–457

Wang L, Li L, Chu S, Shiang K, Li M, Sun H, Xu J, Xiao F J, Sun G, Rossi J J, Ho Y, Bhatia R (2014). MicroRNA-486 regulates normal erythropoiesis and enhances growth and modulates drug response in CML progenitors. Blood, 125(8):1302–1303

Wang Q, Huang Z, Xue H, Jin C, Ju X L, Han J D J, Chen Y G (2008). MicroRNA miR-24 inhibits erythropoiesis by targeting activin type I receptor ALK4. Blood, 111(2): 588–595

Warren A J, Colledge W H, Carlton M B, Evans M J, Smith A J, Rabbitts T H (1994). The oncogenic cysteine-rich LIM domain protein rbtn2 is essential for erythroid development. Cell, 78(1): 45–57

Welch J J, Watts J A, Vakoc C R, Yao Y, Wang H, Hardison R C, Blobel G A, Chodosh L A, Weiss M J (2004). Global regulation of erythroid gene expression by transcription factor GATA-1. Blood, 104(10): 3136–3147

Williams D E, Eisenman J, Baird A, Rauch C, Van Ness K, March C J, Park L S, Martin U, Mochizuki D Y, Boswell H S, Burgess G S, Cosman D, Lyman S D (1990). Identification of a ligand for the *c-kit* proto-oncogene. Cell, 63(1): 167–174

Wu H, Liu X, Jaenisch R, Lodish H F (1995). Generation of committed erythroid BFU-E and CFU-E progenitors does not require erythropoietin or the erythropoietin receptor. Cell, 83(1): 59–67

Xu M J, Sui X, Zhao R, Dai C, Krantz S B, Zhao Z J (2003). PTP-MEG2 is activated in polycythemia vera erythroid progenitor cells and is required for growth and expansion of erythroid cells. Blood, 102(13): 4354–4360

Xu R H, Sampsell-Barron T L, Gu F, Root S, Peck R M, Pan G, Yu J, Antosiewicz-Bourget J, Tian S, Stewart R, Thomson J A (2008). NANOG is a direct target of TGFbeta/activin-mediated SMAD signaling in human ESCs. Cell Stem Cell, 3(2): 196–206

Yang J S, Lai E C (2011). Alternative miRNA biogenesis pathways and the interpretation of core miRNA pathway mutants. Mol Cell, 43(6): 892–903

Yu D, dos Santos C O, Zhao G, Jiang J, Amigo J D, Khandros E, Dore L C, Yao Y, D'Souza J, Zhang Z, Ghaffari S, Choi J, Friend S, Tong W, Orange J S, Paw B H, Weiss M J (2010). miR-451 protects against erythroid oxidant stress by repressing 14-3-3ζ. Genes Dev, 24(15): 1620–1633

Yuan J, Angelucci E, Lucarelli G, Aljurf M, Snyder L M, Kiefer C R, Ma L, Schrier S L (1993). Accelerated programmed cell death (apoptosis) in erythroid precursors of patients with severe beta-thalassemia (Cooley's anemia). Blood, 82(2): 374–377

Zhai P F, Wang F, Su R, Lin H S, Jiang C L, Yang G H, Yu J, Zhang J W (2014). The regulatory roles of microRNA-146b-5p and its target platelet-derived growth factor receptor α (PDGFRA) in erythropoiesis and megakaryocytopoiesis. J Biol Chem, 289(33): 22600–22613

Zhan M, Miller C P, Papayannopoulou T, Stamatoyannopoulos G, Song C Z (2007). MicroRNA expression dynamics during murine and

human erythroid differentiation. Exp Hematol, 35(7): 1015–1025

Zhang L, Flygare J, Wong P, Lim B, Lodish H F (2011). miR-191 regulates mouse erythroblast enucleation by down-regulating Riok3 and Mxi1. Genes Dev, 25(2): 119–124

Zhang L, Sankaran V G, Lodish H F (2012). MicroRNAs in erythroid and megakaryocytic differentiation and megakaryocyte-erythroid progenitor lineage commitment. Leukemia, 26(11): 2310–2316

Zhao H, Kalota A, Jin S, Gewirtz A M (2009). The c-myb proto-oncogene and microRNA-15a comprise an active autoregulatory feedback loop in human hematopoietic cells. Blood, 113(3): 505–516

Zhou D, Liu K, Sun C W, Pawlik K M, Townes T M (2010). KLF1 regulates BCL11A expression and gamma- to beta-globin gene switching. Nat Genet, 42(9): 742–744

Zhu Y, Wang D, Wang F, Li T, Dong L, Liu H, Ma Y, Jiang F, Yin H, Yan W, Luo M, Tang Z, Zhang G, Wang Q, Zhang J, Zhou J, Yu J (2013). A comprehensive analysis of GATA-1-regulated miRNAs reveals miR-23a to be a positive modulator of erythropoiesis. Nucleic Acids Res, 41(7): 4129–4143