Published in final edited form as:
Br J Haematol. 2013 May ; 161(4): 471–483. doi:10.1111/bjh.12276.

MicroRNAs in myeloproliferative neoplasms

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Summary

The chronic myeloproliferative neoplasms (MPN), including polycythaemia vera (PV), essential thrombocythaemia (ET) and primary myelofibrosis (PMF), are clonal stem cell disorders characterized by dysregulated haematopoietic stem cell expansion and production of red cells, white cells and platelets alone or in combination. An acquired mutation *JAK2*^{V617F} can be found in all three disorders and shows many of the phenotypic abnormalities of the diseases in murine models. The disease phenotype is also influenced by other unknown genetic or epigenetic factors. MicroRNAs (miRNA) are 18–24 nucleotide single-stranded non-protein-coding RNAs that function primarily as gene repressors by binding to their target messenger RNAs. There is growing evidence that miRNAs regulate haematopoiesis in both haematopoietic stem cells and committed progenitor cells. Here, we review the field of miRNA biology and its regulatory roles in normal haematopoiesis with an emphasis on miRNA deregulations in MPNs. Continued research into how miRNAs impact *JAK2*^{V617F} clonal expansion, differential haematopoiesis among different MPNs, disease progression and leukaemia transformation will lead to a better understanding of the development of these disorders, their clinical manifestations, and their treatment.

Keywords

microRNA; myeloproliferative neoplasm; polycythaemia vera; essential thrombocythaemia; myelofibrosis

The chronic myeloproliferative neoplasms (MPN), including polycythaemia vera (PV), essential thrombocythaemia (ET) and primary myelofibrosis (PMF), are clonal stem cell disorders characterized by dysregulated stem cell expansion and production of red cells, white cells and platelets alone or in combination, a tendency to extramedullary haematopoiesis, complications of thrombosis and/or haemorrhage, and transformation to

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Author contributions

HZ researched and wrote the manuscript. CC and AR reviewed and edited the manuscript. All authors approved the submitted version of the manuscript.

acute leukaemia or myelofibrosis at variable rates. The discovery of a somatic mutation (V617F) in the negative regulatory domain of the tyrosine kinase Janus Kinase 2 (*JAK2*; Baxter *et al*, 2005; James *et al*, 2005; Kralovics *et al*, 2005; Levine *et al*, 2005; Zhao *et al*, 2005) was the most important advance in MPN since the discoveries 30 years ago that haematopoiesis in these disorders was both autonomous and clonal (Adamson *et al*, 1976) $JAK2^{V617F}$ can be detected in >95% PV patients and in 50–60% of ET and PMF patients (Jones *et al*, 2005). Although murine models have provided unequivocal evidence that $JAK2^{V617F}$ is able to cause MPNs, there is significant heterogeneity in disease phenotypes between different murine lines and even within the same line, suggesting that disease phenotype is affected by other unknown genetic or epigenetic factors (Chen *et al*, 2010).

MicroRNAs (miRNA) are 18–24 nucleotide single-stranded non-protein-coding RNAs that are phylogenetically conserved during evolution and function primarily as gene repressors by binding to the target messenger RNAs (mRNA) to regulate gene expression (Ambros, 2008). There is growing evidence that miRNAs regulate haematopoiesis in both haematopoietic stem cells (HSC) and committed progenitor cells (O'Connell & Baltimore, 2012). Recent progress suggests that deregulated miRNAs may contribute to MPN pathogenesis. Here, we review the field of miRNA biology and its regulatory roles in normal haematopoiesis with an emphasis on miRNA deregulation in myeloproliferative neoplasms.

MicroRNAs biogenesis and function

It is estimated that miRNAs comprise *c*. 1% of the human genome. MiRNAs are encoded either in independent transcription units or within the introns of protein-coding genes (Bartel, 2004). The 'classic' miRNA biogenesis pathway involves the stepwise processing of double-stranded miRNA precursors in the nucleus and then in the cytoplasm (Bartel, 2004; Fig 1). MiRNA genes are transcribed by RNA polymerase II into a long non-coding RNA known as the primary miRNA (pri-miRNA, usually several kilobases in length). This primary transcript folds on itself to form hairpin-loop structures that can be recognized and cleaved by the nuclear microprocessor complex into a miRNA precursor (pre-miRNA, *c*. 60–80 nucleotides). The nuclear microprocessor complex is composed of the RNase III DROSHA (Lee *et al*, 2003; Denli *et al*, 2004) and the DiGeorge Critical Region 8 (DGCR8) protein (Gregory *et al*, 2004; Landthaler *et al*, 2004), which is an RNA-binding protein whose deficiency contributes to the Di-George syndrome where patients develop congenital heart defects, characteristic facial appearance, thymic hypoplasia and immunodeficiency (Shiohama *et al*, 2003). Following its export from the nucleus, the pre-miRNA is cleaved by another RNase III DICER, which results in a 18–24 nucleotide miRNA: miRNA* duplex (Bernstein *et al*, 2001). The duplex is then unwound and one strand is associated with the Argonaute family of proteins which is the central component of the RNA-induced silencing complex (RISC; Kim, 2005). Once incorporated into the RISC, the single-stranded mature miRNA will guide the RISC to interact with the target mRNA via the latter's 3′-untranslated region. Recently, DROSHA-independent or DICER-independent production of miRNAs has been reported (Ruby *et al*, 2007; Cheloufi *et al*, 2010; Cifuentes *et al*, 2010).

Half of the known miRNAs are found in close proximity to other miRNAs and are frequently transcribed together as polycistronic primary transcripts that are processed into

multiple individual mature miRNAs (Lee *et al*, 2002; Stefani & Slack, 2008). Currently only a few miRNA promoters have been identified experimentally (Cai *et al*, 2004; Lee *et al*, 2004). The expression of miRNAs can be regulated at the transcriptional level by specific transcription factors (e.g. TP53, MYC; Johnson *et al*, 2003; He *et al*, 2007; Chang *et al*, 2008) and/or at the post-transcriptional level by mechanisms such as miRNA editing (Johnson *et al*, 2003; Luciano *et al*, 2004; Kawahara *et al*, 2007). Very little is known about the half-lives of most miRNAs or the mechanisms of miRNA decay.

Structural, biochemical, and bioinformatics analyses indicate that a *c*. 7-nucleotide seed sequence at the 5′ end of the miRNA (centred on nucleotides 2–8) is most critical in mediating the interaction with its target while the 3′ end mismatches are the most common (Bartel, 2009). Many different algorithms have been created to predict the targets of specific miRNAs (e.g. MiRanda, TargetScan, RNA22, miRDB, PicTar, and MicroCosm). Most take into account miRNA-mRNA sequence complementarity and miRNA-mRNA duplex thermodynamics, with various algorithms weighing the relative importance of these variables differently. However, the approach is hampered by the fact that the existing algorithms have a high margin of error (i.e. most predicted genes are not real targets and some key targets are not predicted) and different algorithms produce divergent results (Shirdel *et al*, 2011). Therefore, one challenge for research in this field is the reliable identification of the *in vivo* mRNA targets of miRNAs.

In most cases, miRNAs repress their targets by either target RNA degradation (primarily) or translation inhibition (Baek *et al*, 2008; Guo *et al*, 2010a; Fig 1). Overexpression of a single miRNA can result in decreased levels of more than 100 mRNAs (Lim *et al*, 2005). Conversely, the same mRNA can be targeted by multiple miRNAs (Wu *et al*, 2010). Generally, the regulatory impact of miRNA is to fine-tune but not abolish protein expression and miRNA loss of function rarely results in any highly penetrant phenotype in controlled steady-state laboratory conditions (Baek *et al*, 2008; Selbach *et al*, 2008). There are scattered reports of miRNAs that interact with their targets and upregulate the targets (Vasudevan *et al*, 2007). The key features and mechanisms of miRNA function remain an intense focus of current research. The concept that many miRNAs primarily function in response to physiological and pathophysiological stress (e.g. infection, inflammation, DNA damage, oncogene activation) is one that is gaining credence (Leung & Sharp, 2010). Recent studies showed that miRNAs rarely contribute significantly to normal cell development and their dysregulation usually is well tolerated in normal tissues; in contrast, miRNAs can profoundly affect cells and tissues under stress and in disease states (Mendell & Olson, 2012). This functional niche in stress responses suggests that miRNAs can play important roles in the haematopoietic system, which has an extraordinary rapid proliferation rate compared to other cells in human body and is regularly exposed to a variety of stress conditions that impact blood cell development.

MicroRNAs and haematopoiesis

Tight control of gene expression is required for haematopoiesis, which requires multiple cell-fate decisions that govern haematopoietic differentiation. There is growing evidence that miRNAs regulate haematopoiesis in both HSC and committed progenitor cells (Fig 2; Table

I). Here are some vignettes that illustrate the types of regulatory roles miRNAs have in haematopoiesis.

At the stem cell level, the miRNA processing enzyme DICER is essential to maintain a stable pool of HSC (Guo *et al*, 2010b). Some miRNAs are evolutionally conserved and enriched in HSCs to regulate HSC homeostasis. For example, MIR125A and MIR125B expand HSCs by inhibiting apoptosis (Guo *et al*, 2010b; O'Connell *et al*, 2010; Ooi *et al*, 2010). MIR196B is upregulated during the transition from longterm HSCs (LT-HSCs) to short-term HSCs (ST-HSCs) and is then downregulated in more differentiated cells; overexpression of MIR196B causes both differentiation block and reduced HSC capacity in a bone marrow reconstitution assay (Popovic *et al*, 2009; O'Connell *et al*, 2010).

At the progenitor cell level, recent studies showed that the developmental fate of the megakaryocyte-erythroid progenitor (MEP) cell, the common progenitor of the erythroid and megakaryocytic lineages, is regulated in part by miRNAs. For example, MIR150 is expressed in the MEP cell and its overexpression commits MEP toward megakaryocyte differentiation at the expense of erythrocytes (Lu *et al*, 2008). Conversely, MIR145 promotes erythroid differentiation by inhibiting FLI1, which is the key factor to promote megakaryocyte differentiation and inhibit erythroid differentiation of the MEP cells (Kumar *et al*, 2011).

At the more committed haematopoietic cell level, studies showed that there are indeed specific miRNA expressions in different blood cell lineages at different stages of haematopoietic differentiation. For example, MIR221, MIR222, MIR223 and MIR24 are downregulated during erythroid differentiation (Felli *et al*, 2005, 2009; Wang *et al*, 2008); in contrast, MIR144 and MIR451 are highly induced during terminal erythroid differentiation and maturation (Bruchova *et al*, 2007; Zhan *et al*, 2007; Dore *et al*, 2008). Similarly, MIR155 expression is dramatically reduced during megakaryocyte differentiation of the $CD34^+$ HSCs while MIR150 level is upregulated as the MEPs differentiate toward the megakaryocyte lineage (Barroga *et al*, 2008; Lu *et al*, 2008).

For more comprehensive reviews of miRNAs in erythropoiesis, megakaryopoiesis and myelopoiesis, please refer to the excellent reviews by Byon and Papayannopoulou (2012), Zhang *et al* (2012), Edelstein and Bray (2011), Li *et al* (2011) and Pelosi *et al* (2009). It is important to point out that miRNA function is highly dependent on the cell type in which it is induced and that the same miRNA may have very different functions in different cell types and stress conditions, as their effects depend on the mRNAs expressed. This specificity of action may serve as the basis for disease specificity of miRNA-based therapy.

MicroRNA deregulations in myeloproliferative neoplasms

Deregulated miRNAs have been reported in leukaemia, lymphoma and myeloma where they function either as tumour suppressors (e.g. MIR15A and MIR16-1 in chronic lymphocytic leukaemia) or as oncogenes (e.g. in B-cell lymphoma; Calin *et al*, 2004a,b; He *et al*, 2005; O'Donnell *et al*, 2005). Deregulated miRNA profiles have been reported in MPN patient primary samples, mostly peripheral blood mononuclear cells (Bruchova *et al*, 2007, 2008),

granulocytes (Guglielmelli *et al*, 2007; Bruchova *et al*, 2008; Slezak *et al*, 2009), reticulocytes (Bruchova *et al*, 2007, 2008), platelets (Bruchova *et al*, 2008; Girardot *et al*, 2010) and bone marrow megakaryocytes (Hussein *et al*, 2009a,b), as well as in various MPN cell lines (Bruchova-Votavova *et al*, 2010; Girardot *et al*, 2010; Chim *et al*, 2011; Bortoluzzi *et al*, 2012; Lin *et al*, 2012; Table II). The results from these studies revealed differential miRNA expression not only between MPN patients and healthy donors but also among the three disorders. As mentioned earlier, *JAK2*^{V617F} mutation is present in >95% PV patients and in 50–60% of ET and PMF patients (Jones *et al*, 2005). How the same mutation could be responsible for three different MPN phenotypes has never been satisfactorily explained. It is possible that disease-specific miRNA deregulations can contribute to the specific phenotypes of PV, ET, and PMF and can be used to differentiate the three disorders. Some deregulated miRNA expressions were reported to correlate with *JAK2*^{V617F} allele burden or JAK2 activity in either primary patient samples (Girardot *et al*, 2010; Guglielmelli *et al*, 2011a) or *JAK2*^{V617F}-mutated cell lines (Guglielmelli *et al*, 2011a; Lin *et al*, 2012), suggesting that miRNAs may be the downstream targets of abnormal JAK2 signalling. However, many miRNA deregulations were not related to *JAK2*^{V617F} allele burden or JAK2 activity. In our recent study of miRNA expression profiling in PV patients, we did not observe any correlation between the miRNA expression levels and CD34^{+} cell *JAK2*^{V617F} allele burdens (Zhan *et al*, 2012). This suggests that many deregulated miRNAs may act as an independent phenomenon from the abnormal JAK2 signalling in MPN pathogenesis and disease phenotype determination. In this sense, it would be interesting to study both the different and shared miRNA deregulations between *JAK2*^{V617F}-positive and *JAK2*^{V617F}-negative diseases to provide clues to how *JAK2*^{V617F}-negative diseases develop. As the MPNs are stem cell disorders, comprehensive miRNA analysis in MPN stem cells is needed to further delineate their roles in MPN pathogenesis; so far there is only limited data in this area (Bruchova *et al*, 2007; Guglielmelli *et al*, 2007, 2011b; Lin *et al*, 2012; Zhan *et al*, 2012).

Valuable insights regarding the roles of miRNAs in MPN pathogenesis have also been gained from genetically engineered mice models with gain- and loss-of-function alleles of specific miRNAs. (Table II) For example, sustained expression of a single miRNA MIR125B or MIR155, or knock-out of MIR146A in the mouse haematopoietic system could cause the myeloproliferative disorder phenotype (O'Connell *et al*, 2008, 2010; Bousquet *et al*, 2010; Zhao *et al*, 2011). MIR125B expands HSC by inhibiting apoptosis, probably through regulation of the TP53 pathway genes (Le *et al*, 2009; O'Connell *et al*, 2010; Ooi *et al*, 2010). Both MIR155 and MIR146A are upregulated in response to inflammatory stimuli but have opposite actions in granulocyte-monocyte haematopoiesis (Taganov *et al*, 2006; O'Connell *et al*, 2008; Boldin *et al*, 2011; Zhao *et al*, 2011). These transgenic mice studies suggest that both stem cell deregulation and inflammatory stress signalling are important in MPN pathogenesis. This is not surprising as inflammatory stress has a clear impact on blood cell development and blood diseases via the deregulated production of various cytokines and growth factors (Mendell & Olson, 2012; O'Connell & Baltimore, 2012). Indeed, elevated circulating cytokine levels have been reported in PV and PMF patients and predict poorer outcomes(Tefferi *et al*, 2011; Vaidya *et al*, 2012).

Though there has been recent progress (Girardot *et al*, 2010; Lin *et al*, 2012), knowledge regarding the physiologically relevant targets of MPN-associated miRNAs is still lacking.

Many studies only reported the correlation between certain microRNA expressions and their putative targets without functional verification (Guglielmelli *et al*, 2007; Bruchova *et al*, 2008; Hussein *et al*, 2009a). Indeed, one challenge for research in this field is the reliable identification of the *in vivo* mRNA targets and the proteins/pathways that may be influenced by miRNA deregulation and are relevant for MPN pathogenesis.

Given that miRNAs have important roles in the maintenance and development of HSC and in the fine tuning of haematopoietic lineage differentiation, deregulated miRNAs could contribute to MPN stem cell clone expansion and regulate the differential haematopoietic lineage commitment among different MPN phenotypes. We think the following areas would be of particular interest for future miRNA research in MPN.

JAK2^{V617F} clonal expansion

The stem cell compartment in MPN is heterogeneous with the presence of both *JAK2* wild-type clones and *JAK2*^{V617F} mutant clones in most MPN patients. In ET, there is coexistence of the mutant clone and the wild-type clone with no change in the mutant/wild-type cell ratio over long term follow up (Lambert *et al*, 2009). In contrast, in PV, there is usually a gradual increase in the *JAK2*^{V617F} mutant stem/progenitor cell population over time with a decrease in the normal stem cell population (Scott *et al*, 2006; Stein *et al*, 2010). Furthermore, in PV, there are usually more than two distinct homozygous clones and the dominant clone can expand up to *c*. 100-fold of the minor clones (Godfrey *et al*, 2012). Transition from heterozygous to homozygous *JAK2*^{V617F} mutation can be caused by mitotic recombination (Kralovics *et al*, 2005). Although there are conflicting data, most studies showed that the *JAK2*^{V617F} mutation does not provide the mutant haematopoietic stem and progenitor cells with a proliferation advantage (Kralovics *et al*, 2005; Li *et al*, 2010; Mullally *et al*, 2010, 2012; Anand *et al*, 2011). How the *JAK2*^{V617F} mutant clone continues to proliferate and expand is poorly understood.

As mentioned earlier, some miRNAs (e.g. MIR125, MIR196B) are evolutionally conserved and enriched in HSCs to regulate HSC homeostasis (Fig 2). Sustained expression of MIR125 in the mouse haematopoietic system caused the mye-loproliferative disorder phenotype (Bousquet *et al*, 2010; O'Connell *et al*, 2010; Guo *et al*, 2012). Our recent study in PV patient peripheral blood CD34^{+} cells showed that there is downregulation of MIR196B in both male and female PV patients (Zhan *et al*, 2012.) These deregulated stem-progenitor cell miRNAs may have a role in *JAK2*^{V617F} clonal expansion. Research in this field will increase our understanding of the pathogenesis of MPNs and identify potential targets for disease-eradicating therapy.

Haematopoietic lineage commitment among different JAK2^{V617F}-positive MPNs

Although PV, ET and PMF share many characteristics, there are distinctive features of each disorder and each has a unique epidemiology and natural history (Zhan & Spivak, 2009). In particular, PV is characterized by raised red cell mass and sometimes increased platelet and white cell counts, while ET is defined by an elevated platelet count but normal red cell mass. As the erythroid and megakaryocytic lineages are closely associated during differentiation and are generated from a common progenitor cell, additional mechanisms must exist to

regulate erythropoiesis and megakaryopoiesis differently between *JAK2*^{V617F}-positive PV and *JAK2*^{V617F}-positive ET patients (Chen *et al*, 2010).

MiRNAs are involved in the fine tuning of haematopoietic lineage differentiation and the developmental fate of haematopoietic stem/progenitor cells is partly regulated by miRNAs (Zhang *et al*, 2012; Fig 2). Therefore, deregulated miRNAs may modify the expression/signalling of *JAK2*^{V617F} and regulate differential haematopoietic lineage commitment between *JAK2*^{V617F}-positive PV and *JAK2*^{V617F}-positive ET patients. As mentioned above, MIR145 promotes erythrocyte differentiation of the megakaryocyte-erythroid progenitor cells and MIR451 is an erythroid-specific miRNA that is significantly upregulated during erythroid differentiation. Upregulation of MIR451 and MIR145 was previously reported during in vitro erythroid differentiation from PV patient peripheral blood mononuclear cells and in PV patient peripheral blood mononuclear cells respectively (Bruchova *et al*, 2007, 2008). Enforced expression of MIR451 promoted erythroid differentiation of the K562 cells and mice deficient in MIR451 had impaired late erythroblast maturation and impaired stress haematopoiesis (Bruchova-Votavova *et al*, 2010; Rasmussen *et al*, 2010; Table II). Recently, we found that there was upregulation of MIR451 and MIR145 in PV peripheral blood CD34^{+} cells (Zhan *et al*, 2012). These data are consistent with a role for these miRNAs in erythropoiesis, the principal feature of PV. When we examined the nucleated erythroid cells of the Burst Forming Unit-Erythroid (BFU-E) colonies, there appeared to be greater MIR145 and MIR451 expression in PV BFU-E colony cells than in ET (both *JAK2*^{V617F}-positive and *JAK2*^{V617F}-negative) BFU-E colony cells (Zhan *et al*, 2012). This suggests that an erythroid miRNA signature (e.g. upregulation of MIR451 and MIR145) in PV may serve in part as the mechanism for promoting the differential phenotypes between PV and ET. Although it has long been recognized that the *JAK2*^{V617F} allele burden is higher in PV as opposed to ET and *JAK2*^{V617F} mutation in ET patients is associated with higher haemoglobin levels (Campbell *et al*, 2005; Moliterno *et al*, 2008), we did not observe any correlation between the MIR145 or MIR451 expression levels and the CD34^{+} cell allele burden in our study. Further characterization of the roles of miRNAs in the haematopoietic lineage commitment among different MPNs should lead to a better understanding of the development of these diseases, their clinical manifestations, and their treatment.

Disease progression and leukaemia transformation

A major cause of morbidity and mortality in MPNs results from their transformation to acute leukaemia at variable rates. The leukaemic blasts in transformed *JAK2*^{V617F} positive MPNs are frequently negative for the *JAK2*^{V617F} mutation, suggesting that leukaemia probably originates from a malignant clone that has not acquired the *JAK2*^{V617F} mutation, consistent with the notion that *JAK2*^{V617F} is not the disease-initiating event in MPN (Theocharides *et al*, 2007). The exact mechanism of leukaemia transformation in MPN is not clear and this has contributed to the lack of effective treatment in these patients.

MiRNAs are involved in many fundamental processes, such as apoptosis, differentiation and proliferation (Bartel, 2004). More than 50% of miRNA genes are located in cancer-associated genomic regions or in fragile sites, which are frequently amplified or deleted in human cancers, suggesting an important role of miRNAs in malignant transformation(Calin

et al, 2004b). Several miRNAs have been implicated in cancer development by modulating oncogenic (e.g. *MYC*) and tumour suppressor (e.g. *TP53*) pathways (He *et al*, 2005, 2007; O'Donnell *et al*, 2005). Recurrent genetic and epigenetic alterations of miRNAs have been found in some tumours, suggesting that miRNAs can also act as oncogenes or tumour suppressor genes themselves (Ventura & Jacks, 2009). A better understanding of miRNA deregulation in MPN disease progression and leukaemia transformation will provide insight into the mechanisms of MPN tumourigenesis and provide new therapeutic strategies against MPN leukaemia transformation.

Potential microRNA-based therapy in myeloproliferative neoplasms

It has become clear that MPNs, especially PV, are neither rare disorders nor restricted to older individuals (Ruggeri *et al*, 2003). MPNs are more prevalent than chronic myeloid leukaemia (CML) based on the most recent Surveillance Epidemiology and End Results (SEER) database (Howlader *et al*, 2011). In contrast to CML, which has become the remarkable story of scientific discovery that translates into an effective and non-toxic targeted therapy, MPNs represent an underserved group of disorders with significant disease-related morbidity and mortality including thrombosis, haemorrhage, and disease transformation. Although most patients with PV or ET could be well controlled for many years with aspirin, phlebotomies and cytoreductive agents, currently we do not have any targeted therapy that could modify the disease course of MPNs. The identification of the *JAK2*^{V617F} mutation in MPN patients has led to the development of small-molecule inhibitors of JAK2. Clinical trials of these agents revealed their ability to decrease the degree of splenomegaly and to improve systemic symptoms in some MPN patients (Harrison *et al*, 2012; Verstovsek *et al*, 2012). Unfortunately, JAK2 inhibitors do not seem to eradicate the malignant clone (Tefferi, 2012). Over the course of a decade of treating CML with another tyrosine kinase inhibitor imatinib, it has become evident that cure is difficult to achieve if the disease-initiating cells cannot be eliminated. In the case of MPN, the disease-initiating cells lie within the HSC compartment. As miRNAs play an important role in HSC homeostasis, they may contribute to *JAK2*^{V617F}-positive haematopoietic stem/precursor cell maintenance and/or expansion in MPNs and therefore could serve as a target for potential disease-eradicating therapies. Indeed, miRNAs may provide valuable targets as RNA-based inhibition can be designed based on sequence alone. The unique functional niche of miRNA, rendered by its role in physiological and pathophysiological stress response and its dependence on the specific mRNA expressed in the different cell types and conditions, provides the basis for specificity of miRNA-based therapy.

MiRNAs are readily inhibited by antisense oligonucleotides whose specificity, potency, and bioavailability can be enhanced by a variety of modifications. For example, antisense oligonucleotides with the locked nucleic acid (LNA) modification can be delivered systemically by intravenous, intraperitoneal, or subcutaneous injection with sufficient uptake to achieve therapeutic efficacy in the heart, vascular system, and immune system in animal models (Stenvang *et al*, 2008; Obad *et al*, 2011). On the other hand, the activity of miRNAs (e.g. tumour suppressor miRNAs) can be restored using double-stranded miRNA mimics. Efficient systemic delivery of miRNA mimics have been achieved with lipid-based nanoparticle packaging or adeno-associated virus vector in multiple mice tumour models

with significant tumour growth inhibition (Kota *et al*, 2009; Pramanik *et al*, 2011; Trang *et al*, 2011) Perhaps the most attractive aspect of miRNA-based therapy comes from the concept that miRNA dysregulation usually is well-tolerated in normal tissues yet can profoundly affect cells and tissues under pathological stress, which makes miRNA therapy a highly potent means to modulate the disease process while avoiding unwanted toxicity in normal tissues (Leung & Sharp, 2010; Mendell & Olson, 2012).

Conclusions

Despite the current gains in knowledge about miRNA biology in haematopoiesis, our understanding of miRNAs in myeloproliferative neoplasms remains limited. The collective findings suggest that aberrant expression of miRNAs contribute to MPN pathogenesis. Continued research into how miRNAs contribute to $JAK2^{V617F}$ clonal expansion, differential haematopoiesis among different MPNs, disease progression and leukaemia transformation will advance our understanding of MPN pathogenesis and provide insights for new disease-modifying or disease-eradicating therapeutic strategies.

Acknowledgments

Huichun Zhan is currently supported by Veterans Affairs (VA) Career Development Award Grant 10959632 and VA Rehabilitation Research and Development Service Center Grant B9212-C. We are grateful to Dr Jerry L. Spivak from Johns Hopkins University for his valuable advice and critical review during the preparation of the manuscript. We thank Dr Haoyi Zheng from New York University Medical Center for his indispensable help in editing the manuscript. We thank Dr Naomi Galili from Columbia University Medical Center for reviewing the manuscript.

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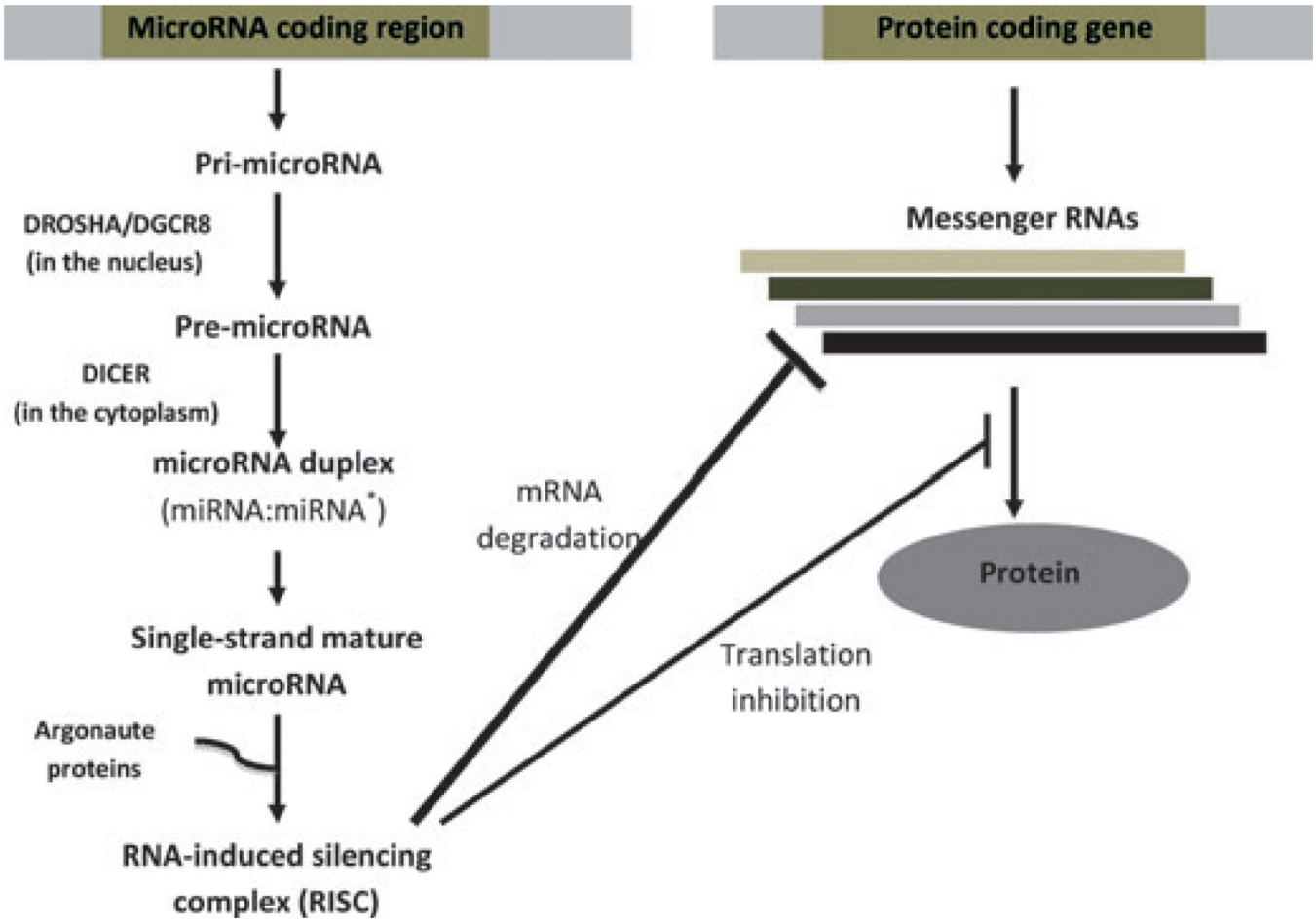

Fig 1.
MicroRNA biogenesis and function.

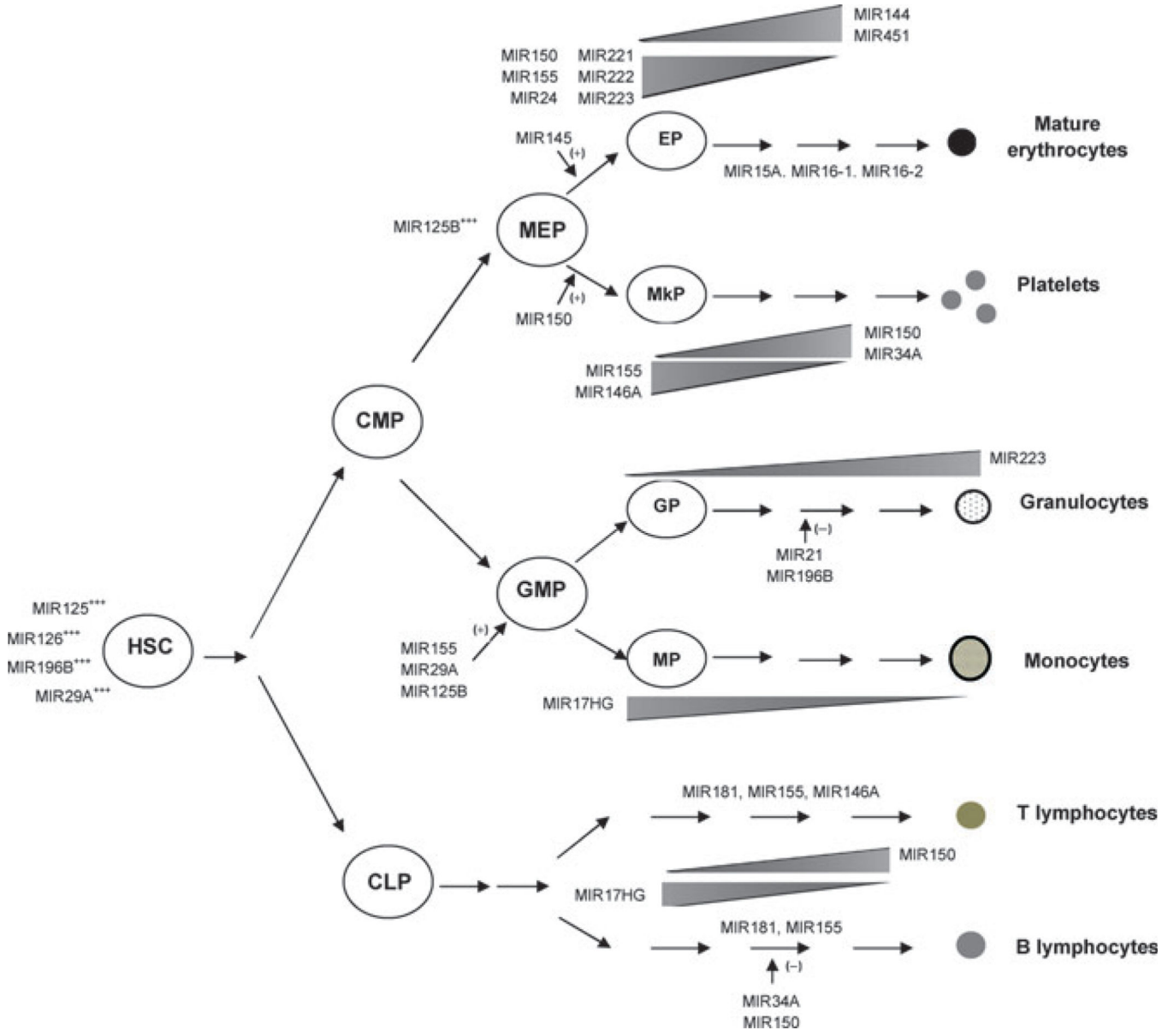

Fig 2.

MicroRNA expression in normal haematopoiesis. HSC, haematopoietic stem cell; CMP, common myeloid progenitor; CLP, common lymphoid progenitor; MEP, megakaryocyte-erythrocyte progenitor; GMP, granulocyte-monocyte progenitor; EP, erythroid progenitor; MkP, megakaryocyte progenitor; GP, granulocyte progenitor; MP, monocyte progenitor.

Table I

MicroRNA expression and function in normal haematopoiesis.

MicroRNA	Expression and function	References
MIR125	Enriched in HSC and expands HSC by inhibiting apoptosis.	Guo *et al* (2010b), O'Connell *et al* (2010), Ooi *et al* (2010)
MIR196B	Upregulated during the transition from long-term HSC to short-term HSC and is then downregulated in more differentiated cells; overexpression of MIR196B causes both differentiation block and reduced HSC capacity in a bone marrow reconstitution assay. Regulates specific HOX family members that control differentiation.	O'Connell *et al* (2010), Popovic *et al* (2009), Velu *et al* (2009), Yekta *et al* (2004)
MIR29	MiR-29A is highly expressed in HSC and downregulated in committed haematopoietic progenitors. Over expression of MIR29A exerts promyeloid differentiation and proliferation effects	Han *et al* (2010)
MIR126	Enriched in HSC and downregulated during progenitor cell differentiation.	Huang *et al* (2011), Shen *et al* (2008)
MIR150	Expressed in MEP cell and is upregulated as the MEPs differentiate toward the megakaryocyte lineage. Overexpression of MIR150 commits MEP toward megakaryocyte differentiation at the expense of erythrocytes. *MYB* is a target gene of MIR150.	Barroga *et al* (2008), Lu *et al* (2008)
	MIR150 is upregulated during B-cell and T cell maturation and controls *MYB* expression during lymphocyte development and response. Premature expression of MIR150 blocks the transition from pro-B to pre-B cell during B-cell maturation.	Monticelli *et al* (2005), Xiao *et al* (2007), Zhou *et al* (2007)
MIR145	Promotes erythroid differentiation by inhibiting FLI1, which is the key factor to promote megakaryocyte differentiation and inhibit erythroid differentiation of MEP cells.	Kumar *et al* (2011), Starczynowski *et al* (2010)
MIR146A	Downregulated during megakaryocytopoiesis. Overexpression impairs megakaryocytic proliferation, differentiation and maturation.	Labbaye *et al* (2008), Starczynowski *et al* (2010)
	Upregulated after immune cell maturation and/or activation. A negative feedback regulator of nuclear factor-κB activation. Prevalently expressed in Treg cells and is critical for their suppressor function. Deletion of MIR146A causes an overproduction of myeloid cells of the granulocyte–monocyte lineage.	Boldin *et al* (2011), Lu *et al* (2010), Taganov *et al* (2006), Zhao *et al* (2011)
MIR451	Upregulated during terminal erythroid differentiation and maturation. MIR451 is a direct transcriptional target of *GATA1* and it targets *gata2* in zebrafish and 14-3-3ζ in mice.	Bruchova *et al* (2007), Dore *et al* (2008), Pase *et al* (2009), Rasmussen *et al* (2010), Yu *et al* (2010), Zhan *et al* (2007)
MIR155	Downregulated during megakaryocyte differentiation. Over expression of MIR155 in HPC impairs megakaryocyte proliferation and differentiation.	Romania *et al* (2008)
	Over expression of MIR155 drives granulocyte/monocyte expansion.	O'Connell *et al* (2008)
	Upregulated in response to B- or T cell activation. Regulates germinal centre reaction and Treg cell differentiation and function.	Lu *et al* (2009), Rodriguez *et al* (2007), Thai *et al* (2007)
MIR221 MIR222	Downregulated during erythroid differentiation. Block erythroid differentiation by targeting *KIT*.	Felli *et al* (2005)
MIR223	Downregulated during erythroid differentiation. Blocks erythroid differentiation by targeting *LMO2*.	Felli *et al* (2009)
	Upregulated during granulocyte differentiation. Knock-down of *Mir223* in transgenic mice shows expanded granulocyte compartment and increased neutrophil activity.	Fazi *et al* (2005), Johnnidis *et al* (2008)
MIR24	Downregulated during erythroid differentiation. MIR24	Wang *et al* (2008)

MicroRNA	Expression and function	References
	inhibits erythropoiesis by targeting *ACVR1B*.	
MIR15A	Upregulated during erythroid differentiation. MYB and MIR15A comprise an autoregulatory loop during erythropoiesis.	Zhao *et al* (2009)
MIR17HG (MIR17-92)	Regulates B cell development at the pro-B to pre-B cell transition. Targets tumour suppressors PTEN and BCL2L11.	Koralov *et al* (2008), Xiao *et al* (2008)
	Downregulated during monocyte differentiation and maturation. Targets *RUNX1*.	Fontana *et al* (2007)
MIR181	Preferentially expressed in haematopoietic tissues (e.g. thymus, bone marrow, spleen) and is upregulated during B cell differentiation.	Chen *et al* (2004)
	MIR181A modulates T cell receptor signalling and regulates T cell sensitivity and selection.	Ebert *et al* (2009), Li *et al* (2007)
MIR34	Over expression of MIR34A blocks B cell development at the pro-B to pre-B cell transition by targeting the transcription factor FOXP1.	Rao *et al* (2010)
	Upregulated during thrombopoietin-induced differentiation of HPC, and its over expression enhanced megakaryocyte differentiation.	Navarro *et al* (2009)

HSC, haematopoietic stem cells; MEP, megakaryocyte-erythroid progenitor; HPC, haematopoietic progenitor cell.

Table II

MicroRNA deregulation in myeloproliferative neoplasms.

Studies from cell lines	Studies from primary patient samples	Studies from mice models
Upregulation of MIR451 and downregulation of MIR150 during erythropoiesis of K562 cells. Enforced expression of MIR451 promoted erythroid differentiation of K562 cells (Bruchova-Votavova *et al*, 2010)	MiRNA deregulations (e.g. downregulation of MIR150, −155 and upregulation of MIR451.) during *in vitro* erythroid differentiation of peripheral blood mononuclear cells from PV patients. Higher MIR451 level in PV CD 34^{+} cells than those in normal controls (Bruchova *et al*, 2007).	Mice deficient for MIR451 display impaired late erythroblast maturation and impaired response to oxidative stress, resulting in erythroid hyperplasia, splenomegaly, and mild anaemia (Rasmussen *et al*, 2010).
MIR28 targets the 3' UTR of the thrombopoietin receptor mRNA and inhibits its translation in Mo-7e cell line. JAK2^{V617F} induces MIR28 expression via the constitutive STAT5 activity in the UT-7 and Ba/F3 cell lines (Girardot *et al*, 2010).	MiRNA deregulations in PV granulocytes, mononuclear cells, platelets, and reticulocytes, e.g. upregulation of MIR143 and MIR145 in PV mononuclear cells, downregulation of MIR150 in PV reticulocytes (Bruchova *et al*, 2008).	Inhibiting MIR16-2 using specific antagomir in mice suppressed erythropoiesis (Guglielmelli *et al*, 2011b).
MiRNA profiling in the *JAK2*^{V617F}-mutated SET2 cell line revealed that 21 miRNAs (e.g. MIR146B-5p, MIR17, MIR19B, MIR92A, MIR181A/B) were highly expressed. Putative targets genes are enriched in the MAPK signalling pathway, TGF-β signalling pathway, mTOR signalling pathway, and Wnt signalling pathway (Bortoluzzi *et al*, 2012)	Overexpression of MIR16-2 in CD34^{+} cell of PV patients. Forced expression of MIR16-2 in normal CD34^{+} cells stimulated erythroid proliferation and differentiation, while inhibiting MIR16-2 in PV CD34^{+} cells reduced erythroid colony formation (Guglielmelli *et al*, 2011b).	Enforced expression of MIR155 in mouse HSC caused profound myeloid proliferation with dysplasia in bone marrow, splenomegaly, and extramedullary haematopoiesis (O'Connell *et al*, 2008).
Differential methylation status of *MIR34A/B/C* in *JAK2*^{V617F}-mutated HEL and SET-2 cell lines.	Upregulation of MIR575 and MIR887 and downregulation of MIR196B and MIR551B in both male and female PV patient peripheral blood CD34^{+} cells.	MIR125B was enriched in mouse HSC and its overexpression caused a dose-dependent myeloproliferative disorder that progressed to a lethal myeloid leukaemia (O'Connell *et al*, 2010).
Treatment with 5-azacitidine unmethylated *MIR34B/C* in HEL cells (Chim *et al*, 2011).	Upregulation of MIR451 and MIR145 in PV patient peripheral blood CD34^{+} cells compared to healthy controls (Zhan *et al*, 2012).	Overexpression of MIR125B in mouse fetal liver cells caused various haematological malignancies including MPN and acute lymphoid leukaemia in transplanted mice (Bousquet *et al*, 2010).
MIR125A-3p and MIR155 levels were affected by JAK2 activity in HEL cell line (Lin *et al*, 2012).	MiRNA deregulations in PMF patient peripheral blood CD34^{+} cells and granulocytes (Guglielmelli *et al*, 2007).	Knock-out of MIR146A in C57BL/6 mice led to splenomegaly with expanded myeloid haematopoiesis and bone marrow myeloproliferation and myelofibrosis (Zhao *et al*, 2011).
	MiR-28 expression is increased in *c*.30% of MPN (PV, ET, and PMF) patient platelets and may correlate with the *JAK2*^{V617V} allele burden. MIR28 inhibits megakaryocyte differentiation of human CD34^{+} cells (Girardot *et al*, 2010).	Over-expression of MIR29A in mouse haematopoietic stem/progenitor cells results in biased myeloid expansion and the development of a myeloproliferative disorder that progresses to acute myeloid leukaemia (Han *et al*, 2010).
	MiRNA deregulations (e.g. downregulation of MIR133A) in MPN patients (4 PV and 2 ET) peripheral blood neutrophils (Slezak *et al*, 2009). MiRNA deregulations in laser-microdissected megakaryocytes from PMF and ET patients (Hussein *et al*, 2009b).	Overexpression of MIR125A led to phenotypes consistent with an atypical MPN with leucocytosis, monocytosis, splenomegaly, and progressive anaemia. The phenotype depends on the sustained expression of MIR125A (Guo *et al*, 2012).
	MiRNA deregulations in laser-microdissected bone marrow megakaryocytes from PV, ET, and PMF patients (Hussein *et al*, 2009a).MiRNA deregulation (e.g. downregulation of MIR34A) in PMF, PV, or ET patient granulocytes compared to controls (Guglielmelli *et al*, 2007).	
	MiRNA microarray analysis in MPN (3 PV, 3 ET, and 1 PMF) peripheral blood CD34+ cells showed 61 miRNAs (e.g. MIR34A, MIR575, MIR146B-5p, MIR29) were significantly deregulated in MPNs and can only be partially attributed to JAK2 activity. MIR433 is significantly elevated in PV CD34^{+} cells and is upregulated during *in vitro* erythroid differentiation of normal CD34^{+} cells. MIR433 negatively regulates haematopoietic proliferation and differentiation in CD34^{+} cells (Lin *et al*, 2012).	

Studies from cell lines	Studies from primary patient samples	Studies from mice models
	Upregulation of MIR145 and MIR451 in PV BFU-E colony cells compared to ET (both *JAK2*^{V617F}-positive and *JAK2*^{V617F}-negative) BFU-E colony cells (Zhan *et al*, 2012).	

PV, polycythaemia vera, ET, essential thrombocythaemia; MPN, myeloproliferative neoplasms; PMF, primary myelofibrosis; HSC, haematopoietic stem cells.