miR-223: infection, inflammation and cancer

● **M. Haneklaus[1],*, M. Gerlic[2,3],*, L. A. J. O'Neill[1] & S. L. Masters[2,3]**

From the [1]Inflammation Research Group and Immunology Research Centre, School of Biochemistry and Immunology, Trinity College Dublin, Dublin, Ireland; [2]The Walter and Eliza Hall Institute of Medical Research; and [3]Department of Medical Biology, The University of Melbourne, Parkville, Vic., Australia

Abstract. Haneklaus M, Gerlic M, O'Neill LAJ, Masters SL (Inflammation Research Group and Immunology Research Centre, School of Biochemistry and Immunology, Trinity College Dublin, Dublin, Ireland; The Walter and Eliza Hall Institute of Medical Research, Parkville, Vic.; and Department of Medical Biology, The University of Melbourne, Parkville, Vic., Australia). miR-223: infection, inflammation and cancer. (Review). *J Intern Med* 2013; **274**: 215–226.

Expression of the microRNA miR-223 is deregulated during influenza or hepatitis B infection and in inflammatory bowel disease, type 2 diabetes, leukaemia and lymphoma. Although this may also be the result of the disease *per se,* increasing evidence suggests a role for miR-223 in limiting inflammation to prevent collateral damage during infection and in preventing oncogenic myeloid transformation. Validated targets for miR-223 that have effects on inflammation and infection include granzyme B, IKKα, Roquin and STAT3. With regard to cancer, validated targets include C/EBPβ, E2F1, FOXO1 and NFI-A. The effect of miR-223 on these targets has been documented individually; however, it is more likely that miR-223 affects multiple targets simultaneously for key processes where the microRNA is important. Such processes include haematopoietic cell differentiation, particularly towards the granulocyte lineage (where miR-223 is abundant) and as cells progress down the myeloid lineage (where miR-223 expression decreases). NF-κB and the NLRP3 inflammasome are important inflammatory mechanisms that are dampened by miR-223 in these cell types. The miRNA can also directly target viruses such as HIV, leading to synergistic effects during infection. Here we review the recent studies of miR-223 function to show how it modulates inflammation, infection and cancer development.

Keywords: cancer, infection, inflammation, miRNA-223.

Introduction

The discovery of microRNAs (miRNAs) revealed a new dimension in post-transcriptional regulation of gene expression. miRNAs are small, noncoding RNA molecules of ~22 nucleotides, which typically repress messenger RNAs (mRNAs) by binding to their 3′ untranslated region (UTR) (reviewed in [1–4]). miRNAs are transcribed as primary miRNA transcripts (pri-miRNAs), which contain a hairpin-like structure that will produce the mature miRNAs [5]. Most miRNAs are transcribed by RNA polymerase II from independent promoters, analogous to typical protein-coding genes [5]. However, transcription can also be dependent on RNA polymerase III when the miRNA is located downstream of repetitive elements such as Alu elements [6]. Alternatively, miRNAs can be embedded within long noncoding RNA or in an intron of a protein-coding gene, which releases the pri-miRNA in the process of splicing [7]. In this case, miRNA transcription is coupled to its host gene. Furthermore, several miRNAs are organized in genomic clusters, which produce a single polycistronic primary transcript that contains the hairpins of several miRNAs. Typically, these miRNAs have related biological functions [8]. To form a mature miRNA, the pri-miRNA hairpin is the substrate for two subsequent cleavage steps by members of the RNase III family. In the nucleus, it is recognized by the RNase III enzyme Drosha to produce the pre-miRNA, a ~70-nt-long precursor molecule, which is then exported to the cytoplasm by exportin-5. Because the structure of the processed pre-miRNA is the signal for nuclear export, the transport is independent of capping or polyadenylation status of the primary transcript [7].

Once in the cytosol, pre-miRNAs are further processed by Dicer, another member of the RNase III family, to produce the mature double-stranded molecule, which consists of the miRNA and its complementary miRNA*. One of the strands is then incorporated into a protein complex termed 'the

---

*These authors contributed equally to this work.

RNA-induced silencing complex (RISC)', which binds to the 3′UTR of mRNAs that contain target sites for the miRNA [7]. The specificity of the interaction is determined by a stretch of six to eight nucleotides in the 5′ end of the miRNA, its so-called seed region, which has perfect complementarity to the target mRNA. The rest of the miRNA sequence is less complementary to the target mRNA, which results in a bulged structure [9]. The central RISC component, a protein of the Argonaute family, can interact with a range of regulatory factors, such as GW182 and Rck/p54, which link the RISC to processing (P) bodies, where factors involved in mRNA degradation and translational repression are concentrated [10]. As a consequence, targeted mRNA molecules can be degraded following deadenylation and decapping and/or translationally repressed by inhibition of initiation and elongation [11, 12]. The relative importance of mRNA degradation versus translation inhibition is still a matter of debate but, given that these are highly coupled processes, it is likely that both are important and influence each other [13].

To date, more than 1500 human miRNAs have been identified as well as hundreds of viral miRNAs, each of which is predicted to have the potential to target dozens of genes. miRNAs have been shown to be important for the regulation of many cellular processes, and individual miRNAs have been found to be involved in more than one setting. Overall, miRNA expression data are very precise in classifying various cancers and providing information regarding lineage and differentiation status [14]. This shows that miRNAs regulate 'cellular identities' making them critical determinants of many biological functions.

In this review, we will consider the functions of only one miRNA, miR-223, in the context of inflammation, infection and cancer.

miR-223 and inflammation

miR-223 is upregulated during granulopoiesis

The miRNA-223 (miR-223) is transcribed from an independent promoter, which shows specific expression in the haematopoietic system [15–17]. A high expression level is seen in cells of the myeloid lineage, with granulocytes showing the highest levels [18] (see Fig. 1). Furthermore, miR-223 is upregulated during retinoic acid (RA)-induced differentiation of promyelocytic leukaemia

cell lines NB4 and HL60, as well as in primary leukaemic cells. This is caused by the induction of miR-223 gene expression by the myeloid transcription factors PU.1 and CAAT/enhancer-binding protein (C/EBP) β [19, 20]. The nuclear factor I-A (NFI-A) can maintain lower levels of miR-223 and stabilize the undifferentiated state of precursor cells as it competes for binding with C/EBPα, another strong inducer of miR-223 transcription [20, 21]. It is interesting that both NFI-A and C/EBPβ are direct targets of miR-223, thus forming negative feedback loops [20, 22]. Fazi *et al.* also showed that overexpression of miR-223 is sufficient to induce markers of differentiation in a myeloid precursor cell line and that inhibition of miR-223 reduces the efficiency of RA-induced differentiation [20]. Taken together, upregulation of miR-223 is clearly an important element of granulopoiesis, which is coordinated by the combined action of different myeloid transcription factors (Fig. 1).

In a recent report by Zardo *et al.* [23], a more surprising role of miR-223 on granulopoiesis was highlighted. The authors found that during differentiation, miR-223 is involved in epigenetically silencing NFI-A. During RA-induced differentiation of leukaemic cell lines, miR-223 appears to bind directly to partly complementary genomic sequences in the NFI-A promoter. As a result, a repressor complex including Ago1 and Dicer together with polycomb proteins can be formed, which is accompanied by the establishment of repressive histone marks, DNA methylation and reduced NFI-A transcription.

A distinct role of miR-223 during granulocyte differentiation was demonstrated by Johnnidis *et al.* [24] by the generation of an miR-223 mutant mouse ($miR\text{-}223^{-/Y}$). Of interest, $miR\text{-}223^{-/Y}$ mice did not fail to produce granulocytes; instead, granulocyte numbers were increased. The authors attributed this to the deregulation of Mef2c, a transcription factor that drives granulocyte–monocyte progenitor proliferation and is targeted by miR-223. Indeed, conditional deletion of Mef2c in the myeloid lineage of $miR\text{-}223^{-/Y}$ animals results in normal numbers of granulocytes. $miR\text{-}223^{-/Y}$ mice were also reported to produce hyperactive neutrophils and present with spontaneous lung inflammation, which was not mediated by Mef2c. Therefore, miR-223 seems to be involved in the regulation of the early and late stages of granulopoiesis, as well as in the homeostasis of mature
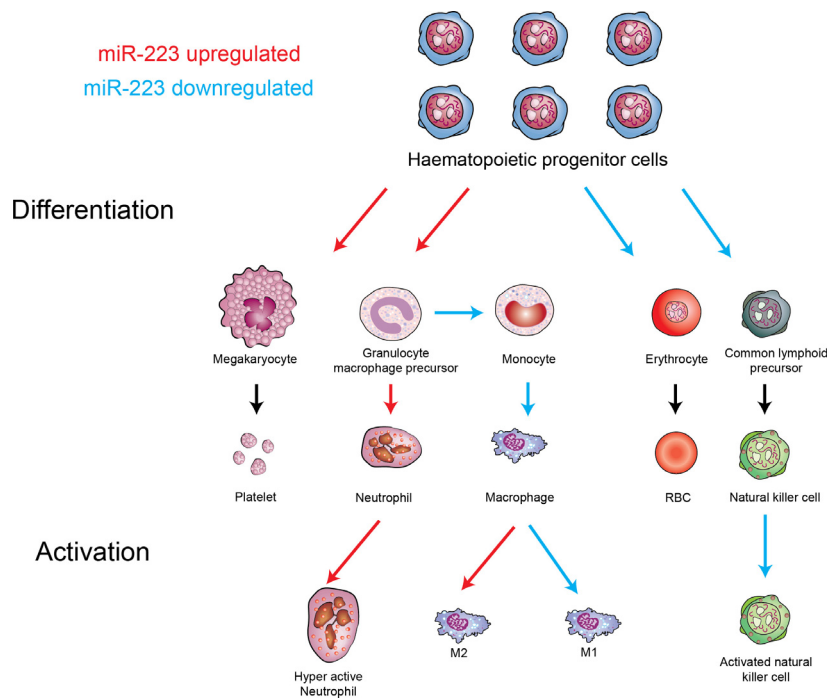
Fig. 1 *Expression of miR-223 during haematopoiesis. miR-223 expression increases as haematopoietic progenitor cells differentiate into red blood cells or natural killer cells, but decreases during differentiation into neutrophils, monocytes, megakaryocytes and eosinophils. Expression is also altered as these cells are further activated (neutrophils and natural killer cells) or polarized (macrophages).*

neutrophils. These findings were independently confirmed by reconstituting mice with bone marrow that expresses a miR-223 sponge construct, which acts as a miR-223 inhibitor. This study showed that impairing miR-223 function in the haematopoietic compartment replicates the phenotype of the *miR-223$^{-/Y}$* mouse [25].

To summarize, miR-223 is upregulated by myeloid transcription factors during granulocyte differentiation. However, this does not seem to be essential for granulopoiesis, because miR-223-deficient mice are able to produce neutrophils, albeit with an abnormal phenotype.

Other roles of miR-223 in haematopoietic differentiation

Besides granulocyte differentiation, miR-223 has been found to be involved in determining the identity of other cell types in the haematopoietic system. Together with other miRNAs, miR-223 fine-tunes the differentiation of myeloid precursors into either granulocytes or monocytes. As described above, miR-223 is strongly induced by

C/EBPα, a transcription factor that drives granulocyte differentiation. As miR-223 targets the NFI-A mRNA, proliferation of myeloid precursors is reduced upon C/EBPα activation, and the differentiation programme can be initiated [20]. Similarly, during osteoclast differentiation from precursors of the myeloid lineage, miR-223 upregulation by PU.1 is important to repress NFI-A, which would otherwise inhibit differentiation [26]. The importance for myeloid differentiation has also been demonstrated using an antagomiR inhibitor of miR-223, which abolishes GM-CSF (granulocyte–macrophage colony-stimulating factor)-induced differentiation of monocytes and PMA (phorbol-12-myristate-13-acetate)-induced differentiation of THP1 cells [27]. Interestingly, the authors also showed that miR-223 is enriched in microvesicles shed from differentiated macrophages and that these vesicles are sufficient to induce differentiation in monocytes. An increasing number of studies show that microvesicles (exosomes or microparticles) are a means of intercellular communication [28] that can even act over long distances because they can be found in the circulation. Even

though blocked differentiation by miR-223 inhibition implies a positive role of the miRNA on macrophage differentiation, we and others have shown that miR-223 is downregulated in macrophages compared with monocytes [24, 29, 30] (see Fig. 1). Nevertheless, the strong effect of miR-223 inhibition described above suggests that a low functional level of the miRNA is essential for monocyte differentiation.

Additionally, miR-223 has been found to negatively regulate erythroid [31] and positively regulate megakaryocyte differentiation by targeting LIM domain only 2 (LMO2) [32]. As C/EBPβ represses LMO2 expression and C/EBPβ is also a target of miR-223, these constitute a group of factors that collectively balance their own expression. With regard to the regulation of proliferation, LMO2 is probably the main effector [22]. Most recently, miR-223 expression was found to increase during eosinophil differentiation, such that eosinophil production is increased in miR-223$^{-/-}$ mice, probably due to effects on IGF1R (insulin-like growth factor 1 receptor) [33].

In summary, miR-223 expression changes considerably during cellular differentiation. It increases during osteoclast, megakaryocyte and eosinophil differentiation and decreases during erythrocyte and macrophage differentiation. The relevance of this change in expression has not been thoroughly investigated in each case, but it is clear that miR-223 is part of a regulatory network with several central factors, such as NFI-A, C/EBPβ and LMO2, which shape the cellular phenotypes.

Roles of miR-223 in immune cell function and activation

When maintained on a high-fat diet, *miR-223*$^{-/Y}$ mice revealed an additional inflammatory phenotype with more severe adipose tissue inflammation and insulin resistance [34]. This was accompanied by increased infiltration of macrophages and secretion of pro-inflammatory cytokines in the adipose tissue. Furthermore, the infiltrating macrophages expressed higher levels of markers of the classically activated pro-inflammatory phenotype (M1), suggesting a defect in macrophage polarization. On a molecular level, this deregulation may be partly due to the novel miR-223 target Pknox1. By targeting Pknox1, miR-223 helps to steer the activation pattern of macrophages towards the alternative M2 phenotype. In the context of obesity-induced adipose tissue inflammation, this is

beneficial as it reduces adverse metabolic phenotypes.

This is in agreement with recent findings by us and others regarding macrophage differentiation. As monocytes differentiate into macrophages (or dendritic cells), NLRP3, an important protein for the processing of the IL-1β and IL-18, is upregulated, although its mRNA levels are downregulated [30, 35]. This coincides with lower expression of miR-223 in mature myeloid cells. As miR-223 can target the NLRP3 3′UTR, it can prevent translation early in the myeloid lineage. Given the high miR-223 expression levels, the miRNA is likely to be an important regulator of the NLRP3 inflammasome in neutrophils [35]. Other groups have also found that miR-223 may influence IL-1β production, in this case due to an effect on STAT3 [36]. miR-223 downregulation was found to promote the production of IL-1β and IL-6 at a transcriptional level. Furthermore, miR-223 cooperated with miR-15a and miR-16 to enhance noncanonical NF-κB signalling during macrophage differentiation. These miRNAs were all found to target the inhibitor of NF-κB kinase α (IKKα). As the levels of all three miRNA decrease during macrophage differentiation, repression of IKKα is relieved and NF-κB signalling increases [29]. miR-223 expression also changes when other cell types are activated, for example in natural killer (NK) cells treated with IL-15. Here, decreased miR-223 levels result in increased expression of its target granzyme B, an effector of cytotoxic lymphocytes [37].

miR-223 is not only involved in the differentiation of a variety of immune cells, especially macrophages, but can also influence their activation pattern. For example, it can modulate macrophage polarization, as well as inflammasome and NF-κB signalling.

Role of miR-223 in inflammatory disorders

Global miRNA expression analyses revealed that miR-223 is deregulated in many inflammation-related disorders. For example, it is overexpressed in the synovium and peripheral T cells of patients with rheumatoid arthritis [38, 39], in peripheral blood mononuclear cells of patients with osteoarthritis [40] and in the colonic mucosa of patients with inflammatory bowel disease (IBD) [41]. Consequently, lentivirus-mediated silencing of miR-223 can suppress collagen-induced arthritis in mice [42]. In mouse models of IBD, miR-223 was

overexpressed in the colon and peripheral blood lymphocytes [43]. A link was proposed between the novel target Roquin, a negative regulator of IL-17 production in lymphocytes, and IBD pathogenesis. IL-17 is an important inflammatory cytokine; therefore, higher levels of miR-223 result in increased IL-17-mediated inflammation.

miR-223 is also deregulated in type 2 diabetes [44], which is consistent with an emerging body of evidence suggesting that this is an inflammatory disease with pathogenesis driven at least in part by pro-inflammatory cytokines. On the other hand, miR-223 is consistently upregulated in the insulin-resistant heart where it increases Glut4 expression to promote glucose uptake [45]. It is possible that increased miR-223 expression in the insulin-resistant heart may overcompensate for the systemic reduction in miR-223 seen during insulin resistance. In individuals with type 2 diabetes, increased miR-223 and Glut4-dependent glucose uptake may not be possible in the periphery, but in the heart this may have the opposite effect and increase metabolic processes that could be detrimental; this possibility is suggested by the fact that cardiac arrest is the primary cause of mortality in this disease.

Overall, evidence suggests a central position of miR-223 in maintaining the homeostasis of a wide range of processes and cell types in the immune system that is not merely restricted to haematopoietic differentiation. As miR-223 is the most abundant miRNA in peripheral blood microvesicles, it could even act as a systemic homeostatic factor [46]. The variety of functions of miR-223 has been linked to the suppression of many different target genes. Table 1 shows all the currently validated targets of miR-223; however, this is certainly not a complete list. The distinct expression pattern of miR-223 in different cell types, during differentiation and activation, provides interesting opportunities for the regulation of its targets in inflammatory pathologies.

miR-223 and infection

As discussed above, miR-223 regulates the differentiation of several key players of the innate immune response (e.g. neutrophils, monocytes and granulocytes), and therefore, it is likely that miR-223 will play an important role in the early stages of infection. Indeed, Johnnidis *et al.* showed enhanced killing of neutrophils co-cultured with *Candida albicans* in miR-223 mutant mice

(miR-$223^{-/Y}$), indicating increased fungicidal activity in these animals. Furthermore, miR-$223^{-/Y}$ mice exhibited elevated tissue destruction after endotoxin challenge [24]. Endotoxin challenge is a model of sepsis, a severe form of infection associated with inflammatory clinical symptoms such as fever, chills, malaise and high inflammatory cytokine levels. In the light of these data from miR-223 mutant mice, the observation that miR-223 expression is decreased during sepsis suggests that this miRNA could have a role in the disease pathology rather than serving as a biomarker alone [47]. Again this reflects the importance of miR-223 in negative regulation of the inflammatory process during infection.

miR-223 and viral infection

To date, understanding of the H1N1 influenza infection (the Spanish flu), considered to be the worst pandemic infection of the twentieth century, remains incomplete. Recently, MG Katze and colleagues found that miR-223 and other microRNAs were differentially expressed in response to influenza virus infection [48]. More specifically, miR-223 was strongly upregulated in r1918 infection and only moderately upregulated in control influenza infection. Severe acute respiratory syndrome (SARS), a twenty-first-century pandemic infection with typical clinical features of pulmonary fibrosis and lung failure that led to significant mortality, is caused by the coronavirus *SARS-CoV*. Although *SARS-CoV* cannot infect or replicate in fully differentiated cells, it is possible to study the virus in infected bronchoalveolar stem cells [49]. Using this method, Mallick *et al.* showed that miR-223 amongst other miRNAs is downregulated during infection. Furthermore, they demonstrated potential targets of miR-223 in both the virus (N protein) and the host (CCR1), although these targets remain to be validated. In another study, it was found that miR-223 is elevated in serum from patients with chronic type B hepatitis, to a similar extent as in patients with hepatocellular carcinoma (HCC), suggesting it may be a marker of liver injury [50]. Although it is not clear how higher levels in the serum relate to the expression levels of this miRNA in cells, it is tempting to speculate from the finding that miR-223 is downregulated in HCC and that it may also be downregulated in chronic type B hepatitis. As discussed above, miR-223 may act as a negative regulator of inflammation, which could explain the chronic inflammatory response in the liver if miR-223 is repressed.

Table 1 *Validated targets of miR-223*

Gene	Disease	Pathway	Cell type	Species	Reference
Artemin	Oesophageal carcinoma	Invasion/metastasis	Oesophageal cancer cell lines	H	[71]
C/EBPβ	Leukaemia	Differentiation/ proliferation	Erythromyeloblast	H	[22]
E2F1	AML	Cell cycle	AML blast	H	[55]
EPB41L3	Gastric cancer	Metastasis	Cancer cell	H	[70]
Fbxw7/ Cdc4	Leukaemia, gastric cancer, oesophageal squamous cell carcinoma	Cell cycle regulation, proliferation	Erythroblast, gastric cancer cell, oesophageal squamous cell carcinoma	H, M	[56, 77, 78]
FOXO1	Cancer	Proliferation	Cancer cell lines	H	[67]
Gag-pol	HIV infection	HIV latency	CD4+ T cell	H	[51]
Granzyme B	Inflammation	NK cell activation	NK cell	M	[37]
HSP90B1	Cancer	Cell survival	Osteosarcoma cell line	H	[66]
IGF1R	Inflammation, cancer	Neutrophil activation, eosinophil differentiation, cell survival/proliferation	Neutrophil, eosinophil, cancer cell lines, T-ALL cell	H, M	[24, 33, 65]
IKKα	Inflammation	NF-κB	Monocytes	H	[29]
LMO2	Leukaemia/lymphoma	Erythroid and megakaryocyte differentiation	Megakaryocyte–erythroid progenitor	H	[22, 31, 32]
Mef2c	Granulocytosis, tumour immunology	Granulocyte progenitor proliferation, myeloid-derived suppressor cell differentiation	Granulocyte progenitor, myeloid progenitor	M	[24, 74]
NFI-A	Leukaemia/lymphoma	Myeloid differentiation	Myeloid progenitor	H	[20]
NFI-A promoter	Leukaemia/lymphoma	Granulopoiesis, epigenetic silencing	Myeloid progenitor	H	[23]
NLRP3	Inflammation	NLRP3 inflammasome	Monocyte/macrophage	H, M	[30, 35]
Pknox1	Adipose tissue inflammation	Macrophage polarization	Macrophage	M	[34]
Rasa1	Cancer	Cell cycle regulation	HeLa cell	H	[65]
RHOB	Cancer	Cytoskeleton remodelling/motility	Cancer cell lines	H	[79]
Roquin	Inflammation	IL-17A production	Th17 cell	M	[43]
STAT3	Inflammation	NF-κB	Macrophage	M	[36]
STMN1	HCC	Microtubule dynamics	Hepatocyte	H	[68]

H, human; M, mouse.

As miRNAs target RNA in the cytoplasm, it was speculated that they could act as antiviral immune defence mechanisms against RNA viruses. Indeed, miR-223 expression correlates with susceptibility of T cells as well as monocytes/macrophages with HIV-1 infection [51, 52]. Furthermore, together

with other miRNAs, miR-223 was found to target the 3′ ends of HIV-1 messenger RNAs. In resting CD4+ T cells (where HIV replication is silenced), miR-223 expression was increased compared with the level in activated CD4+ T cells. Inhibition of miR-223 alone or in combination with other miR-NAs resulted in HIV-1 production from resting CD4+ T cells [51]. The physiological relevance of this finding is still questionable as resting CD4+ T cells are not the main reservoir of HIV (<1%). In another study, it was shown in macrophages that miR-223 and other cellular HIV-1 interfering factors are induced by type I interferons, which could be one of the mechanisms of their antiviral activity [53].

Given the pronounced effects on the immune system as a whole, we can expect that in the near future, more studies will be reported regarding the role of miR-223 and infection.

miR-223 in cancer

miR-223 and haematological malignancy

Given the prominent role of miR-223 in the immune system, it is not surprising that it is commonly found to be deregulated in leukaemia and lymphomas. AML1/ETO, the most common fusion protein in acute myeloid leukaemia (AML), reduces miR-223 expression via chromatin remodelling [54]. As described above, miR-223 induces differentiation of myeloid progenitor cells by targeting NFI-A. As a result, AML1/ETO-expressing cells maintain an undifferentiated state with increased proliferative potential. Because repression of miR-223 transcription is achieved by epigenetic silencing, treatment with demethylating agents can induce differentiation in these cancer cells, providing potential therapeutic opportunities.

Additionally, miR-223 has been found to affect the cell cycle in AML by targeting the transcription factor E2F1 [55]. Regulation is part of a negative feedback loop, in which E2F1 can induce expression of C/EBPα, which then induces miR-223. The miRNA can in turn repress E2F1, and E2F1 can bind to the miR-223 promoter and repress its transcriptional activity. miR-223 levels are reduced in AML; therefore, E2F1-mediated cell cycle progression is increased, and reintroducing miR-223 can inhibit proliferation.

Furthermore, Xu and co-workers found that miR-223 was repressed in erythroblasts from a cyclin

$E^{T74A\ T393A}$ transgenic mouse, which showed increased levels of cyclin E [56]. The authors demonstrated that miR-223 targets the tumour suppressor Fbxw7/Cdc4, which is the substrate-recognizing part of the SCF^{Fbw7} E3 ubiquitin ligase complex. Because one of the proteins targeted for degradation by Fbxw7 is cyclin E, the interaction between miR-223 and Fbxw7 closes a negative feedback loop. As described above, E2F1 can inhibit the transcription of miR-223, which might be the mediator of the repressive function of cyclin E.

In contrast to AML, miR-223 is highly expressed in T-cell acute lymphoblastic leukaemia (T-ALL) and contributes to T-ALL development in a mouse model [57]. However, glucocorticoids, which induce apoptosis in T-ALL, also increase miR-223 expression in glucocorticoid-sensitive cell lines, which indicates that increased miR-223 levels do not always support oncogenesis [58]. Similarly, inhibition of oncogenic Notch signalling in T-ALL cells led to an increase in miR-223, which implicates Notch as another physiological mechanism to regulate miR-223 in cancer cells [59]. As for glucocorticoid treatment, an increase in miR-223 correlated with reduced oncogenesis. However, altering miR-223 levels alone was not sufficient to alter cell growth in these cells.

With regard to other haematological malignancies, miR-223 was downregulated in chronic lymphoid leukaemia [60–62], whereas it was overexpressed in a subset of T-ALL with myeloid features [63] and in Epstein–Barr virus (EBV)-positive diffuse large B-cell lymphoma [64]. Interestingly, we found that EBV expresses a miRNA, miR-BART15, which has an almost identical seed region to miR-223 and may target some of the same genes [30]. We therefore speculate that EBV miR-BART15 may promote EBV-associated malignancies through some of the above mechanisms.

miR-223 and nonhaematological malignancy

Increasing evidence also highlights a role for miR-223 in solid cancers, substantiating its involvement in basic cellular processes. A general role for miR-223 in cell cycle regulation was validated by overexpressing miR-223 in HeLa cells, which suppressed proliferation and tumour growth of the cells when injected into nude mice [65]. This suppression was mainly caused by reducing expression of the miR-223 target IGF1R and

downstream Akt/mTOR/p70S6K signalling, which was also seen in leukaemia and hepatoma cell lines. The same relationship has been observed in the osteosarcoma cell line MG63, where miR-223 also targets the heat-shock protein 90B1 (HSP90B1) [66]. However, the importance of this particular target for the miR-223 phenotype was not established. In another study, the targeting of the transcription factor FOXO1 by miR-223 as an alternative route to influence proliferation was highlighted [67]. miR-223 was shown to be repressed in HCC, which leads to the upregulation of stathmin1 (STMN1), a microtubule regulatory protein that is frequently overexpressed in cancers [68].

Regulation of miR-223 could also be part of the phenotype caused by the p53 gain-of-function mutation p53^{R175H}, which is associated with poor prognosis and chemoresistance. Masciarelli *et al.* showed that this mutant of p53 represses the miR-223 promoter in cooperation with the transcription factor ZEB1 in colon and breast cancer cell lines [69]. This could play a part in drug resistance, as exogenous miR-223 expression increased sensitivity to DNA-damaging agents. The authors attributed this effect to the miR-223 target STMN1, because siRNA-mediated knockdown of this protein has the same effect as miR-223 overexpression. Thus, mutant p53 reduces miR-223 expression, which in turn leads to an upregulation of STMN1 and increased chemoresistance.

Separate studies indicate opposing roles for miR-223 in regulating migration and invasion, two important characteristics of metastatic tumour cells. Screening for miRNA expression revealed that miR-223 is particularly overexpressed in metastatic gastric cancer cells [70]. Expression of miR-223 in nonmetastatic cell lines increased their migration and invasion, whereas the phenotype reverted following inhibition in metastatic cells. Li *et al.* showed that the metastasis-promoting transcription factor Twist can drive miR-223 transcription. The invasive phenotype of modulating miR-223 levels can be recapitulated by altering the level of the novel miR-223 target EPB41L3, a cytoskeletal linker protein involved in motility and adhesion. In stark contrast, miR-223 seems to be suppressing migration and invasiveness in oesophageal cancer lines, potentially by targeting the oncogene Artemin [71]. It remains to be determined whether this discrepancy can be explained by the different cell origins. Furthermore, in recurrent ovarian cancer, miR-223 is overexpressed compared with primary tumours, which could indicate a metastatic signature [72].

miR-223 and the tumour microenvironment

Global expression profiling shows that miR-223 is fairly specific for the haematopoietic lineage. Nevertheless, it has pronounced effects on proliferation and invasiveness in other cell types. Therefore, the question arises, 'How can such effects be explained in a physiological setting?' One possibility is trafficking of miRNA between cells in microvesicles, which is emerging as an important mechanism by which cells exchange regulatory factors as described above [28]. Indeed, miR-223 appears to be actively transferred from IL4-stimulated tumour-associated macrophages to co-cultured breast cancer cells, which leads to increased invasiveness in the cancer cells [73]. Thus, miR-223 could act as a signal in the crosstalk between tumour and immune cells in the tumour microenvironment.

Communication between cancer cells and the immune system can also be reversed, as miR-223 has been implicated in mediating immune evasion mechanisms by tumours. Cancer cells can induce suppressive myeloid cells, which accumulate in secondary lymphoid organs and suppress tumour-directed T-cell responses. In mouse models, this induction requires the downregulation of miR-223 and consequent upregulation of its target Mef2c in the suppressor cells [74]. Consequently, reconstitution of irradiated and cancer-bearing mice with bone marrow cells that overexpress miR-223 or lack Mef2c suppresses tumour growth, whereas miR-223 inhibition and Mef2c overexpression has the opposite effect.

Regarding its physiological functions, miR-223 has a high potential to influence carcinogenesis. Its role in regulating differentiation is very important for cancers of haematopoietic origin, where dedifferentiation is a common feature, as it increases the proliferative potential. miR-223 can alter several of the hallmarks of cancer that were defined by Hanahan and Weinberg [75], such as regulating cell cycle, cell survival, metastasis and immune evasion. Given its predominance in microvesicles from various cell types, it can play an important role in the crosstalk between cancer cells and the microenvironment. In many cases, miR-223 seems to function as a tumour-suppressive miRNA,

Table 2 *Diseases in which miR-223 expression is altered*

Disease	Tissue/cell type	miR-223 expression	Effect	Reference
Inflammation				
Mouse model of obesity	Adipose tissue	↓	Inflammation and immune cell recruitment	[34]
Rheumatoid arthritis	Synovium, T cell	↑		[38, 39]
Osteoarthritis	PBMC	↑		[40]
IBD	T cell	↑		[41]
IBD mouse model (IL-10-/-)	Colon, lymphocyte	↑	Target Roquin (negative regulator of IL-17)	[43]
Type 2 diabetes	Plasma	↓		[44]
Insulin-resistant heart	Heart	↑		[45]
Infection				
Sepsis	Serum	↓		[47]
Increased tissue destruction after endotoxin challenge		↓		[24]
H1N1 influenza infection	Lung	↑	Induced by r1918 variant more than seasonal influenza	[48]
SARS	Bronchoalveolar stem cell	↓	Potentially targets both the virus (N protein) and the host (CCR1)	[49]
Chronic type B hepatitis	Serum	↑		[50]
HIV-1 infection	Activated CD4+ T cell	↓	Targets the 3′ ends of HIV-1 messenger RNAs	[51]
Cancer				
AML	Bone marrow	↓	Affects the cell cycle in AML by targeting the transcription factor E2F1	[55]
T-ALL	Leukaemic cell	↑		[58] [63]
Chronic lymphoid leukaemia	B cell	↓		[60–62]
EBV-positive diffuse large B-cell lymphoma	B cell	↑		[64]
HCC	HCC cell	↓	Upregulation of stathmin1 (STMN1; a microtubule regulatory protein)	[68]
Metastatic gastric cancer	Gastric carcinoma cell	↑	Increases migration and invasion by altering the level of EPB41L3 (a cytoskeletal linker protein involved in motility and adhesion)	[70]
Oesophageal cancer	Oesophageal cancer tissue	↓	Suppresses migration and invasiveness, potentially by targeting the oncogene Artemin	[71]

PMBC, peripheral blood mononuclear cell.

targeting predominantly oncogenic effectors and being silenced in cancer cells. However, in other cases, miR-223 is overexpressed. This could reflect varying cellular requirements in different stages of tumour development and different tumour origins, for example in the primary tumour mass versus metastatic cells. More generally, it probably reflects the complexity of miRNA regulation. With the large number and diversity of targets, it seems unlikely that a single miRNA will have exclusively tumour-suppressive or oncogenic effects. The ultimate outcome will depend on the cell type, the tumour microenvironment and the network of other expressed effectors.

Conclusion

Based on the evidence reported to date, it is clear that miR-223 has an impact on different cellular processes, ranging from cell cycle regulation and invasiveness to haematopoietic differentiation and immune cell function. The changes in miR-223 expression in different haematopoietic cell types as well as genetic interference using knockout and miRNA sponge methods convincingly support a central role in differentiation, particularly in the myeloid lineage. Recently, evidence has pointed to an important role of miR-223 in altering macrophage polarization and activation. Given its role in basic cellular functions such as proliferation and survival, it is not surprising that miR-223 has a potential to alter cancer cell phenotypes. This has been observed not only in haematological malignancies but also in solid tumours. These findings together with the pronounced effects of miR-223 on the immune response suggest that this miRNA is part of the exciting link between inflammation and cancer [76].

As a consequence of its cellular functions, there is a wide range of pathologies in which miR-223 expression is deregulated (Table 2). It is important to recognize that, due to cellular specificity of miR-223 expression, altered miR-223 levels may simply reveal an imbalance in the number of cells that express high levels of this miRNA. Nevertheless, pooling these data makes it possible to speculate about the role of validated miR-223 targets (Table 1) in the pathologies in which miR-223 is deregulated. For example, E2F1, Foxo1, C/EBPβ and RHOB are implicated as miR-223 targets in cancer; however, these proteins are also extensively involved in inflammation and infection and might contribute to the effect of miR-223 in these settings. Furthermore, granzyme B, NLRP3, Pknox1 and Roquin have been discussed above in the context of inflammation, but they may also account for the functions of miR-223 in the development of cancer. Continued effort to understand and relate phenotypes and molecular observations will provide better insight into the biology of miR-223.

Conflict of interest statement

The authors have no conflict of interests to declare.

References

1 Ambros V. The functions of animal microRNAs. *Nature* 2004; **431:** 350–5.
2 Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; **116:** 281–97.
3 He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. *Nat Rev Genet* 2004; **5:** 522–31.
4 Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet* 2008; **9:** 102–14.
5 Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. *Nat Rev Genet* 2010; **11:** 597–610.
6 Borchert GM, Lanier W, Davidson BL. RNA polymerase III transcribes human microRNAs. *Nat Struct Mol Biol* 2006; **13:** 1097–101.
7 Winter J, Jung S, Keller S, Gregory RI, Diederichs S. Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nat Cell Biol* 2009; **11:** 228–34.
8 Hayashita Y, Osada H, Tatematsu Y *et al.* A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. *Cancer Res* 2005; **65:** 9628–32.
9 Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009; **136:** 215–33.
10 Eulalio A, Behm-Ansmant I, Izaurralde E. P bodies: at the crossroads of post-transcriptional pathways. *Nat Rev Mol Cell Biol* 2007; **8:** 9–22.
11 Eulalio A, Huntzinger E, Izaurralde E. Getting to the root of miRNA-mediated gene silencing. *Cell* 2008; **132:** 9–14.
12 Meijer HA, Kong YW, Lu WT *et al.* Translational repression and eIF4A2 activity are critical for microRNA-mediated gene regulation. *Science* 2013; **340:** 82–5.
13 Djuranovic S, Nahvi A, Green R. A parsimonious model for gene regulation by miRNAs. *Science* 2011; **331:** 550–3.

14 Lu J, Getz G, Miska EA *et al.* MicroRNA expression profiles classify human cancers. *Nature* 2005; **435:** 834–8.

15 Landgraf P, Rusu M, Sheridan R *et al.* A mammalian microRNA expression atlas based on small RNA library sequencing. *Cell* 2007; **129:** 1401–14.

16 Lim LP, Glasner ME, Yekta S, Burge CB, Bartel DP. Vertebrate microRNA genes. *Science* 2003; **299:** 1540.

17 Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. *Science* 2004; **303:** 83–6.

18 Ramkissoon SH, Mainwaring LA, Ogasawara Y *et al.* Hematopoietic-specific microRNA expression in human cells. *Leuk Res* 2006; **30:** 643–7.

19 Fukao T, Fukuda Y, Kiga K *et al.* An evolutionarily conserved mechanism for microRNA-223 expression revealed by microRNA gene profiling. *Cell* 2007; **129:** 617–31.

20 Fazi F, Rosa A, Fatica A *et al.* A minicircuitry comprised of microRNA-223 and transcription factors NFI-A and C/EBP-alpha regulates human granulopoiesis. *Cell* 2005; **123:** 819–31.

21 Eyholzer M, Schmid S, Schardt JA, Haefliger S, Mueller BU, Pabst T. Complexity of miR-223 regulation by CEBPA in human AML. *Leuk Res* 2010; **34:** 672–6.

22 Sun W, Shen W, Yang S, Hu F, Li H, Zhu TH. miR-223 and miR-142 attenuate hematopoietic cell proliferation, and miR-223 positively regulates miR-142 through LMO2 isoforms and CEBP-beta. *Cell Res* 2010; **20:** 1158–69.

23 Zardo G, Ciolfi A, Vian L *et al.* Polycombs and microRNA-223 regulate human granulopoiesis by transcriptional control of target gene expression. *Blood* 2012; **119:** 4034–46.

24 Johnnidis JB, Harris MH, Wheeler RT *et al.* Regulation of progenitor cell proliferation and granulocyte function by microRNA-223. *Nature* 2008; **451:** 1125–9.

25 Gentner B, Schira G, Giustacchini A *et al.* Stable knockdown of microRNA *in vivo* by lentiviral vectors. *Nat Methods* 2009; **6:** 63–6.

26 Sugatani T, Hruska KA. Impaired micro-RNA pathways diminish osteoclast differentiation and function. *J Biol Chem* 2009; **284:** 4667–78.

27 Ismail N, Wang Y, Dakhlallah D *et al.* Macrophage microvesicles induce macrophage differentiation and miR-223 transfer. *Blood* 2013; **121:** 984–95.

28 Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* 2007; **9:** 654–9.

29 Li T, Morgan MJ, Choksi S, Zhang Y, Kim YS, Liu ZG. MicroRNAs modulate the noncanonical transcription factor NF-kappaB pathway by regulating expression of the kinase IKKalpha during macrophage differentiation. *Nat Immunol* 2010; **11:** 799–805.

30 Haneklaus M, Gerlic M, Kurowska-Stolarska M *et al.* Cutting edge: miR-223 and EBV miR-BART15 regulate the NLRP3 inflammasome and IL-1beta production. *J Immunol* 2012; **189:** 3795–9.

31 Felli N, Pedini F, Romania P *et al.* MicroRNA 223-dependent expression of LMO2 regulates normal erythropoiesis. *Haematologica* 2009; **94:** 479–86.

32 Yuan JY, Wang F, Yu J, Yang GH, Liu XL, Zhang JW. MicroRNA-223 reversibly regulates erythroid and megakaryocytic differentiation of K562 cells. *J Cell Mol Med* 2009; **13:** 4551–9.

33 Lu TX, Lim EJ, Besse JA *et al.* MiR-223 deficiency increases eosinophil progenitor proliferation. *J Immunol* 2013; **190:** 1576–82.

34 Zhuang G, Meng C, Guo X *et al.* A novel regulator of macrophage activation: miR-223 in obesity-associated adipose tissue inflammation. *Circulation* 2012; **125:** 2892–903.

35 Bauernfeind F, Rieger A, Schildberg FA, Knolle PA, Schmid-Burgk JL, Hornung V. NLRP3 inflammasome activity is negatively controlled by miR-223. *J Immunol* 2012; **189:** 4175–81.

36 Chen Q, Wang H, Liu Y *et al.* Inducible microRNA-223 down-regulation promotes TLR-triggered IL-6 and IL-1beta production in macrophages by targeting STAT3. *PLoS ONE* 2012; **7:** e42971.

37 Fehniger TA, Wylie T, Germino E *et al.* Next-generation sequencing identifies the natural killer cell microRNA transcriptome. *Genome Res* 2010; **20:** 1590–604.

38 Fulci V, Scappucci G, Sebastiani GD *et al.* miR-223 is overexpressed in T-lymphocytes of patients affected by rheumatoid arthritis. *Hum Immunol* 2010; **71:** 206–11.

39 Shibuya H, Nakasa T, Adachi N *et al.* Overexpression of microRNA-223 in rheumatoid arthritis synovium controls osteoclast differentiation. *Mod Rheumatol* 2012; [Epub ahead of print].

40 Okuhara A, Nakasa T, Shibuya H *et al.* Changes in microRNA expression in peripheral mononuclear cells according to the progression of osteoarthritis. *Mod Rheumatol* 2012; **22:** 446–57.

41 Fasseu M, Treton X, Guichard C *et al.* Identification of restricted subsets of mature microRNA abnormally expressed in inactive colonic mucosa of patients with inflammatory bowel disease. *PLoS ONE* 2010; **5:** e13160.

42 Li YT, Chen SY, Wang CR *et al.* Amelioration of collagen-induced arthritis in mice by lentivirus-mediated silencing of microRNA-223. *Arthritis Rheum* 2012; **10:** 3240–5.

43 Schaefer JS, Montufar-Solis D, Vigneswaran N, Klein JR. Selective upregulation of microRNA expression in peripheral blood leukocytes in IL-10-/- mice precedes expression in the colon. *J Immunol* 2011; **187:** 5834–41.

44 Zampetaki A, Kiechl S, Drozdov I *et al.* Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. *Circ Res* 2010; **107:** 810–17.

45 Lu H, Buchan RJ, Cook SA. MicroRNA-223 regulates Glut4 expression and cardiomyocyte glucose metabolism. *Cardiovasc Res* 2010; **86:** 410–20.

46 Hunter MP, Ismail N, Zhang X *et al.* Detection of microRNA expression in human peripheral blood microvesicles. *PLoS ONE* 2008; **3:** e3694.

47 Wang JF, Yu ML, Yu G *et al.* Serum miR-146a and miR-223 as potential new biomarkers for sepsis. *Biochem Biophys Res Commun* 2010; **394:** 184–8.

48 Li Y, Chan EY, Li J *et al.* MicroRNA expression and virulence in pandemic influenza virus-infected mice. *J Virol* 2010; **84:** 3023–32.

49 Mallick B, Ghosh Z, Chakrabarti J. MicroRNome analysis unravels the molecular basis of SARS infection in bronchoalveolar stem cells. *PLoS ONE* 2009; **4:** e7837.

50 Xu J, Wu C, Che X *et al.* Circulating microRNAs, miR-21, miR-122, and miR-223, in patients with hepatocellular carcinoma or chronic hepatitis. *Mol Carcinog* 2011; **50:** 136–42.

51 Huang J, Wang F, Argyris E *et al.* Cellular microRNAs contribute to HIV-1 latency in resting primary CD4+ T lymphocytes. *Nat Med* 2007; **13:** 1241–7.

52 Wang X, Ye L, Hou W *et al.* Cellular microRNA expression correlates with susceptibility of monocytes/macrophages to HIV-1 infection. *Blood* 2009; **113:** 671–4.

53 Cobos Jimenez V, Booiman T, de Taeye SW *et al.* Differential expression of HIV-1 interfering factors in monocyte-derived macrophages stimulated with polarizing cytokines or interferons. *Sci Rep* 2012; **2:** 763.

54 Fazi F, Racanicchi S, Zardo G *et al.* Epigenetic silencing of the myelopoiesis regulator microRNA-223 by the AML1/ETO oncoprotein. *Cancer Cell* 2007; **12:** 457–66.

55 Pulikkan JA, Dengler V, Peramangalam PS *et al.* Cell-cycle regulator E2F1 and microRNA-223 comprise an autoregulatory negative feedback loop in acute myeloid leukemia. *Blood* 2010; **115:** 1768–78.

56 Xu Y, Sengupta T, Kukreja L, Minella AC. MicroRNA-223 regulates cyclin E activity by modulating expression of F-box and WD-40 domain protein 7. *J Biol Chem* 2010; **285:** 34439–46.

57 Mavrakis KJ, Van Der Meulen J, Wolfe AL *et al.* A cooperative microRNA-tumor suppressor gene network in acute T-cell lymphoblastic leukemia (T-ALL). *Nat Genet* 2011; **43:** 673–8.

58 Rainer J, Ploner C, Jesacher S *et al.* Glucocorticoid-regulated microRNAs and mirtrons in acute lymphoblastic leukemia. *Leukemia* 2009; **23:** 746–52.

59 Gusscott S, Kuchenbauer F, Humphries RK, Weng AP. Notch-mediated repression of miR-223 contributes to IGF1R regulation in T-ALL. *Leuk Res* 2012; **36:** 905–11.

60 Visone R, Rassenti LZ, Veronese A *et al.* Karyotype-specific microRNA signature in chronic lymphocytic leukemia. *Blood* 2009; **114:** 3872–9.

61 Stamatopoulos B, Meuleman N, Haibe-Kains B *et al.* microRNA-29c and microRNA-223 down-regulation has *in vivo* significance in chronic lymphocytic leukemia and improves disease risk stratification. *Blood* 2009; **113:** 5237–45.

62 Calin GA, Liu CG, Sevignani C *et al.* MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. *Proc Natl Acad Sci USA* 2004; **101:** 11755–60.

63 Chiaretti S, Messina M, Tavolaro S *et al.* Gene expression profiling identifies a subset of adult T-cell acute lymphoblastic leukemia with myeloid-like gene features and over-expression of miR-223. *Haematologica* 2010; **95:** 1114–21.

64 Imig J, Motsch N, Zhu JY *et al.* microRNA profiling in Epstein-Barr virus-associated B-cell lymphoma. *Nucleic Acids Res* 2011; **39:** 1880–93.

65 Jia CY, Li HH, Zhu XC *et al.* MiR-223 suppresses cell proliferation by targeting IGF-1R. *PLoS ONE* 2011; **6:** e27008.

66 Li G, Cai M, Fu D *et al.* Heat shock protein 90B1 plays an oncogenic role and is a target of microRNA-223 in human osteosarcoma. *Cell Physiol Biochem* 2012; **30:** 1481–90.

67 Wu L, Li H, Jia CY *et al.* MicroRNA-223 regulates FOXO1 expression and cell proliferation. *FEBS Lett* 2012; **586:** 1038–43.

68 Wong QW, Lung RW, Law PT *et al.* MicroRNA-223 is commonly repressed in hepatocellular carcinoma and potentiates expression of Stathmin1. *Gastroenterology* 2008; **135:** 257–69.

69 Masciarelli S, Fontemaggi G, Di Agostino S *et al.* Gain-of-function mutant p53 downregulates miR-223 contributing to chemoresistance of cultured tumor cells. *Oncogene* 2013; [Epub ahead of print].

70 Li X, Zhang Y, Zhang H *et al.* miRNA-223 promotes gastric cancer invasion and metastasis by targeting tumor suppressor EPB41L3. *Mol Cancer Res* 2011; **9:** 824–33.

71 Li S, Li Z, Guo F *et al.* miR-223 regulates migration and invasion by targeting Artemin in human esophageal carcinoma. *J Biomed Sci* 2011; **18:** 24.

72 Laios A, O'Toole S, Flavin R *et al.* Potential role of miR-9 and miR-223 in recurrent ovarian cancer. *Mol Cancer* 2008; **7:** 35.

73 Yang M, Chen J, Su F *et al.* Microvesicles secreted by macrophages shuttle invasion-potentiating microRNAs into breast cancer cells. *Mol Cancer* 2011; **10:** 117.

74 Liu Q, Zhang M, Jiang X *et al.* miR-223 suppresses differentiation of tumor-induced CD11b(+) Gr1(+) myeloid-derived suppressor cells from bone marrow cells. *Int J cancer* 2011; **129:** 2662–73.

75 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144:** 646–74.

76 Trinchieri G. Cancer and inflammation: an old intuition with rapidly evolving new concepts. *Annu Rev Immunol* 2012; **30:** 677–706.

77 Li J, Guo Y, Liang X *et al.* MicroRNA-223 functions as an oncogene in human gastric cancer by targeting FBXW7/hCdc4. *J Cancer Res Clin Oncol* 2012; **138:** 763–74.

78 Kurashige J, Watanabe M, Iwatsuki M *et al.* Overexpression of microRNA-223 regulates the ubiquitin ligase FBXW7 in oesophageal squamous cell carcinoma. *Br J Cancer* 2012; **106:** 182–8.

79 Sun G, Li H, Rossi JJ. Sequence context outside the target region influences the effectiveness of miR-223 target sites in the RhoB 3′UTR. *Nucleic Acids Res* 2010; **38:** 239–52.

Correspondence: Seth L. Masters, 1G Royal Pde, Parkville, Vic. 3052, Australia.
(e-mail: masters@wehi.edu.au).