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Review Article
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The Crosstalk between Micro RNA and Iron Homeostasis

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Abstract

Iron is a micronutrient essential for fundamental cellular processes. Iron deficiency has been proven to be the leading course of some blood diseases. Though essential, iron overload may contribute to the generation of free radicals capable for cell damaging. As such, the maintenance and control of iron homeostasis is critical to prevent either iron deficiency or iron overload toxicity. Iron homeostasis is largely coordinated by a family of Iron Regulatory Proteins (IRP) that functions to control iron uptake, storage, transport, and utilization. More recently, iron metabolism has also been implicated in the modulation of microRNA (miRNA), a class of small non-coding RNA recognized as the major regulation mechanism for gene expression at post-transcriptional level. Vice versa, miRNAs have been demonstrated to regulate the expression of genes associated with iron acquisition (transferrin receptor and divalent metal transporter), iron export (ferroportin), iron storage (ferritin), iron utilization (ISCU), and coordination of systemic iron homeostasis (HFE and hemojevelin), bridging the crosstalk between miRNAs regulation and iron homeostasis. Herein we briefly summarize recent advances in the inter-regulation between miRNAs and maintenance of iron homeostasis. It will enhance our understanding of mechanisms by which cells respond to changes in iron demand and/or iron availability to control cellular iron homeostasis, and how miRNAs regulate iron homeostasis.

Keywords: MicroRNA; Iron homeostasis; Crosstalk; RNAi, Metabolic pathway; PCBP2

miRNA/RNAi Pathway

MicroRNAs (miRNAs) are a class of non-protein-coding RNAs with length of 18-25 nucleotides (nt) [1,2]. They are involved in nearly every aspect of development and cell physiology, and contribute to pathogenesis of some diseases by the alteration of mRNA expression levels or some specific cellular signals [3-5]. MiRNA genes are frequently localized within intronic regions but are also expressed from non-protein coding transcripts localized in intergenic regions [6,7]. Generally, mature miRNAs are generated by two sequentially biochemical processing steps. First, the pri-miRNAs, which are transcribed initially by RNA polymerase II from 5'-capped, spliced and polyadenylated, range from 100 to 1000nt in length and bear one or more hairpin structures in mammals [8,9]. In most cases, the first processing step takes place inside the nucleus, where the pri-miRNAs are recognized and cleaved by Drosha and its partner DGCR8 [10-13]. Drosha is an RNA endonuclease III in mammals. DGCR8 is a RNA-binding protein that is essential for maturation of miRNAs. They cleaves pri-miRNAs to ~70 nt stem-loop-structured precursor miRNAs (pre-miRNAs) [14]. The pre-miRNAs are then transported into cytoplasm by exportin-5/RanGTP for secondary processing [15,16]. Dicer, another member of the RNase III family, further cleaves the pre-miRNAs to generate mature miRNAs along with its co-factors TAR RNA binding protein 2 (TRBP) and PACT [17,18]. The mature form of miRNAs must be loaded into RNA-Induced Silencing Complex (RISC) to guide it to its target mRNA sequence, and functionally regulate gene expression at post-transcriptional levels. MiRNAs recognize miRNA Responsive Elements (MREs), sequences with partial complementary localized within the 3' Untranslated Region (UTR) of mRNAs. Depending on the level of complementarily between miRNA and MRE, this interaction either limits mRNA translation or decreases mRNA stability [11]. In addition, mRNAs containing identical MREs in their 3'UTR will co-regulate each other's expression by competing for a limited miRNA pool [19-21]. Likewise, MRE-containing pseudogenes and long non-coding (lnc) RNAs may act as 'sponges' for miRNAs. Under some circumstances, the expression of a specific miRNA remains unaltered, but the distribution of miRNAs on their target mRNAs changes hence imposing an additional layer of post-transcriptional control on gene expression [22-24].

Iron Homeostasis and Regulatory Network

Iron is an essential micronutrient required as a cofactor for normal cellular processes including oxygen transport, heme biosynthesis, DNA synthesis, cell proliferation, cytochrome p450 enzyme activity, and hypoxic response reactions [25,26]. Iron can quickly transform between a reduced ferrous Fe (II) and oxidative ferric Fe (III) state a property which is important for many catalytic processes. Iron homeostasis is well controlled, but its disorder such as iron deficiency or iron overload can lead to cytotoxicity, cell growth arrest and apoptosis, respectively [27,28]. It is well known that iron deficiency is the leading cause of anemia, interfering significantly with normal cognitive development in children. Conversely, the iron overload observed in common diseases like hemochromatosis and thalassemia results in liver and heart failure [29]. The redox activity of iron can lead to the production of hydroxyl radicals, the most toxic Reactive Oxygen Species (ROS), damaging lipid membranes, proteins, and nucleic acids. Iron regulatory proteins (IRPs) are the iron regulatory proteins that control post-transcriptionally iron homeostasis in most mammals [25]. Trans-acting IRPs interact with ferritin and Transferrin Receptor (TfR) Iron-Responsive Elements (IREs) through their metal availability sensors. IREs are conserved hairpin structures found in the UTRs of mRNA, and control either mRNA stability or initiation of translation. Expression of the genes involved in maintenance of cellular iron homeostasis is predominantly controlled post-transcriptionally by the binding of IRPs 1/2 to IREs. In

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iron-deficient conditions, IRE/IRP complexes form within an mRNA 5'UTR to inhibit translation, whereas IRP binding to IREs in the 3'UTR of TfR1 and the Divalent Metal Transporter 1 (DMT1) prevents mRNA degradation. Mice lacking both copies of the IRP1 and the IRP2 genes die at the embryonic stage, indicating that the IRE/IRP regulatory network is essential, at least for early development [30]. Therefore, cellular iron homeostasis is carefully maintained by an exquisite system of IRPs through sensing the cellular iron levels and coordinating the expression of targets of importance for balancing iron export and uptake with intracellular storage and utilization [31,32].

Ferritins (Fn) are the main iron storage proteins and essential for metabolisms in mammals. Poly (rC)-binding protein 1 (PCBP1) was found to bind to ferritin in vivo and facilitate the loading of iron into ferritin [33]. Depletion of PCBP1 inhibits iron loading and increases cytosolic iron pools, suggesting that PCBP1 functions as a cytosolic iron chaperone in the delivery of iron to ferritin. It was also noted that PCBP2, a paralog of PCBP1, could also potentially contribute to ferritin iron loading [33].

Ferroportin (FPN) is a transmembrane iron efflux channel protein, and highly expressed on cells that release iron, such as hepatocytes, macrophages and enterocytes. FPN functions as the main iron exporter in mammalian cells and plays a critical role in the maintenance of iron balance [34-36]. FPN is regulated at multiple levels–transcriptionally by heme post-transcriptionally by the IRP system and post-translationally by the iron regulatory hormone hepcidin [29,37-42]. During iron deficiency, IRPs inhibit the translation of FPN by binding to the IRE located in the 5' UTR of FPN mRNA, leading to lower FPN protein levels, decreased export of iron, and cellular iron retention [29].

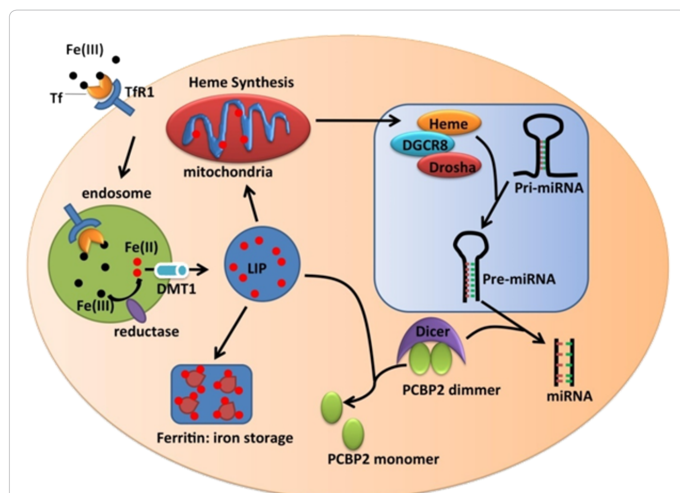

Figure 1: miRNA processing is regulated by Iron Homeostasis

The ubiquitously expressed transferrin receptor-1 (TfR1) controls cellular uptake of iron-bound transferrin (Tf). The Tf-iron-TfR1 complex is taken up into the cell via endocytosis and the iron is released from the endosome by the nonIRE isoform of Divalent Metal Transporter 1 (DMT1). In the cytoplasm iron is incorporated within the cellular labile iron pool (LIP), and is stored by ferritin or is utilized by mitochondria for heme synthesis and Fe–S cluster biogenesis. Heme is critical for the processing of miRNA primary transcripts (pri-miRNAs) and binds to the microprocessor complex composed of DGCR8 and Drosha. miRNA precursors (pre-miRNA) are exported to the cytoplasm where cellular iron levels affect dimerization of poly(C) binding protein 2 (PCBP2), association of PCBP2 and DICER, binding of PCBP2 and pre-miRNAs, and processing of DICER-conferred processing of pre-miRNAs to mature miRNAs. The dimerized PCBP2 is the functional form of PCBP2, and the increased cellular iron level leads to enhanced monomerization of PCBP2, disassociation of DICER and PCBP2, lost binding of PCBP2 and pre-miRNAs and consequently to inhibition of pre-miRNAs processing into mature miRNAs.

Hepcidin, an antimicrobial peptide hormone, is produced by the liver in response to hypoxia, systemic iron availability, erythroid iron demand and inflammatory cues. Hepcidin was found to control systemic iron homeostasis by interacting with its target FPN [43,44]. Upon hepcidin binding, FPN is internalized and degraded [42]. Although hepcidin levels are controlled by systemic iron requirements, iron levels are not directly sensed by hepcidin. This is because hepcidin expression is balanced either by upstream activators, such as Hfe [45,46], transferrin receptor 2 (TfR2), hemojuvelin (Hjv) or by inhibitors such as Smad family member 7 and the transmembrane protease, serine 6 (TMPRSS6) [47-53]. Hepcidin deficiency or hepcidin resistance due to FPN mutations eventually leads to systemic iron overload in the form of Hereditary Hemochromatosis (HH) resulting in significant tissue damage and multi-organ failure with limited therapeutic options [54-57].

Transferrins (Tf) are iron-binding glycoproteins that control the level of free iron in biological fluids. The transferrin-bound iron (Tf-Fe2) in the plasma is taken up by most mammalian cell types satisfy their iron needs via transferrin receptor 1 (TfR1). The complex is then internalized by clathrin-dependent endocytosis. Subsequent acidification of early endosomes promotes the release of iron from transferring. Fe (III) is reduced to Fe (II) by members of the STEAP family of metalloreductases for transport into the cytoplasm via the endosomally expressed DMT1. TfR1 and apo-transferrin are recycled to the cell surface [58]. Although TfR1 is ubiquitously expressed, the transferrin cycle is most critical for the massive iron demand of erythroid precursor cells (Figure 1).

miRNA Processing is Regulated by Iron Homeostasis

Heme-mediated control of miRNA biogenesis

Iron appears to play a critical role in miRNA processing via its physiological role as the functional component in heme. This potential role for iron to participate in miRNA biogenesis was first demonstrated when DGCR8 was identified as a heme-binding protein [59]. In reconstituted pri-miRNA processing assays, heme binds to and enhances DGCR8 dimerization, which is required for increased activity of this essential miRNA processing cofactor [60-63]. During this process, heme binds to DGCR8 via its Fe (III) iron co-factor. By contrast, Fe (II) heme is unable to enhance miRNA processing, and reduction of the ferric heme iron in DGCR8 greatly increases the rate of heme dissociation, suggesting that the oxidation state of iron in heme affects heme-mediated regulation of DGCR8, and the rate and efficiency of pri-miRNA processing [63]. Additional studies demonstrated that heme-free DGCR8 was less active than heme-bound DGCR8 and suggests that an impaired ability to synthesize heme as a result of inadequate iron could decrease pri-miRNA processing [59] (Figure 1). Heme excess also produces changes in miRNA profiles, which likely contribute to the modulation of targeted mRNA expressions [64].

Iron-mediated control of miRNA biogenesis

Iron was found to be significantly synergistic in up regulating Reactive Oxygen Species (ROS) abundance, and miRNA-125b and miRNA-146a expression. Treatment of iron stressed cells with the antioxidant Phenyl Butyl Nitrone (PBN) or the metal chelator-antioxidant Pyrollidine Dithiocarbamate (PDTC), abrogated induction of these miRNAs [65]. It suggests that iron may modulate the biogenesis of miRNAs by oxidative stress or other signal pathway. When study

HBV replication in human hepatocytes, it was found that cellular microRNA expression profiles were significantly different in iron-treated cells compared with untreated control cells [66]. In many cases, exposure to iron changed microRNA expression in opposite directions. Introduction in cells of sequences representing such differentially expressed microRNAs, e.g., hsa-miR-125a-5p and -151-5p, even reproduced effects on virus replication of iron. This knowledge should suggest that iron may involve in Drosha/DGCR8/heme-mediated processing of microRNAs.

Recently, we have identified iron chelators as a class of enhancers for the miRNA/RNAi pathway in a way that these chelators could promote the processing of both miRNA precursors and short hairpin RNAs (shRNAs) [67]. We found that cytosolic iron, but not heme, regulated the activity of the miRNA pathway through poly(C)-binding protein 2 (PCBP2), but not its analogue PCBP1. This specificity is particularly intriguing given the high similarity between these two proteins [68]. Indeed, an earlier proteomic analysis of Argonaute-containing mRNA-protein complexes in human cells suggested that PCBP2 (hnRNP E2), but not PCBP1, is associated with Argonaute 1 protein-containing complex with Dicer activity [69]. PCBP2 is associated with Dicer and promotes the processing of miRNA precursors. Cytosolic iron could modulate the association between PCBP2 and Dicer, as well as the multimerization of PCBP2 and its ability to bind to miRNA precursors, altering the processing of miRNA precursors. Our findings indicate that the role of iron homeostasis in the regulation of miRNA biogenesis. Altered miRNA expression might contribute directly to the molecular pathogenesis of human diseases associated with disrupted iron homeostasis. When cytosolic iron is low, PCBP2 could multimerize, bind to miRNA precursors, and present them to Dicer for more efficient miRNA processing. Excess cytosolic iron will interfere with the binding of PCBP2 to miRNA precursors as well as its association with Dicer, and lead to the reduced production of mature miRNAs [67] (Figure 1).

miRNAs	Target genes	References
miR-485-3p	FPN	[74]
miR-320	TfR1	[81]
miR-22	TfR1	[81]
miR-Let-7d	DMT1B	[87]
	BACH1	[88]
miR-210	Ferrochelatase	[106]
	TfR1	[110]
	ISCU 1/2	[101,110]
miR-200a	TfR1	[81]
miR-200b	FtH1	[116]
miR-122	Hfe and Hjv	[92]
miR-221	TfR2	[91]
miR-222	TfR2	[91]
miR-214	Lactoferrin	[120]
miR-584	Lactoferrin	[121]
miR-31	TfR1	[82]
miR-194	FPN	[82]
miR-19a	DMT1	[82]
miR-133a	FnL	[82]
miR-141	TfR1	[82]
miR-145	TfR1	[82]
miR-149	DMT1	[82]
miR-182	TfR1	[82]
miR-758	TfR1	[82]
miR-196	BACH1	[89]

FPN: Ferroportin; TfR: transferrin receptor; DMT1: Divalent Metal Transporter 1; Hjv: hemojuvelin; ISCU: iron-sulfur cluster assembly proteins; FtH: Ferritin heteropolymers heavy chains; IRP: iron regulatory proteins; IRE: iron-responsive elements; FnL: Ferritin light chain.

Table 1: Targets of miRNA associated with mammalian iron metabolism.

miRNAs-Mediated Control of Iron Homeostasis

miRNAs have emerged as a class of noncoding genes involved in regulating a wide variety of biological processes. Here we review that several miRNAs have been found to regulate targets with key roles in iron homeostasis (Table 1).

miRNA regulates cellular iron homeostasis by targeting ferroportin

The monitoring of local iron levels by cellular microRNAs leads to dynamic responses to spatial and temporal fluctuations. While both microRNAs and IRPs are iron-responsive and target a group of mRNAs, they may also respond to different sets of non-iron environmental conditions and regulate distinct sets of target mRNAs to allow for diversity and fine-tuning of gene regulation. The targeting of FPN by microRNAs in the 3'UTR allows for the possibility of iron-dependent regulation of subsets of FPN mRNAs known to lack the 5' UTR [70,71]. Additionally, high-throughput techniques to probe the microRNA-mRNA interactome offer powerful complementary approaches to identify the in vivo target mRNAs associated with Ago2 during different iron states [72,73]. Such exploration of iron-responsive microRNAs and their respective targets will lead to a more comprehensive pathway that demonstrates an integrated role for microRNAs in the regulation of cellular iron homeostasis. Since FPN is known to be repressed by the IRP/IRE system under the iron-deficient condition, it is suggested that a potential cooperative relationship between RNA-Binding Proteins (RBPs) and microRNAs in the regulation of FPN. miR-485-3p is an important post-transcriptional regulator of endogenous FPN. Through gain-of-function and loss-of function studies, miR-485-3p is found to directly target the 3' UTR of FPN and modulate cellular iron homeostasis [74]. The cooperative contribution of RBPs, including both IRPs and the microRNA guided RISC, to the post-transcriptional regulation of target RNAs constitutes a major regulatory layer of gene expression [75,76]. RBPs can function to promote or inhibit microRNA target availability and binding, leading to the enhancement or inhibition of mRNA stability and translation [77]. In the case of FPN, it is possible that the IRP/IRE 5'UTR interaction can be further stabilized and fine-tuned by the microRNA-mediated RISC on the 3'UTR. This enable a more dynamic and fine-tuned expression over a wide range of iron conditions.

miRNAs control cellular iron uptake by transferrin cycle

Recent data suggest that transferrin cycle is controlled by miRNAs at two different steps. It was recognized that TfR1 expression is increased in neoplastic cells to satisfy their increased iron requirements for cellular proliferation [78,79]. Conversely, differentiation of neoplastic cells, such as the human leukemia cell line HL-60, into themonocytic/macrophage lineage represses TfR1 expression and increases levels of miRNAs predicted to bind to the 3'UTR of TfR1 (miR-320, miR-200a, and miR-22) [80]. Of these, miR-320 represses the activity of luciferase reporter vectors fused to sequences of the TfR1 3'UTR that contain the miR-320-specific MRE. Likewise, enforced miR-320 expression in the lung carcinoma A549 cell line decreases TfR1 surface expression and slows down cell cycle progression and cell growth. Because the additional treatment of cells with a soluble iron salt reverses the growth inhibitory effect, it is inferred that decreased TfR1 expression in miR-320 overexpressing cells may lower iron availability and inhibit

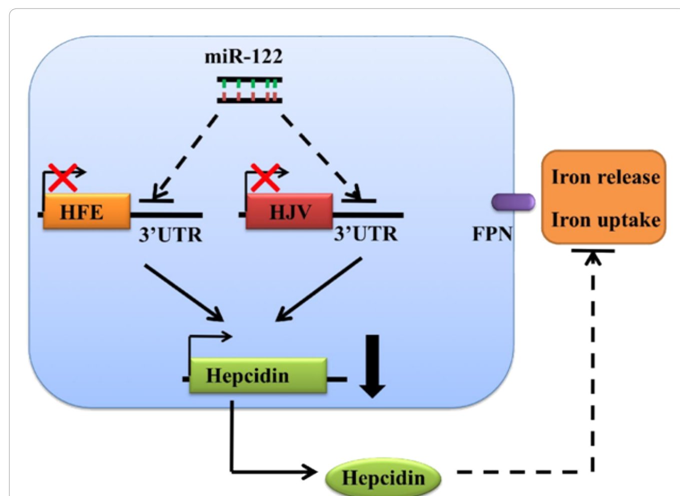

Figure 2: Role of miR-122 in iron homeostasis

miR-122 is critical for the control of systemic iron homeostasis by targeting 3'UTR of Hfe and Hjv, which encode proteins crucial for the hepcidin hormone. Hepcidin protein interacts with iron exporter, ferroportin, and inhibits systemic iron uptake and iron release.

cell proliferation [81]. In early stage of colorectal cancer (CRC), IRP2 can be engaged in the mRNA stabilization of TfR1. However, in more advanced stages of CRC, mRNA level of TfR1 is related to miR-31 level. Ferroportin concentration is significantly associated with miR-194 level, causing the reduction of TfR1amount in tumor tissues of patients with more advanced stages of CRC [82].

In addition to miRNA-dependent regulation of TfR1, miRNAs are involved in the regulation of transferrin cycle as well by controlling iron release from the endosome via DMT1. The gene coding for DMT1gives rise to four variant mRNA transcripts that either differ at their 5'end as a result of alternative promoter usage (DMT1A and 1B protein isoforms) or at the 3'end due to alternative splicing [83]. The alternative 3'UTR sequences differ as to whether they contain an IRE sequence motif, whereby only the IRE-containing isoforms are controlled in response to cellular iron levels by IRP binding [84]. All different DMT1 isoforms are capable of transporting iron [85]. Notably, the DMT1 splice variant lacking the IRE sequence (DMT1B-nonIRE) is abundantly expressed in erythroid cells where it is responsible for iron export across the membrane of acidified endosomes into the cytoplasm following iron-bound transferrin uptake [85,86]. Although the DMT1B-nonIRE isoform plays a fundamental role during erythroid precursor differentiation, the regulatory mechanism underlying its expression remained unclear. A recent study in erythroid cells revealed that the expression of the DMT1B-nonIRE isoform is fine-tuned by the miRNA Let-7d, providing an alternative regulation mode for this isoform that cannot respond to iron availability through the IRE/IRP regulatory system [87]. Iron storage may also be indirectly affected by miRNA as both miR-196 and miR-let-7d target the heme-regulated transcriptional repressor Bach1 resulting in a de-repression of Bach1 targets such as HMOX1 and ferritin [88,89]. Although ferritin transcription may be reduced via Bach1, the capacity for miR-let-7d-dependent repression of Bach1 to de-repress ferritin expression and synthesis remains unknown [90].

TfR2 cannot be regulated by IRPs because of the lack on the IRE elements at the 3'-UTR of its mRNA. For this reason, a different regulation mechanism can be supposed. It was showed that TfR2 expression is regulated by miR-221 in a PD cellular model. TfR2 and miR-221 are inversely correlated in SH-SY5Y cells during the treatment with MPP+. Moreover, overexpression of miR-221 decreases the expression of TfR2 at the mRNA and protein levels [91]. TfR2 increase is involved in iron cellular uptake, and this could justify the excess of iron influx. The complex Tf/TfR2 let iron enters the cell by endocytosis. It is well known that the subsequent acidification of endosome causes the release of iron from transferrin.

miR-122-mediated regulation of systemic iron

MiRNA profiling using mouse models of iron overload shows that miR-122, a miRNA abundantly and selectively expressed in the liver, is downregulated in a murine disease model of Hfe-mediated Hereditary Hemochromatosis (HH) as well as in liver biopsies from HH patients with homozygous C282Y mutations [92]. Liver is the major iron storage site and the endocrine organ responsible for the regulation of systemic iron homeostasis. In appropriately low hepcidin activity as a result of mutations in Hfe, Hjv, TfR2, or hepcidin itself cause the iron overload disorder HH. The liver enriched miR-122 has been found to be critical for the control of systemic iron homeostasis in mice by targeting Hfe and Hjv, which encode proteins crucial for the hepcidin hormone response to systemic iron availability (Figure 2). Previously there were 2 groups had specifically silenced miR-122 in mice [93,94]. They observed that mRNA levels of Hfe and Hjv were up-regulated in the livers of mice silenced for miR-122 and wondered whether miR-122 plays a role in regulating iron homeostasis. To investigate this they profiled the expression of miRNAs in the livers of Hfe-/- mice and wild-type mice with iron overload caused by iron-dextran injection. They found miR-122 was down-regulated in Hfe-/- mice. No down-regulation of miR-122 was observed in wild-type mice with iron overload.

MiR-122 depleted mice are hallmarked by decreased systemic iron levels, which result in an inadequate iron supply to the erythron that mildly impairs hematopoiesis [92]. Specifically, miR-122 inhibition increases reticulocyte counts, decreases the mean corpuscular volume (MCV) of erythrocytes, and reduces the reticulocyte hemoglobin content. Additionally, the iron content of the liver (site of iron storage), the plasma (site of iron transport), and the spleen (site of iron recycling) were reduced in miR-122-inhibited mice. The decrease in plasma iron levels resulted in reduced transferring iron binding capacity (TIBC) 3 weeks after antimiR-122 injection. The finding that miR-122 regulates systemic iron homeostasis adds to a growing list of liver functions that are controlled by miR-122. For example, miR-122 levels are decreased in advanced liver diseases such as cirrhosis and hepatocellular carcinoma pathologies known to be exacerbated by increased liver iron levels [95-98].

miRNA-210 decreases heme by targeting ferrochelatase

MiR-210 is highly expressed in human and murine erythroid cells and in the spleen of mice with hemolytic anemia [99]. Erythrocytes require iron to perform their duty as oxygen carriers. Recent reports have shown that Therefore, miR-210 might reports have indicated that the expression of miR-210 is induced by hypoxic conditions [100]. Play an important role in the connection of iron and oxygen. Several hypoxia-induced miR-210 directly targets iron-sulfur cluster scaffold proteins (ISCU 1/2) important proteins for the biogenesis and integrity of iron-sulfur clusters and consequently proper functioning of the electron transport chain [101-105]. Through modulation of ISCU, miR-210 has been shown to alter mitochondrial respiratory complex activity and mitochondrial function [101-103]. Repression of iron-sulfur

clusters increases the functionality of IRP1 as an RNA binding protein and indirectly alters IRP1-dependent regulation [101,104].

It is shown that miR-210 levels are increased with iron chelation through a hypoxia-inducible factor (HIF)-dependent pathway [106]. MiR-210 is upregulated and target of hypoxia-inducible factors 1 and 2 in renal cancer, and correlates negatively with its gene target ISCU at the protein and mRNA level [107]. It was supported by another study which showed that miR-210 was increased in hypoxic fibroblasts and high-altitude placentas. The increased miR-210 was related with decreased expression of its targets, Iron-Sulfur Cluster Scaffold (ISCU) and Cytochrome C Oxidase Assembly Protein (COX10) [108]. So we speculate that a signaling axis HIF-1α/microRNA - 210/ISCU may modulate gene expression and iron homeostasis.

Hypoxia enhances the generation of Reactive Oxygen Species (ROS). There is a positive feed-forward loop between ROS generation and miR-210 [109]. ROS generation could regulate miR-210 expression, and increase proliferation/migration of ASCs. MiR-210 itself increases ROS generation by downregulation of ISCU2.

Modulation of miR-210 alters cellular heme and the activity of both mitochondrial and cytosolic heme containing proteins. These effects occur through modulation of ferrochelatase by binding to the 3` UTR of its respective mRNA. Overexpression of ferrochelatase reverses the effects of miR-210 on heme and ferrochelatase. Because overexpression of ISCU1/2 lacking the 3` UTR does not alter miR-210–mediated changes in heme and ferrochelatase, the changes are independent of ISCU. MiR-210 targets two essential mRNAs for iron homeostasis, encoding TfR and ISCU. Furthermore, the distribution of miR-210-expressing cells in inoculated tumor cells could be observed in the chronic hypoxic regions. These indicated that iron-deficiency-inducible miR-210 controls the expression of two iron regulatory proteins to optimize the survival and proliferation rate of cancer cells located in the chronic hypoxic regions [110].

miR-210 down-regulates ISCU and the Fe-S cluster to mediate the energy metabolic shift from aerobic oxidative phosphorylation to anaerobic glycolysis [101]. Concurrently, reduction of the Fe-S cluster activates IRP1, and subsequently the expression of TfR is enhanced, resulting in the elevated uptake of the iron. To reduce the cellular iron concentration, miR-210 directly suppresses the expression of TfR. Thus, miR-210 regulates iron homeostasis and avoids intracellular iron toxicity. Compared with normal cells, cancer cells require a large amount of iron; thus, they generally proliferate at a larger rate than their normal counterparts. Hence, iron chelators exert their anti-proliferative effects on tumors [111]. Moreover, down-regulation of TfR decreased cellular proliferation and altered expression of genes involved in cell cycle control [112]. Reduction of ISCU increases the binding activity of IRP1, but does not change its expression level. Thus, the effect of up-regulation of TfR by reduced ISCU depends on the amount of IRP1 protein. There are limitations to the up-regulation of TfR by the indirect pathway. Then, in the case of a further increase of miR-210, direct suppression of TfR is superior to its up-regulation by the indirect pathway. Therefore, exogenous transfection of miR-210 causes TfR suppression, and reducing cellular proliferation [113,114]. These observations suggest that precise regulation of miR-210 expression level is vital for maintaining the iron homeostasis, leading to the survival and proper cellular proliferation of cancer cells.

miR-200b-mediated regulation of ferritin

Ferritin heteropolymers consist of 24 subunits of heavy (FtH1) and light (FtL) chains that bind iron from the cytoplasmic "Labile Iron Pool" (LIP) that is not exported or utilized [115]. Only the FtH1 subunit exerts ferroxidase activity that is necessary for iron deposition into the nanocage, while FtL facilitates iron nucleation and increases the turnover of the ferroxidase site. Ferritin detoxifies excess iron in a redox inactive form to prevent iron-mediated cell and tissue damage; it also constitutes an iron store whose mobilization involves both proteasomal and lysosomal degradation of ferritin. A recent study showed that human breast cancer cells with an aggressive mesenchymal phenotype express substantially higher mRNA and protein levels of FtH1 and FtL and lower LIP compared to breast cancer cells with an epithelial and less aggressive phenotype [116]. A large fraction of FtH1 in these breast cancer cells was associated with the chromatin-bound nuclear fraction, a finding that lacks explanation. Increased FtH1 levels correlated with low expression of miR-200b, a miRNA predicted to bind to the 3'UTR of FtH1. Unexpectedly, miR-200b transfection also decreased FtL protein levels, although the FtL 3'UTR is not predicted to bind to miR-200b. It needs to be determined whether miR-200b-mediated ferritin regulation is related to previous clinical findings that patients with higher circulating plasma ferritin levels show worse treatment outcome compared to patients with lower ferritin values [116].

Concluding Remarks

The crosstalk between miRNA and iron homeostasis is critically maintained for many essential cellular functions. Distortion of systematic or cellular iron homeostasis, including iron deficiency and iron overload, causes frequent disorders such as cancer, neurodegeneration, anemia and hereditary hemochromatosis. As hall markers in the cases of disorders, miRNA expression is altered. Furthermore, Iron homeostasis is tightly regulated by miRNAs at the level of cellular uptake of iron-bound transferrin, iron storage by ferritin, and the hepatic control of systemic iron levels via hepcidin. These findings suggest that miRNAs control large regulatory networks that link micro-environmental stress, such as oxidative stress and hypoxia to the regulation of iron metabolism.

Many *in vivo* and *in vitro* investigations have shown that cancer cells are more sensitive to Fe deprivation compared with normal cells because of their marked Fe requirements depriving cancer cells of their essential iron is an approach for cancer treatment [117,118]. The etiology of neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, and other neurodegenerative diseases, is not yet well understood. Accumulating evidence has pointed to iron dependent oxidative stress, elevated levels of iron, and depletion of antioxidants in the brain as major pathogenic factors [118]. Indeed, iron chelators have shown efficacy in a variety of cellular and animal models of cancer and neurodegenerative diseases. More recently, RNAi technology has been under rapid development to treat human diseases, and several pilot RNAi clinical studies are underway to test the therapeutic utility of RNAi-based approaches [119]. The identification of iron chelator as an enhancer of shRNA-mediated RNAi could give a push to this development. It is hoped that combining these two approaches might have a synergistic effect and make for better therapeutic interventions.

As noted above, iron homeostasis is micromanaged by miRNAs. miR-122 binding sites in the 3'-UTRs of Hfe and Hjv are functional [92]. It would be interesting to see whether there are functional binding sites for other miRNAs in genes involved in iron metabolism in humans such as HFE and HJV. Which of these sites or whether of these sites are functional may be of some interest? It is quite possible that genetic variation in miRNAs binding sites in human transcripts or variation

in miRNAs itself may be responsible for phenotypic variations in iron parameters in both patients with iron overload disorders or the general population. Besides, miRNAs could be essential for maintaining other metal homeostasis in mammals; for example, copper, zinc, and cadmium. Dysregulation of metal homeostasis-related miRNAs may contribute to various diseases. Further analyses are required on how these miRNAs can be affected by genetic and epigenetic mechanisms in the physiological and pathological microenvironments.

References

1. Ambros V (2004) The functions of animal microRNAs. Nature 431: 350-355.
2. Bartel DP (2009) MicroRNAs: target recognition and regulatory functions. Cell 136: 215-233.
3. Bartley AN, Yao H, Barkoh BA, Ivan C, Mishra BM, et al. (2011) Complex patterns of altered MicroRNA expression during the adenoma-adenocarcinoma sequence for microsatellite--stable colorectal cancer. Clinical cancer research : an official journal of the American Association for Cancer Research 17: 7283 -7293.
4. Davis BN, Hilyard AC, Nguyen PH, Lagna G, Hata A (2010) Smad proteins bind a conserved RNA sequence to promote microRNA maturation by Drosha. Mol Cell 39: 373-384.
5. Davis BN, Hilyard AC, Lagna G, Hata A (2008) SMAD proteins control DROSHA-mediated microRNA maturation. Nature 454: 56-61.
6. Kim YK, Kim VN (2007) Processing of intronic microRNAs. EMBO J 26: 775-783.
7. Saini HK, Griffiths-Jones S, Enright AJ (2007) Genomic analysis of human microRNA transcripts. Proc Natl Acad Sci U S A 104: 17719-17724.
8. Carthew RW, Sontheimer EJ (2009) Origins and Mechanisms of miRNAs and siRNAs. Cell 136: 642-655.
9. Kim VN, Han J, Siomi MC (2009) Biogenesis of small RNAs in animals. Nat Rev Mol Cell Biol 10: 126-139.
10. Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, et al. (2004) The Microprocessor complex mediates the genesis of microRNAs. Nature 432: 235-240.
11. Denli AM, Tops BB, Plasterk RH, Ketting RF, Hannon GJ (2004) Processing of primary microRNAs by the Microprocessor complex. Nature 432: 231-235.
12. Han J, Lee Y, Yeom KH, Kim YK, Jin H, et al. (2004) The Drosha-DGCR8 complex in primary microRNA processing. Genes Dev 18: 3016-3027.
13. Wang Y, Medvid R, Melton C, Jaenisch R, Blelloch R (2007) DGCR8 is essential for microRNA biogenesis and silencing of embryonic stem cell self-renewal. Nat Genet 39: 380-385.
14. Lee Y, Ahn C, Han J, Choi H, Kim J, et al. (2003) The nuclear RNase III Drosha initiates microRNA processing. Nature 425: 415-419.
15. Bohnsack MT, Czaplinski K, Gorlich D (2004) Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA 10: 185-191.
16. Okada C, Yamashita E, Lee SJ, Shibata S, Katahira J, et al. (2009) A high-resolution structure of the pre-microRNA nuclear export machinery. Science 326: 1275-1279.
17. Lee Y, Hur I, Park SY, Kim YK, Suh MR, et al. (2006) The role of PACT in the RNA silencing pathway. EMBO J 25: 522-532.
18. Maniataki E, Mourelatos Z (2005) A human, ATP-independent, RISC assembly machine fueled by pre-miRNA. Genes Dev 19: 2979-2990.
19. Karreth FA, Tay Y, Perna D, Ala U, Tan SM, et al. (2011) In vivo identification of tumor- suppressive PTEN ceRNAs in an oncogenic BRAF-induced mouse model of melanoma. Cell 147: 382-395.
20. Tay Y, Kats L, Salmena L, Weiss D, Tan SM, et al. (2011) Coding-independent regulation of the tumor suppressor PTEN by competing endogenous mRNAs. Cell 147: 344-357.
21. Sumazin P, Yang X, Chiu HS, Chung WJ, Iyer A, et al. (2011) An extensive microRNA-mediated network of RNA-RNA interactions regulates established oncogenic pathways in glioblastoma. Cell 147: 370-381.
22. Poliseno L, Salmena L, Zhang J, Carver B, Haveman WJ, et al. (2010) A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. Nature 465: 1033-1038.
23. Cesana M, Cacchiarelli D, Legnini I, Santini T, Sthandier O, et al. (2011) A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. Cell 147: 358-369.
24. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP (2011) A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell 146: 353-358.
25. Rouault TA (2006) The role of iron regulatory proteins in mammalian iron homeostasis and disease. Nat Chem Biol 2: 406-414.
26. Theil EC, Goss DJ (2009) Living with iron (and oxygen): questions and answers about iron homeostasis. Chem Rev 109: 4568-4579.
27. Aisen P, Enns C, Wessling-Resnick M (2001) Chemistry and biology of eukaryotic iron metabolism. Int J Biochem Cell Biol 33: 940-959.
28. Hentze MW, Muckenthaler MU, Galy B, Camaschella C (2010) Two to tango: regulation of Mammalian iron metabolism. Cell 142: 24-38.
29. Muckenthaler MU, Galy B, Hentze MW (2008) Systemic iron homeostasis and the iron-responsive element/iron-regulatory protein (IRE/IRP) regulatory network. Annu Rev Nutr 28: 197-213.
30. Smith SR, Cooperman S, Lavaute T, Tresser N, Ghosh M, et al. (2004) Severity of neurodegeneration correlates with compromise of iron metabolism in mice with iron regulatory protein deficiencies. Ann N Y Acad Sci 1012: 65-83.
31. Andrews NC (2008) Forging a field: the golden age of iron biology. Blood 112: 219-230.
32. Wallander ML, Leibold EA, Eisenstein RS (2006) Molecular control of vertebrate iron homeostasis by iron regulatory proteins. Biochim Biophys Acta 1763: 668-689.
33. Shi H, Bencze KZ, Stemmler TL, Philpott CC (2008) A cytosolic iron chaperone that delivers iron to ferritin. Science 320: 1207-1210.
34. McKie AT, Marciani P, Rolfs A, Brennan K, Wehr K, et al. (2000) A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. Mol Cell 5: 299-309.
35. Donovan A, Lima CA, Pinkus JL, Pinkus GS, Zon LI, et al. (2005) The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. Cell Metab 1: 191-200.
36. Abboud S, Haile DJ (2000) A novel mammalian iron-regulated protein involved in intracellular iron metabolism. J Biol Chem 275: 19906-19912.
37. Delaby C, Pilard N, Puy H, Canonne--Hergaux F (2008) Sequential regulation of ferroportin expression after erythrophagocytosis in murine macrophages: early mRNA induction by haem, followed by iron--dependent protein expression. Biochem J 411: 123-131.
38. Marro S, Chiabrando D, Messana E, Stolte J, Turco E, et al. (2010) Heme controls ferroportin1 (FPN1) transcription involving Bach1, Nrf2 and a MARE/ARE sequence motif at position -7007 of the FPN1 promoter. Haematologica 95: 1261-1268.
39. Hentze MW, Muckenthaler MU, Andrews NC (2004) Balancing acts: molecular control of mammalian iron metabolism. Cell 117: 285-297.
40. Lymboussaki A, Pignatti E, Montosi G, Garuti C, Haile DJ, et al. (2003) The role of the iron responsive element in the control of ferroportin1/IREG1/MTP1 gene expression. J Hepatol 39: 710-715.
41. De Domenico I, Ward DM, Langelier C, Vaughn MB, Nemeth E, et al. (2007) The molecular mechanism of hepcidin-mediated ferroportin down-regulation. Mol Biol Cell 18: 2569-2578.
42. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, et al. (2004) Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 306: 2090-2093.
43. Krause A, Neitz S, Mägert HJ, Schulz A, Forssmann WG, et al. (2000) LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. FEBS Lett 480: 147-150.
44. Park CH, Valore EV, Waring AJ, Ganz T (2001) Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J Biol Chem 276: 7806-7810.
45. Muckenthaler M, Roy CN, Custodio AO, Miñana B, deGraaf J, et al. (2003) Regulatory defects in liver and intestine implicate abnormal hepcidin and Cybrd1 expression in mouse hemochromatosis. Nat Genet 34: 102-107.

46. Bridle KR, Frazer DM, Wilkins SJ, Dixon JL, Purdie DM, et al. (2003) Disrupted hepcidin regulation in HFE-associated haemochromatosis and the liver as a regulator of body iron homoeostasis. Lancet 361: 669-673.

47. Camaschella C, Roetto A, Calì A, De Gobbi M, Garozzo G, et al. (2000) The gene TFR2 is mutated in a new type of haemochromatosis mapping to 7q22. Nat Genet 25: 14-15.

48. Fleming RE, Ahmann JR, Migas MC, Waheed A, Koeffler HP, et al. (2002) Targeted mutagenesis of the murine transferrin receptor-2 gene produces hemochromatosis. Proc Natl Acad Sci U S A 99: 10653-10658.

49. Papanikolaou G, Samuels ME, Ludwig EH, MacDonald ML, Franchini PL, et al. (2004) Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. Nature genetics 36: 77-82.

50. Huang FW, Pinkus JL, Pinkus GS, Fleming MD, Andrews NC (2005) A mouse model of juvenile hemochromatosis. J Clin Invest 115: 2187-2191.

51. Niederkofler V, Salie R, Arber S (2005) Hemojuvelin is essential for dietary iron sensing, and its mutation leads to severe iron overload. J Clin Invest 115: 2180-2186.

52. Mleczko-Sanecka K, Casanovas G, Ragab A, Breitkopf K, Müller A, et al. (2010) SMAD7 controls iron metabolism as a potent inhibitor of hepcidin expression. Blood 115: 2657-2665.

53. Silvestri L, Pagani A, Nai A, De Domenico I, Kaplan J, et al. (2008) The serine protease matriptase-2 (TMPRSS6) inhibits hepcidin activation by cleaving membrane hemojuvelin. Cell Metab 8: 502-511.

54. Fernandes A, Preza GC, Phung Y, De Domenico I, Kaplan J, et al. (2009) The molecular basis of hepcidin-resistant hereditary hemochromatosis. Blood 114: 437-443.

55. Lee PL, Beutler E (2009) Regulation of hepcidin and iron-overload disease. Annu Rev Pathol 4: 489-515.

56. Drakesmith H, Schimanski LM, Ormerod E, Merryweather-Clarke AT, Viprakasit V, et al. (2005) Resistance to hepcidin is conferred by hemochromatosis-associated mutations of ferroportin. Blood 106: 1092-1097.

57. Sham RL, Phatak PD, Nemeth E, Ganz T (2009) Hereditary hemochromatosis due to resistance to hepcidin: high hepcidin concentrations in a family with C326S ferroportin mutation. Blood 114: 493-494.

58. Ohgami RS, Campagna DR, Greer EL, Antiochos B, McDonald A, et al. (2005) Identification of a ferrireductase required for efficient transferrin-dependent iron uptake in erythroid cells. Nat Genet 37: 1264-1269.

59. Faller M, Matsunaga M, Yin S, Loo JA, Guo F (2007) Heme is involved in microRNA processing. Nat Struct Mol Biol 14: 23-29.

60. Barr I, Smith AT, Senturia R, Chen Y, Scheidemantle BD, et al. (2011) DiGeorge critical region 8 (DGCR8) is a double-cysteine-ligated heme protein. J Biol Chem 286: 16716-16725.

61. Faller M, Toso D, Matsunaga M, Atanasov I, Senturia R, et al. (2010) DGCR8 recognizes primary transcripts of microRNAs through highly cooperative binding and formation of higher-order structures. RNA 16: 1570-1583.

62. Senturia R, Faller M, Yin S, Loo JA, Cascio D, et al. (2010) Structure of the dimerization domain of DiGeorge critical region 8. Protein Sci 19: 1354-1365.

63. Barr I, Smith AT, Chen Y, Senturia R, Burstyn JN, et al. (2012) Ferric, not ferrous, heme activates RNA-binding protein DGCR8 for primary microRNA processing. Proc Natl Acad Sci U S A 109: 1919-1924.

64. Bonkovsky HL, Hou W, Steuerwald N, Tian Q, Li T, et al. (2013) Heme status affects human hepatic messenger RNA and microRNA expression. World J Gastroenterol 19: 1593-1601.

65. Pogue AI, Percy ME, Cui JG, Li YY, Bhattacharjee S, et al. (2011) Up-regulation of NF-kB-sensitive miRNA-125b and miRNA-146a in metal sulfate-stressed human astroglial (HAG) primary cell cultures. J Inorg Biochem 105: 1434-1437.

66. Park SO, Kumar M, Gupta S (2012) TGF-Î² and iron differently alter HBV replication in human hepatocytes through TGF-Î²/BMP signaling and cellular microRNA expression. PLoS One 7: e39276.

67. Li Y, Lin L, Li Z, Ye X, Xiong K, et al. (2012) Iron homeostasis regulates the activity of the microRNA pathway through poly(C)-binding protein 2. Cell Metab 15: 895-904.

68. Makeyev AV, Liebhaber SA (2002) The poly(C)-binding proteins: a multiplicity of functions and a search for mechanisms. RNA 8: 265-278.

69. Höck J, Weinmann L, Ender C, Rüdel S, Kremmer E, et al. (2007) Proteomic and functional analysis of Argonaute-containing mRNA-protein complexes in human cells. EMBO Rep 8: 1052-1060.

70. Cianetti L, Segnalini P, Calzolari A, Morsilli O, Felicetti F, et al. (2005) Expression of alternative transcripts of ferroportin-1 during human erythroid differentiation. Haematologica 90: 1595-1606.

71. Zhang DL, Hughes RM, Ollivierre-Wilson H, Ghosh MC, Rouault TA (2009) A ferroportin transcript that lacks an iron--responsive element enables duodenal and erythroid precursor cells to evade translational repression. Cell metab 9: 461-473.

72. Hafner M, Landthaler M, Burger L, Khorshid M, Hausser J, et al. (2010) Transcriptome--wide identification of RNA-binding protein and microRNA target sites by PAR-CLIP. Cell 141: 129-141.

73. Chi SW, Zang JB, Mele A, Darnell RB (2009) Argonaute HITS-CLIP decodes microRNA-mRNA interaction maps. Nature 460: 479-486.

74. Sangokoya C, Doss JF, Chi JT (2013) Iron-responsive miR-485-3p regulates cellular iron homeostasis by targeting ferroportin. PLoS Genet 9: e1003408.

75. Keene JD (2007) RNA regulons: coordination of post-transcriptional events. Nat Rev Genet 8: 533-543.

76. Tenenbaum SA, Christiansen J, Nielsen H (2011) The post-transcriptional operon. Methods Mol Biol 703: 237-245.

77. Leung AK, Sharp PA (2010) MicroRNA functions in stress responses. Mol Cell 40: 205-215.

78. Magro G, Cataldo I, Amico P, Torrisi A, Vecchio GM, et al. (2011) Aberrant expression of TfR1/CD71 in thyroid carcinomas identifies a novel potential diagnostic marker and therapeutic target. Thyroid : official journal of the American Thyroid Association 21: 267-277.

79. Kwok JC, Richardson DR (2002) The iron metabolism of neoplastic cells: alterations that facilitate proliferation? Crit Rev Oncol Hematol 42: 65-78.

80. Rovera G, Santoli D, Damsky C (1979) Human promyelocytic leukemia cells in culture differentiate into macrophage-like cells when treated with a phorbol diester. Proc Natl Acad Sci U S A 76: 2779-2783.

81. Schaar DG, Medina DJ, Moore DF, Strair RK, Ting Y (2009) miR-320 targets transferrin receptor 1 (CD71) and inhibits cell proliferation. Exp Hematol 37: 245-255.

82. Hamara K, Bielecka-Kowalska A, Przybylowska-Sygut K, Sygut A, Dziki A, et al. (2013) Alterations in expression profile of iron-related genes in colorectal cancer. Mol Biol Rep 40: 5573-5585.

83. Hubert N, Hentze MW (2002) Previously uncharacterized isoforms of divalent metal transporter (DMT)-1: implications for regulation and cellular function. Proc Natl Acad Sci U S A 99: 12345-12350.

84. Gunshin H, Allerson CR, Polycarpou-Schwarz M, Rofts A, Rogers JT, et al. (2001) Iron-dependent regulation of the divalent metal ion transporter. FEBS Lett 509: 309-316.

85. Tchernitchko D, Bourgeois M, Martin ME, Beaumont C (2002) Expression of the two mRNA isoforms of the iron transporter Nramp2/DMTI in mice and function of the iron responsive element. Biochem J 363: 449-455.

86. Canonne-Hergaux F, Zhang AS, Ponka P, Gros P (2001) Characterization of the iron transporter DMT1 (NRAMP2/DCT1) in red blood cells of normal and anemic mk/mk mice. Blood 98: 3823-3830.

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

88. Hou W, Tian Q, Steuerwald NM, Schrum LW, Bonkovsky HL (2012) The let-7 microRNA enhances heme oxygenase-1 by suppressing Bach1 and attenuates oxidant injury in human hepatocytes. Biochim Biophys Acta 1819: 1113-1122.

89. Hou W, Tian Q, Zheng J, Bonkovsky HL (2010) MicroRNA-196 represses Bach1 protein and hepatitis C virus gene expression in human hepatoma cells expressing hepatitis C viral proteins. Hepatology 51: 1494-1504.

90. Hintze KJ, Katoh Y, Igarashi K, Theil EC (2007) Bach1 repression of ferritin and thioredoxin reductase1 is heme-sensitive in cells and in vitro and coordinates expression with heme oxygenase1, beta-globin, and NADP(H) quinone (oxido) reductase1. J Biol Chem 282: 34365-34371.

91. Asci R, Vallefuoco F, Andolfo I, Bruno M, De Falco L, et al. (2013) Trasferrin receptor 2 gene regulation by microRNA 221 in SH-SY5Y cells treated with MPP(+) as Parkinson's disease cellular model. Neurosci Res 77: 121-127.

92. Castoldi M, Vujic Spasic M, Altamura S, Elmén J, Lindow M, et al. (2011) The liver-specific microRNA miR-122 controls systemic iron homeostasis in mice. J Clin Invest 121: 1386-1396.

93. Krützfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, et al. (2005) Silencing of microRNAs in vivo with 'antagomirs'. Nature 438: 685-689.

94. Elmén J, Lindow M, Silahtaroglu A, Bak M, Christensen M, et al. (2008) Antagonism of microRNA-122 in mice by systemically administered LNA-antimiR leads to up-regulation of a large set of predicted target mRNAs in the liver. Nucleic Acids Res 36: 1153-1162.

95. Padgett KA, Lan RY, Leung PC, Lleo A, Dawson K, et al. (2009) Primary biliary cirrhosis is associated with altered hepatic microRNA expression. J Autoimmun 32: 246-253.

96. Kutay H, Bai S, Datta J, Motiwala T, Pogribny I, et al. (2006) Downregulation of miR-122 in the rodent and human hepatocellular carcinomas. J Cell Biochem 99: 671-678.

97. Girard M, Jacquemin E, Munnich A, Lyonnet S, Henrion-Caude A (2008) miR-122, a paradigm for the role of microRNAs in the liver. J Hepatol 48: 648-656.

98. Kew MC (2009) Hepatic iron overload and hepatocellular carcinoma. Cancer Lett 286: 38-43.

99. Kosaka N, Sugiura K, Yamamoto Y, Yoshioka Y, Miyazaki H, et al. (2008) Identification of erythropoietin-induced microRNAs in haematopoietic cells during erythroid differentiation. Br J Haematol 142: 293-300.

100. Camps C, Buffa FM, Colella S, Moore J, Sotiriou C, et al. (2008) hsa-miR-210 Is induced by hypoxia and is an independent prognostic factor in breast cancer. Clinical cancer research : an official journal of the American Association for Cancer Research 14: 1340-1348.

101. Chan SY, Zhang YY, Hemann C, Mahoney CE, Zweier JL, et al. (2009) MicroRNA-210 controls mitochondrial metabolism during hypoxia by repressing the iron-sulfur cluster assembly proteins ISCU1/2. Cell Metab 10: 273-284.

102. Chen Z, Li Y, Zhang H, Huang P, Luthra R (2010) Hypoxia-regulated microRNA-210 modulates mitochondrial function and decreases ISCU and COX10 expression. Oncogene 29: 4362-4368.

103. Favaro E, Ramachandran A, McCormick R, Gee H, Blancher C, et al. (2010) MicroRNA-210 regulates mitochondrial free radical response to hypoxia and krebs cycle in cancer cells by targeting iron sulfur cluster protein ISCU. PLoS One 5: e10345.

104. Tong WH, Rouault TA (2006) Functions of mitochondrial ISCU and cytosolic ISCU in mammalian iron-sulfur cluster biogenesis and iron homeostasis. Cell Metab 3: 199-210.

105. Ye H, Rouault TA (2010) Human iron-sulfur cluster assembly, cellular iron homeostasis, and disease. Biochemistry 49: 4945-4956.

106. Qiao A, Khechaduri A, Kannan Mutharasan R, Wu R, Nagpal V, et al. (2013) MicroRNA-210 decreases heme levels by targeting ferrochelatase in cardiomyocytes. J Am Heart Assoc 2: e000121.

107. McCormick RI, Blick C, Ragoussis J, Schoedel J, Mole DR, et al. (2013) miR-210 is a target of hypoxia-inducible factors 1 and 2 in renal cancer, regulates ISCU and correlates with good prognosis. Br J Cancer 108: 1133-1142.

108. Colleoni F, Padmanabhan N, Yung HW, Watson ED, Cetin I, et al. (2013) Suppression of mitochondrial electron transport chain function in the hypoxic human placenta: a role for miRNA-210 and protein synthesis inhibition. PLoS One 8: e55194.

109. Kim JH, Park SG, Song SY, Kim JK, Sung JH (2013) Reactive oxygen species-responsive miR-210 regulates proliferation and migration of adipose-derived stem cells via PTPN2. Cell Death Dis 4: e588.

110. Yoshioka Y, Kosaka N, Ochiya T, Kato T (2012) Micromanaging Iron Homeostasis: hypoxia-inducible micro-RNA-210 suppresses iron homeostasis-related proteins. J Biol Chem 287: 34110-34119.

111. Richardson DR, Kalinowski DS, Lau S, Jansson PJ, Lovejoy DB (2009) Cancer cell iron metabolism and the development of potent iron chelators as anti-tumour agents. Biochim Biophys Acta 1790: 702-717.

112. O'Donnell KA, Yu D, Zeller KI, Kim JW, Racke F, et al. (2006) Activation of transferrin receptor 1 by c-Myc enhances cellular proliferation and tumorigenesis. Mol Cell Biol 26: 2373-2386.

113. Chitambar CR, Massey EJ, Seligman PA (1983) Regulation of transferrin receptor expression on human leukemic cells during proliferation and induction of differentiation. Effects of gallium and dimethylsulfoxide. J Clin Invest 72: 1314-1325.

114. Neckers LM, Trepel JB (1986) Transferrin receptor expression and the control of cell growth. Cancer Invest 4: 461-470.

115. Arosio P, Levi S (2010) Cytosolic and mitochondrial ferritins in the regulation of cellular iron homeostasis and oxidative damage. Biochim Biophys Acta 1800: 783-792.

116. Shpyleva SI, Tryndyak VP, Kovalchuk O, Starlard-Davenport A, Chekhun VF, et al. (2011) Role of ferritin alterations in human breast cancer cells. Breast Cancer Res Treat 126: 63-71.

117. Richardson DR (2002) Iron chelators as therapeutic agents for the treatment of cancer. Crit Rev Oncol Hematol 42: 267-281.

118. Whitnall M, Richardson DR (2006) Iron: a new target for pharmacological intervention in neurodegenerative diseases. Semin Pediatr Neurol 13: 186-197.

119. Vaishnaw AK, Gollob J, Gamba-Vitalo C, Hutabarat R, Sah D, et al. (2010) A status report on RNAi therapeutics. Silence 1: 14.

120. Liao Y, Du X, Lönnerdal B (2010) miR-214 regulates lactoferrin expression and pro-apoptotic function in mammary epithelial cells. J Nutr 140: 1552-1556.

121. Liao Y, Lönnerdal B (2010) miR-584 mediates post-transcriptional expression of lactoferrin receptor in Caco-2 cells and in mouse small intestine during the perinatal period. Int J Biochem Cell Biol 42: 1363-1369.