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The role of microRNAs in metal carcinogen-induced cell malignant transformation and tumorigenesis

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Abstract

MicroRNAs (miRNAs), an important component of epigenetic mechanisms of carcinogenesis, have been shown to play crucial roles in cancer initiation, metastasis, prognosis and responses to drug treatment and may serve as biomarkers for early diagnosis of cancer and tools for cancer therapy. Metal carcinogens, such as arsenic, cadmium, hexavalent chromium and nickel, are well-established human carcinogens causing various cancers upon long term exposure. However, the mechanism of metal carcinogenesis has not been well understood, which limits our capability to effectively diagnose and treat human cancers resulting from chronic metal carcinogen exposure. Over recent years, the role of miRNAs in metal carcinogenesis has been actively explored and a growing body of evidence indicates the critical involvement of miRNAs in metal carcinogenesis. This review aims to discuss recent studies showing that miRNAs play important roles in metal carcinogen-induced cell malignant transformation and tumorigenesis. Some thoughts for future further studies in this field are also presented.

Keywords

MicroRNA (miRNA); epigenetics; metal; metal carcinogenesis; arsenic; cadmium; chromium; nickel

1. Introduction

1.1. miRNA biogenesis, function and regulation

MicroRNAs (miRNAs or miRs), are a large family of small non-coding RNA molecules that negatively regulate protein-coding gene expression post-transcriptionally (Bartel, 2004;

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Hobert, 2008). miRNAs are initially transcribed either mono- or polycistronically by RNA polymerase II (RNA Pol II) in the nucleus to yield primary transcripts (termed primary miRNAs, or pri-miRNAs) that are hundreds to thousands of nucleotides long (Lee et al., 2004; Cai et al., 2004). Pri-miRNAs are then polyadenylated, capped, and subjected to a microprocessing event carried out by the type III RNase Drosha with its cofactor DiGeorge Syndrome Critical Region 8 (DGCR8) to reduce them to ~70 nucleotide precursor miRNAs (pre-miRNAs) (Lee et al., 2003; Han et al., 2004). The pre-miRNA then forms a complex with exportin-5 and Ran-GTP, a GTP-binding nuclear protein, which then exports the pre-miRNA into the cytosol. Once in the cytosol the pre-miRNA is then subjected to another processing event by another type III RNase Dicer resulting in a ~21-22 nucleotide miRNA duplex (Hutvagner et al., 2001; Grishok et al., 2001). Following miRNA duplex unwinding, one strand (the passenger strand or the star strand) is usually degraded, while the other strand (the guide strand or the mature miRNA) is associated with the Argonaute protein and subsequently incorporated into the RNA-induced silencing complex (RISC) (Meister, 2013). The mature miRNA/RISC complex is then able to regulate the expression of target genes.

Typically, miRNAs elicit their regulatory function by base pairing with the 3' untranslated regions (3' UTR) of their target messenger RNAs (mRNAs) through their seed sequences. However instances of miRNAs interacting with other parts of the mRNA have also been reported (Zhou and Rigoutsos, 2014; Qin et al., 2010). The seed sequence is the second to eighth nucleotide of the 5' end of the mature miRNA that gives the miRNA specificity towards their target mRNAs. The binding of a miRNA to its target mRNA can result in mRNA destabilization and degradation, translational inhibition or direct cleavage (Pillai et al., 2007; Bartel, 2009; Karginov et al., 2010; Bracken et al., 2011). Destabilization and degradation of mRNA is a common form of protein-coding gene expression regulation, which usually is mediated by the same imperfect miRNA:mRNA base pairing that causes translational inhibition (Eichhorn et al., 2014; Baek et al., 2008; Selbach et al., 2008). However, direct cleavage of the target mRNA typically requires more extensive base pairing (Karginov et al., 2010; Bracken et al., 2011). Even though the seed sequence of a miRNA is the most prominent characteristic that regulates miRNA:mRNA interactions, there are examples of miRNAs having weak seed sequence binding and better overall complementarity to the mRNA which can lead to the inhibition of gene expression (Bartel, 2009; Brennecke et al., 2005; Carroll et al., 2014). A brief schematic description of miRNA biogenesis and mechanism of down-regulating gene expression is presented in Figure 1.

Since the seed sequence of a miRNA can be the same for multiple miRNAs and that an individual miRNA can target many genes, miRNAs have been thought to regulate at least two-thirds of all protein coding genes in humans (Friedman et al., 2009). It is not surprising that experimental evidence has shown that miRNAs are involved in almost all aspects of cellular functions and many important biological processes. For example, numerous studies have demonstrated that miRNAs play crucial roles in cell proliferation (Piccoli et al., 2015) and differentiation (Lazare et al., 2014), cell death (Su et al., 2015), immunological functions (Chen et al., 2013), stem cell functions (Shenoy and Bluelloch, 2014), angiogenesis (Santulli, 2015), and cancer development and progression (Frixia et al., 2015; Humphries and Yang, 2015; Oom et al., 2014; Iorio and Croce, 2012). The importance of miRNAs in many biological processes is further evidenced by the high evolutionary conservation of individual

miRNA sequences and the process of miRNA biogenesis among many different species of organisms (Mattick, 2003; Taft et al., 2007).

Similar to protein-coding genes, the expression of miRNAs can also be regulated through genetic and epigenetic mechanisms. Studies indicate that modifications to the promoter regions of miRNAs can alter their expression. Hypermethylation of CpG islands and acetylation of promoter regions have been shown to decrease and increase the expression of miRNAs, respectively (Hou et al., 2011). Furthermore, the promoter regions of miRNAs can also be bound by certain transcription factors to regulate their expression. In addition to promoter modifications and interactions that regulate miRNA expression levels, the mature miRNAs can also be sequestered in the cytosol by long intergenic noncoding RNAs (lincRNAs). These lincRNAs that sequester mature miRNAs and reduce their activity are also referred to as miRNA sponges or competing endogenous RNAs (ceRNAs). More information about ceRNAs can be found in recent excellent reviews (Karthi and Subramanian, 2014; Sanchez-Mejias and Tay, 2015).

1.2. miRNAs and cancer

Calin et al. (2002) were the first to show that miRNAs are dysregulated in cancer. Since this discovery many studies have shown that miRNA expression is dysregulated in cancer through many different mechanisms, such as amplifications or deletions (Croce, 2009; Iorio and Croce, 2012). Further studies demonstrated that miRNAs are involved in cancer initiation and metastasis and dysregulated miRNAs may act as either oncogenes or tumor suppressors in cancer and are often referred to as oncomirs (Esquela-Kerscher and Slack, 2006). In addition, numerous studies also showed that circulating miRNAs in the blood are stable and may function as potential biomarkers for cancer diagnosis and prediction of cancer prognosis (Mitchell et al., 2008; Chim et al., 2008; Lawrie et al., 2008; Chen et al., 2008)..

Given the important roles of miRNAs in almost all aspects of cell functions and variety of biological processes, it is likely that miRNAs may play crucial roles in regulating cellular responses to chemical carcinogen exposure and thus are critically involved in chemical carcinogen-induced cell malignant transformation and tumorigenic process. Many studies have shown that the expression and function of cellular miRNAs are deregulated upon chronic exposure to chemical carcinogens (Izzotti and Pulliero, 2014; Pogribny et al., 2015). This review aims to discuss the growing body of evidence showing that the dysregulated miRNAs play important roles in a particular class of chemical carcinogens, metal carcinogen-induced cell malignant transformation and tumorigenesis.

2. The role of miRNAs in metal carcinogen-induced cell malignant transformation and tumorigenesis

Metal carcinogens such as arsenic, cadmium, hexavalent chromium, and nickel, are usually atomic dense metallic elements that can be toxic or poisonous even at low concentrations (Duffus, 2002). Exposure to metal carcinogens through air, soil, water, and food has been shown to increase the risk of cancer (Hu, 2002; Langie et al., 2015), however, the underlying

mechanisms of metal carcinogenesis have not been well understood. An increasing body of evidence has shown that miRNA dysregulation plays an important role in metal carcinogen-induced cell transformation and tumorigenesis.

2.1. Arsenic

Arsenic is one of the most common environmental pollutants and one of the most well-known human carcinogens. General population exposure to arsenic is mainly through consuming contaminated drinking water, and long term arsenic exposure significantly increases the risk of developing lung, skin, bladder and other cancers (Smith et al., 1992; Frumkin and Thun, 2001; IARC, 2004; Tapio and Grosche, 2006; Celik et al., 2008; Tokar et al., 2010a). Unlike many typical carcinogens, arsenic is not considered as a strong genotoxic carcinogen and it is generally accepted that the epigenetic mechanism and other non-genotoxic mechanisms may play crucial roles in arsenic carcinogenesis (Yang and Frenkel, 2002; Ren et al., 2011). Dysregulation of miRNAs, an important component of epigenetic mechanism of carcinogenesis, has been shown over recent years to be critically involved in arsenic-induced cell malignant transformation and tumorigenesis.

2.1.1. miR-200 family—The miR-200 family consists of five members located on two different chromosomes, *miR-200b*, *-200a*, *-429* located on chromosome 1 and *miR-200c* and *-141* on chromosome 12 in humans. The miR-200 family has been the focus of much research over the years because of their capability to inhibit epithelial-to-mesenchymal transition (EMT) (Bracken et al., 2008; Burk et al., 2008; Gregory et al., 2008; Korpál et al., 2008; Park et al., 2008) and the potential of the miR-200 members to suppress cancer metastasis (Gibbons et al., 2009; Li et al., 2014; Humphries et al., 2014; Humphries and Yang, 2015;).

Research in our lab has been the first to show the critical role of miRNA dysregulation in the effects of chronic arsenic exposure on cells (Wang et al., 2011). A 16-week continuous exposure of immortalized human bronchial epithelial cells (HBECs), that either had normal p53 expression or p53 expression stably knocked down (p53^{low}HBECs), with a low dose of arsenite resulted in drastic changes only in p53^{low}HBECs. These changes include a morphological shift from epithelial to a spindle-like mesenchymal morphology, a loss of the epithelial marker gene E-cadherin expression, and increases of the mesenchymal markers vimentin, zinc-finger E-box-binding homeobox factor 1 (ZEB1) and 2 (ZEB2) expression indicating the occurrence of epithelial-to-mesenchymal transition. Furthermore, a significant increase of colony formation in soft agar was only observed in arsenite-exposed p53^{low}HBECs (As-p53^{low}HBECs), and subcutaneous injection of As-p53^{low}HBECs into nude mice formed tumors whereas passage-matched control p53^{low}HBECs and arsenite-exposed p53-normal HBECs did not. These findings demonstrate that chronic arsenite exposure causes malignant transformation of p53^{low}HBECs.

To determine if miRNAs play a role in arsenite-induced cell malignant transformation, a miRNA microarray was performed (Wang et al., 2011). Results from the microarray showed that six miRNAs (the five miR-200 family members and miR-205) were downregulated more than two-fold in As-p53^{low}HBECs compared to passage-matched control

p53^{low}HBECs. Further QPCR analysis confirmed the significant down-regulation of miR-200 family in As-p53^{low}HBECs. To determine if the loss of miR-200 plays a role in arsenite-induced cell transformation and tumor formation, miR-200b was stably re-expressed in As-p53^{low}HBECs. Stable expression of miR-200b in As-p53^{low}HBECs (As-p53^{low}HBECs-GFP-200b) resulted in restored E-cadherin expression, an epithelial-like morphology, and a decreased expression of ZEB1 and ZEB2. Furthermore, miR-200b stable expression in As-p53^{low}HBECs significantly decreased their soft agar colony formation, and completely blocked subcutaneous tumor formation when inoculated into nude mice. To further determine whether stably expressing miR-200 in parental p53^{low}HBECs is capable of reducing or preventing cell transformation by chronic arsenic exposure, miR-200b was stably expressed in parental p53^{low}HBECs and subjected the cells to a similar chronic low dose of arsenite exposure. It was found that stable expression of miR-200b prevented cellular transformation by chronic arsenite exposure (Wang et al., 2011). Taken together, these results indicate that miR-200b down-regulation plays a causal role in arsenite-induced cell malignant transformation and tumorigenesis. In addition, our further follow-up studies also demonstrated that down-regulation of miR-200b plays critical roles in promoting arsenic-transformed cell migration, invasion and tumor angiogenesis (Wang et al., 2012b, 2013, 2014b). Moreover, significant down-regulation of miR-200c level in arsenic-transformed human bronchial epithelial (HBE) cells was recently also observed by other group (Xu et al., 2015a).

Since arsenic exposure has previously been shown to cause DNA methylation (Cui et al., 2006; Arita and Costa, 2009; Hernandez et al., 2009; Reichard and Puga, 2010), we looked at promoter hypermethylation as a possible mechanism for miR-200 down-regulation by chronic arsenic exposure. A melt curve PCR analysis found that both the *miR-200b/200a/429* and *miR-200c/141* cluster promoter regions were highly methylated in arsenite-transformed cells (As-p53^{low}HBECs) (Wang et al., 2011). Furthermore, treatment of As-p53^{low}HBECs with the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine (5Aza) increased the expression of miR-200b and -200c, as well as E-cadherin (Wang et al., 2011). This data suggests that increased DNA methylation plays an important role in chronic arsenic exposure-caused down-regulation of miR-200 family expression.

Similarly, another recent study reported an important role of miR-200 down-regulation in arsenic-induced human urothelial cell transformation. Michailidi et al. (2015) found that chronic treatment of immortalized human urothelial cells (HUC1) with arsenic resulted in an EMT-like change in morphology at 4, 6, 8, and 10 months. Concurrent with these morphological changes, arsenic-treated cells also had a dramatic increase in the PI3K-Akt signaling pathway, as well as a decrease in E-cadherin at 6 and 10 months and an increase of vimentin at 10 months. Due to the EMT-like morphological changes, Michailidi et al. (2015) analyzed the expression of the EMT-regulating miR-200 family as well as miR-205. It was found that miR-200a, -200b, and -200c were significantly reduced at months 6, 8 and 10 in the arsenic-treated HUC1 cells compared to untreated HUC1 cells.

Moreover, Michailidi et al. (2015) further determined whether this phenotype also occurred in an arsenic-exposed human population. It was found that urine levels of miR-200a, -200b, -200c, and -205 was reduced in the arsenic-exposed population. Further analysis on urine

levels of miR-200 in humans exposed to different levels of arsenic in water revealed that the level of miR-200c was inversely associated with the levels of arsenic exposure (Michailidi et al., 2015). These findings suggest that down-regulation of miR-200 family members may also play an important role in arsenic exposure-caused human cancers.

2.1.2 miR-21—miR-21 has been reported to be one of the most commonly up-regulated miRNAs in various human cancers functioning as a key regulator of carcinogenic process, and has been considered as a novel target in cancer therapeutics (Selcuklu et al., 2009; Krichevsky and Gabriely, 2009; Pan et al., 2010; Kumarswamy et al., 2011; Wang et al., 2014a). It is interesting that recent studies also show that miR-21 is critically involved in arsenic-induced cell malignant transformation and tumorigenesis.

Ling et al. (2012) first reported the involvement of miR-21 in arsenic-induced transformation of human embryo lung fibroblast cells (HELFs). After continuously treating HELF cells with arsenite for 30 passages (15 weeks), Ling et al. found HELF cells were transformed as evidenced by increased soft agar colony formation by the arsenite-treated cells. The expression levels of miR-21 were assessed at 10, 20, and 30 passages of arsenite treatment. At each of the time points, the miR-21 expression level was significantly higher in cells treated with arsenite than that in passage-matched control cells. Furthermore, treatment of HELF cells with arsenite resulted in increased miR-21 expression starting at 3 hrs post-treatment. The authors then determined the mechanism by which arsenite treatment up-regulates miR-21 expression. It was found that arsenite treatment increased the formation of reactive oxygen species (ROS), which triggered the extracellular signal-regulated kinase (ERK)-nuclear factor- κ B (NF- κ B) pathway activation. The activated NF- κ B directly binds to the miR-21 promoter region, resulting in increased miR-21 expression.

To determine a potential role of miR-21 up-regulation in arsenite-induced transformation of HELF cells, Ling et al. (2012) further analyzed the expression levels of known targets of miR-21 and found that the level of Spry, a negative regulator of the Ras/MEK/ERK pathway, was decreased in arsenite-treated cells. Given the well-established role of ERK/NF- κ B pathway in cancer, these findings suggest that miR-21 up-regulation plays a critical role in arsenite-induced transformation of HELF cells.

Further studies from the same laboratory showed that miR-21 up-regulation also plays a role in arsenite-induced transformation of human lung epithelial cells. Luo et al. (2013) found that the expression level of miR-21 was increased in arsenite-transformed human bronchial epithelial cells (HBECs). Mechanistic studies revealed that arsenite treatment increases the secretion of a pro-inflammatory cytokine interleukin-6 (IL-6), which in turn causes activation of signal transducer and activator of transcription 3 (STAT3), a transcription factor. Activated STAT3 subsequently increased the expression of miR-21 as inhibition of STAT3 blocked the arsenite-induced increase of miR-21 expression (Luo et al., 2013). The important role of miR-21 in arsenic-induced cell transformation and tumorigenesis was further demonstrated by recent studies showing that exosomal miR-21 derived from arsenite-transformed cell promotes cell proliferation (Xu et al., 2015b) and that knockdown of miR-21 expression reduces tumor angiogenesis in xenograft tumors produced by inoculation

of arsenic-transformed cells (Zhao et al., 2013). Together, these studies indicate that miR-21 plays a multifaceted role in arsenite-induced cellular transformation and tumorigenesis.

2.1.3 Other miRNAs—In addition to the studies discussed above showing the important roles of miR-200 and miR-21 in arsenic-induced human lung cell transformation and tumorigenesis, a recent study using another human lung epithelial cell line (BEAS-2B) showed that the expression level of miR-199a-5p was down-regulated more than 100-fold in arsenic-transformed cells (He et al., 2014). In contrast, stably expressing miR-199a-5p in arsenic-transformed cells significantly reduced mouse xenograft tumor growth and tumor angiogenesis. Further mechanistic studies revealed that arsenic exposure down-regulates miR-199a-5p expression by generating ROS (He et al., 2014). Another recent study showed that miR-191 was significantly up-regulated in arsenic-transformed human bronchial epithelial (HBE) cells and inhibition of miR-191 significantly reduced their transformed phenotypes (Xu et al., 2015a). Together, these studies suggest that the dysregulation of multiple miRNA expression levels may play important roles in arsenic-caused human lung cell malignant transformation.

In addition to causing lung cancer, arsenic exposure also increases the risk of developing skin cancer (Smith et al., 1992). Chronic arsenic exposure is capable of inducing human skin cell malignant transformation although the underlying mechanism has not been clearly defined (Pi et al., 2008). Jiang et al. (2014) recently reported that the levels of let-7 family miRNAs were significantly reduced in arsenic-transformed human keratinocytes HaCaT cells. Re-expression of let-7c, a member of the let-7 family of miRNAs, significantly reduced the malignant phenotypes of arsenic-transformed HaCaT cells. Mechanistic studies suggest that arsenic treatment down-regulates let-7 expression by increasing DNA methylation as treatment with 5-aza-2-deoxycytidine, an inhibitor of DNA methyltransferases, prevents the arsenic-caused decreases of let-7 levels. Consistent with the fact that oncogenic Ras is the target of the let-7 family (Johnson et al., 2005; Choudhury and Li, 2012), Jiang et al. (2014) observed that the Ras/NF- κ B pathway is activated in arsenic-transformed HaCaT cells, and re-expression of let-7c inhibits the Ras/NF- κ B pathway. The findings from this study suggest that down-regulation of let-7 family of miRNAs may play an important role in arsenic-caused transformation of human keratinocytes.

Previous studies showed that chronic arsenic exposure causes malignant transformation of human prostate epithelial cells (Achanzar et al., 2002; Tokar et al., 2010b). A recent study analyzed the miRNA expression profiles of transformed and non-transformed human prostate epithelial RWPE-1 cells (Ngalame et al., 2014). Compared to controls, arsenic-transformed RWPE-1 (CAsE-PE) cells had 29 and arsenic-transformed prostate stem (As-CSC) cells had 13 differentially expressed miRNAs with the majority of them being downregulated. Of the downregulated miRNAs, many of them correlated with an increased expression of *RAS* and *RAN* oncogenes in CAsE-PE (miR-134, -373, -155, -138, -205, -181d, -181c, let-7b, let-7i, let-7e, let-7c) and As-CSC (miR-143, -34c-5p, and -205) cells, respectively (Ngalame et al., 2014). The authors concluded that arsenic induces malignant cellular transformation of human prostate cells by dysregulating the cellular miRNA profiles, which leads to an increase in the activation of oncogenic pathways such as *RAS*. Indeed, a recent follow-up study from the same group showed that restoration of miR-143,

one of the significantly down-regulated miRNAs in arsenic-transformed prostate stem cells (As-CSC), greatly reduced multiple malignant phenotypes in the As-CSCs (Ngalame et al., 2015).

2.2 Nickel

Nickel is a metal that is commonly used to form alloys with iron, copper, chromium and zinc. Although commonly found in the earth's crust, nickel is released into the environment mainly from the industrial plants that make alloys or from power plants and trash incinerators (ATSDR, 2005). Nickel exposure is most commonly through inhalation of contaminated air or smoking tobacco. Chronic nickel exposure leads to increased lung and nasal cancers and it is generally accepted that epigenetic mechanisms and other non-genotoxic mechanisms may play critical roles in nickel carcinogenesis (Andersen et al., 1996; Grimsrud et al., 2002; Arita and Costa, 2009; Cameron et al., 2011; Sun et al., 2013).

Ji et al. (2008) found that the expression level of DNA methyltransferase 1 (DNMT1) was up-regulated in nickel (NiS)-transformed human bronchial epithelial (16HBE) cells, which is associated with the silenced expression of O⁶-methylguanine DNA methyltransferase (MGMT), an important DNA damage repair gene. To determine the mechanism by which nickel exposure up-regulates DNMT1 expression level, the authors investigated whether the expression of miRNAs that are capable of down-regulating DNMT1 are reduced in nickel-transformed cells (Ji et al. 2013). By using miRNA target prediction computer program analysis, Ji et al. (2013) identified a number of miRNAs, which show sequence complementarity to the 3'-UTR of DNMT1, including miR-148a, -148b and -152. Further Q-PCR analysis revealed that the expression level of miR-152 was significantly down-regulated in nickel-transformed cells compared to passage-matched control cells. Ectopic expression of miR-152 in nickel-transformed cells greatly reduced the level of DNMT1. Interestingly, treatment with 5-Aza, an inhibitor of DNMT, or knockdown of DNMT1 using shRNA, significantly up-regulated the expression of miR-152 in nickel-transformed cells. These observations indicate that there is a double-negative loop for regulating the expression of miR-152 and DNMT1 in nickel-transformed cells. The authors further determined that re-expression of miR-152 in nickel-transformed cells significantly reduced cell proliferation and colony formation in a 14-day colony formation assay. Collectively, these findings suggest that miR-152 down-regulation may play an important role in nickel-induced human bronchial epithelial cell transformation.

To study the role of miRNA dysregulation in nickel-induced cell transformation and tumorigenesis, Zhang and colleagues (2013a) took a different approach by direct intramuscular injection of Ni₃S₂ into Wistar rats. Thirty two weeks after injection, muscle tumors formed by nickel treatment were collected for miRNA analysis to establish a miRNA library. From this miRNA library, miR-222 expression level was found to be significantly higher in nickel-induced tumor tissues than normal muscle tissue. Moreover, the authors found that the expression level of miR-222 is also significantly up-regulated in nickel-transformed human bronchial epithelial cells (16HBE), which produced tumors upon inoculation into nude mice. The findings from these *in vitro* cell culture and *in vivo* animal

model studies suggest that miR-222 up-regulation may play an important role in nickel-induced cell transformation and tumorigenesis.

In addition, Zhang and colleagues (2013b) also found that miR-203 is significantly down-regulated in nickel-transformed 16HBE cells. Overexpressing miR-203 in nickel-transformed 16HBE cells significantly decreased cell proliferation, colony formation and mouse xenograft tumor growth. Mechanistic studies revealed hypermethylation of CpGs in the miR-203 promoter and first exon area as well. Treatment with 5-AzadC, a DNA methyltransferase inhibitor, significantly increased miR-203 levels in nickel-transformed cells. Interestingly, treatment with a histone deacetylase inhibitor (TSA) also significantly up-regulated miR-203 levels in nickel-transformed cells. These findings suggest that chronic nickel exposure decreases miR-203 levels through DNA methylation and other deregulated epigenetic mechanisms; and miR-203 down-regulation could play a critical role in nickel-induced cell transformation and tumorigenesis. Further mechanistic studies showed that miR-203 down-regulation leads to up-regulation of its target gene ABL1, a well-established oncogene, and up-regulation of ABL1 may promote nickel-induced cell transformation and tumorigenesis (Zhang et al., 2013b).

As mentioned above, miR-21 is one of the most commonly up-regulated miRNAs in various human cancers and acts as a key regulator of carcinogenic process (Selcuklu et al., 2009; Krichevsky and Gabriely, 2009; Kumarswamy et al., 2011). A recent study reported that nickel treatment is able to increase miR-21 expression levels in a dose-dependent manner in human lung cancer cells (Chiou et al., 2015), suggesting that miR-21 may play a role in nickel carcinogenesis. Chiou et al. (2015) also observed that patients' lung cancer tissue nickel levels are associated with miR-21 expression levels. Moreover, Kaplan–Meier plot analysis showed that the high-nickel/high-miR-21 patient subgroup has significantly shorter overall survival and relapse free survival periods than the low-nickel/low-miR-21 patient subgroup. Mechanistically, it was determined that nickel exposure induces miR-21 expression through activation of the EGFR/NF- κ B signaling pathway.

2.3 Chromium

Chromium can be found in three different states (0, III, and VI) and it is chromium VI [Cr(VI)] that has carcinogenic effect (ATSDR, 2012b). Human Cr(VI) exposure can be through air, water, food, and skin contact with contaminated soil (ATSDR, 2012b).

Although Cr(VI) is a well-known human carcinogen that increases the risk for lung cancer, the underlying mechanism has not yet been fully elucidated. Since Cr(VI) is generally considered a strong genotoxic carcinogen, much less research has been done on the nongenotoxic mechanism of Cr(VI) carcinogenesis. As a result, the role of miRNA dysregulation in Cr(VI)-induced cell transformation and tumorigenesis has rarely been investigated. One recent study has shown that miR-143 was significantly down-regulated in Cr(VI)-transformed human bronchial epithelial BEAS-2B cells (He et al., 2013). As discussed above, the expression level of miR-143 was also found to be significantly decreased in arsenic-transformed human prostate stem cells and miR-143 re-expression greatly reduced malignant phenotypes of arsenic-transformed cells (Ngalame et al., 2014, 2015). Similarly, re-expression of miR-143 in Cr(VI)-transformed BEAS-2B cells

significantly reduced their malignant phenotypes as evidenced by decreased mouse xenograft tumor angiogenesis and tumor growth (He et al., 2013). Moreover, the authors also found that miR-143 levels were significantly lower in human lung cancer A549 and H2195 cells than that in immortalized non-tumorigenic human bronchial epithelial BEAS-2B cells. These findings suggest that down-regulation of miR-143 may play an important role in Cr(VI)-induced cell transformation and lung cancer. A further mechanistic study showed that down-regulation of miR-143 promotes Cr(VI)-induced cell transformation probably through increasing the expression of insulin-like growth factor-1 receptor (IGF-I-R) and insulin receptor substrate-1 (IRS1) expression, which in turn activates ERK/hypoxia-induced factor 1 α /NF- κ B signaling pathway (He et al., 2013).

2.4 Cadmium

Cadmium is a metal that is usually extracted as a byproduct during the production of other metals such as zinc, lead, or copper. Once extracted and purified, cadmium is mostly used in batteries, in the making of color pigments, and in the coating and plating of goods (ATSDR, 2012a). Cadmium is typically emitted into the soil, water, and air by metal mining and refining as well as through the use of fertilizers and fossil fuel combustion. Therefore, human exposure risk exists through air, food, and water (ATSDR, 2012a), and human cadmium exposure through air causes lung cancer (Waalkes, 2003; Huff et al., 2007; Hartwig, 2013).

While it is generally believed that epigenetic mechanisms play an important role in cadmium carcinogenesis (Arita and Costa, 2009; Wang et al., 2012a), little is known about the role of miRNA deregulation in cadmium-induced cell transformation and tumorigenesis. A recent study showed that a number of miRNAs are differentially expressed in cadmium (CdCl₂)-transformed 16HBE cells (Liu et al., 2015). Further bioinformatics analysis suggested that differentially expressed miRNAs are predicted to target genes involved in cell cycle regulation, p53 signaling, Wnt signaling, etc. Although whether miRNA deregulation plays a role in cadmium-induced cell transformation and tumorigenesis was not experimentally investigated, this study identified a few candidate miRNAs to further study their involvement in cadmium carcinogenesis.

3. Conclusions and Future Studies

As an important mechanism of epigenetic regulation of gene expression, miRNAs have been increasingly recognized as an important class of players in cancer initiation, metastasis, and responses to cancer therapeutics. Studies on the dysregulation of a variety of miRNAs during chronic metal carcinogen exposure have increased our understanding of the crucial roles that they may play in metal carcinogenesis. Even though limitations exist, the findings from many studies discussed above clearly show that miRNAs are critically involved in metal carcinogen-induced cell malignant transformation and tumorigenesis. However, further studies are needed for better defining the role of miRNAs in metal carcinogenesis, and translating the knowledge for potential clinical applications to benefit the diagnosis and treatment of human cancers resulting from metal carcinogen exposure.

First, current studies have almost exclusively been carried out in *in vitro* cell culture and mouse xenograft tumor models, which provide invaluable information for understanding the mechanism of metal carcinogenesis but may not fully reflect changes of miRNAs in metal carcinogen exposure-induced cancers in *in vivo* situations. Future studies need to further demonstrate the important role of miRNA dysregulation in metal carcinogenesis using animal models that develop various cancers upon metal carcinogen exposure. Second, although the role of miRNAs in metal carcinogenesis has in general been fairly actively explored, not all metal carcinogens are well studied. The majority of current studies have investigated the role of miRNA dysregulation in arsenic- and nickel-induced cell transformation and tumorigenesis. However, very few studies have been done to understand the role of miRNAs in hexavalent chromium- and cadmium-induced cell transformation and tumorigenesis. More studies are needed to determine whether miRNA deregulation is critically involved in chromium and cadmium carcinogenesis. Third, while many studies showed the dysregulation of certain miRNAs during metal carcinogen exposure and that manipulating the level of deregulated miRNAs in cells already transformed by metal carcinogens could change their malignant phenotypes, very few studies have been done to show whether manipulating the levels of deregulated miRNAs in parental non-transformed cells is able to prevent metal-carcinogen-induced cell transformation and tumor formation. More studies are needed to further determine whether miRNA deregulation plays a causal role in metal-carcinogen-induced cell transformation and tumorigenesis. Forth, further studies in animals and humans are needed to explore the potential value of dysregulated miRNAs as biomarkers for early diagnosis of cancers caused by metal carcinogen exposure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. A brief schematic description of miRNA biogenesis and the mechanism of miRNA down-regulating protein-coding gene expression

MiRNAs are transcribed in the nucleus by RNA Polymerase II (RNA Pol II) into primary-miRNA (pri-miRNA). The pri-miRNA is polyadenylated and capped, and then processed by Drosha and its binding partner DiGeorge syndrome critical region 8 (DGCR8) to generate the precursor-miRNA (pre-miRNA). This pre-miRNA is then exported out of the nucleus by exportin-5. In the cytosol, the pre-miRNA is processed via Dicer into a miRNA duplex. The miRNA duplex is then unwound into its guide strand (mature miRNA) and passenger strand (star strand). While the passenger strand is usually degraded, the mature miRNA then associates with the Argonaute protein and is subsequently loaded into the RNA-induced silencing complex (RISC). The mature miRNA/RISC complex goes to find its target mRNA based upon the miRNA seed sequence. The interaction of miRNA/RISC complex with the target mRNA can cause mRNA destabilization and degradation, cleavage, or inhibition of translation of the mRNA.