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FREE RADICALS MEDICINE (X SHI, SECTION EDITOR)

Progress and Prospects of Reactive Oxygen Species in Metal Carcinogenesis

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Abstract Carcinogenesis induced by environmental metal exposure is a major public health concern. The exact mechanisms underlying metal carcinogenesis remain elusive. In the past few decades, the relationship between metal-induced generation of reactive oxygen species (ROS) and the mechanism of metal carcinogenesis has been established. The carcinogenic process is a very complex one. In the early stage of metal carcinogenesis or cell transformation, high levels of ROS are oncogenic by causing DNA damage, genetic instability, epigenetic alteration, and metabolic reprogramming, leading to malignant transformation. In the second stage of metal carcinogenesis or the cancer development of metal-transformed cells, low levels of ROS are carcinogenic by promoting apoptosis resistance. The metal-transformed cells have the property of autophagy deficiency, resulting in accumulation of p62 and constitutive activation of NF-E2-related factor 2 (Nrf2) and leading to higher levels of antioxidants, decreased levels of ROS, apoptosis resistance, inflammation, and angiogenesis. This review summarizes the most recent development in the field of metal carcinogenesis with emphasis on the difference in cellular events between early (cell transformation) and late (after cell transformation) stages of metal carcinogenesis.

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Introduction

Metal carcinogenesis is a worldwide health concern [1–4]. Metals such as arsenic, cadmium, hexavalent chromium, and nickel are known human carcinogens. They are widely present in our living environment, found in food, soil, and water. Industrial processes and widespread usage have led to increased environmental human exposures to these metals [2, 5–7]. Epidemiological studies have demonstrated that exposure to these metals has carcinogenic effects on animals as well as humans [8]. Chronic exposure to these metals has been linked to skin [9], lung [10], gastrointestinal [11], liver [12], kidney [13], and prostate cancers [14]. Despite well-recognized carcinogenic potentials, the exact mechanisms of metal carcinogenesis remain elusive.

Among various mechanisms proposed, the reactive oxygen species (ROS) generated by carcinogenic metals are believed to be very important [15, 16]. ROS are chemically reactive molecules containing oxygen. ROS can be oxygen-containing free radicals or non-radicals, which include peroxides, superoxide, hydroxyl radical, and singlet oxygen. Carcinogenic metal-generated ROS could lead to a series of consequences [17]. These consequences include autophagy, apoptosis, angiogenesis, inflammation, genetic instability, cancer stem cells, epigenetic alteration, and metabolic reprogramming. These aberrant cellular processes contribute to cell malignant transformation and tumorigenesis. This review summarizes the most recent development in the role of ROS in metal carcinogenesis.

ROS and Metal Carcinogenesis

ROS are generated in a number of reactions essential to life and are recognized as double-edged sword. On one hand, ROS are able to play functional roles. For example, at normal physiological concentrations, ROS can act as secondary messengers and activate some intercellular and intracellular signaling pathways [18]. These pathways are involved in proliferation, apoptosis, autophagy, inflammation, and other biological processes [19]. On the other hand, ROS are toxic to cells. Unpaired electron endows free radicals highly reactive and thereby is able to damage biomolecules, such as lipid, protein, and DNA.

Although the mechanism of metal carcinogenesis remains to be investigated, ROS are considered to be important [20]. Metal ions produce intracellular ROS via both direct reactions with cellular molecules and indirect reactions through stimulation of the cells [21]. Most carcinogenic metals have been shown to produce a whole spectrum of ROS, such as the superoxide anion radical ($O_2\cdot^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical (·OH) [21]. ROS induce intracellular oxidative stress, which could damage macro biomolecules and eventually contribute to a variety of diseases including cancer. Furthermore, oxidative stress can directly or indirectly be involved in autophagy dysfunction, chronic inflammation, apoptosis resistance, genetic instability, epigenetic alteration, and metabolic reprogramming, all of which are documented contributors to metal-mediated carcinogenesis.

ROS Play a Major Role in Metal-Induced Malignant Transformation

ROS are considered to be the most important mechanism involved in metal carcinogenesis [20]. There are multiple reports addressing the oncogenic effects of ROS in normal cells [10]. Our studies have shown that cells treated with chromium generate ROS through induction of NADPH oxidase (NOX) and cause malignant transformation of these cells [10]. Both short-term and long-term exposures to chromium promote generation of ROS and the expression of NOX subunits, such as $p47^{phox}$ and $p67^{phox}$ [22]. Our studies have also shown that inhibition of ROS by catalase (antioxidant against H_2O_2) and superoxide dismutase (SOD, antioxidant against $O_2\cdot^-$) decreases arsenic-induced cell transformation [23]. Catalase or SOD decreases the number of colonies, indicating that ROS are necessary in malignant transformation process. It should be noted that the inhibition of each individual ROS species (H_2O_2 or $O_2\cdot^-$) by respective antioxidant enzyme caused a significant decrease in arsenic-induced transformation [23]. It is expected that up-regulations of all related antioxidant enzymes by their general upstream regulator will cause a much higher degree of inhibition. Our studies showed that inhibition of ROS overproduction by stable overexpression of antioxidant enzymes, such as SOD or catalase, or stable down-regulation of NOX activation by $p47^{phox}$ short hairpin RNA (shRNA) abolished chromium-induced malignant transformation and tumor formation [22]. These results indicate that ROS are generated in metal exposure and are one of important mechanisms for metal-induced cell transformation.

Decreased ROS Generation in Metal-Transformed Cells

Since ROS have been viewed as major contributors to cell carcinogenic process [24], it is very interesting that the basal ROS levels in the transformed cells are low [15]. Our studies have shown that transformed cells exhibit a sharply decreased capability of ROS generation [15]. The decreased production of ROS contributes to some of the malignant features, such as fast growth, apoptosis resistance, and anchorage-independent growth [25]. Low basal ROS levels show an increased survival of transformed cells. The decreased production of ROS could be responsible by increased expression of antioxidant enzymes (to be discussed in a later section). In arsenic-transformed cells, levels of antioxidants are higher than those in parent non-transformed cells, which contribute to decreased ROS generation and apoptosis resistance [15]. Inhibition of catalase could increase ROS level and then rescue the apoptosis capability of these cells [26]. Our previous study showed that nuclear factor kappa B (NF-κB) family proteins are up-regulated in transformed cells [15]. The higher level of SOD2 in these transformed cells is an important contributing factor for the up-regulation of this transcription factor. Taken together, it appears that metal-induced ROS generation is oncogenic by inducing cell transformation in the early stage of metal carcinogenesis. Decreased ROS generation is oncogenic by resulting in apoptosis resistance and increased cell survival (to be discussed in detail in a later section) in the late stage (tumorigenesis).

Autophagy Deficiency in Metal-Transformed Cells

Autophagy serves as a stress adaptation and is a protective mechanism in certain circumstances. Autophagy is thought to play a dual role in carcinogenesis. On one hand, autophagy promotes tumor cell survival [27]. On the other hand, autophagy plays a key role in maintenance of cellular homeostasis and in protection against cell transformation. In healthy cells, such a homeostatic activity constitutes a barrier against various degenerative processes that may affect healthy cells, including malignant transformation. Thus, autophagy exhibits cancer-suppressive effects. Carcinogenic metals are able to induce autophagy in normal cells [26, 28]. The activation of

autophagy in cadmium- [29] or arsenic-exposed [28] cells is likely to be the cell self-defense mechanism against metal-induced oxidative damage. The potency of metal in autophagy induction is very different in normal and transformed cells. Metals have limited function to induce autophagy in transformed cells. In arsenic-transformed cells, there are only a limited number of autolysosomes generated by fuse between autophagosomes and lysosomes, suggesting that arsenic-transformed cells are autophagy impaired [30]. A similar result was observed in cadmium-transformed cells [29]. Autolysosomes are not generated significantly in cadmium-transformed cells compared with parent non-transformed cells [29]. These results show that metal-transformed cells have a property of autophagy deficiency. Autophagy-deficient cells accumulate damaged mitochondria, which likely contributes to accumulation of mutation and tumor-initiating capability. Our published and unpublished data showed that transformed cells exhibit an increase of p62 because autophagy deficiency limited the cells to eliminate this protein [29]. The accumulation of p62 increases the level of NF-E2-related factor 2 (Nrf2) (to be discussed in the next section). The accumulation of p62/Nrf2 in transformed cells may further contribute to up-regulated antioxidant enzymes, antiapoptotic proteins, and inflammatory factors and angiogenesis proteins, which could play important roles in metal carcinogenesis. Our ongoing project shows that autophagy deficiency may originate from the inhibition of transcription factor EB (TFEB). TFEB is a master gene for lysosomal biogenesis [31]. Under arsenic exposure, TFEB is inhibited and followed by lysosomal biogenesis aberrant. The lack of lysosome may block autophagy flux. This blockage of autophagy flux could stabilize p62 and hypoxia-inducible factor 1 α (HIF-1α) to promote arsenic-induced carcinogenesis.

Metal-Induced p62 Accumulation and Constitutive Nrf2 Activation

Nrf2 is a key transcription factor that regulates antioxidant proteins to neutralize ROS and to restore cellular redox balance [32, 33]. It has been reported that Nrf2 has both antioncogenic and pro-oncogenic functions, which play a dual role in metal carcinogenesis. In normal cells, Nrf2 is an inducible stress response factor in a ROS-dependent mechanism [22]. Enhancement of inducible Nrf2 activity, which lessens oxidative or mutagenic stress, appears to be beneficial during pre-malignant states [34, 35]. The activation of inducible Nrf2 may inhibit metal-induced malignant transformation by up-regulation of its target antioxidants. On the other hand, constitutively activated Nrf2 can be oncogenic by protecting cancer cells against oxidative stress and chemotherapeutic agents [36, 37]. Constitutive activation of Nrf2 has been identified in several types of human cancer cell lines and tumors [34, 38, 39] as well as arsenic- [30] and cadmium-transformed cells [29]. Our published and unpublished data indicate that the constitutively expressed Nrf2 in metal-transformed cells may lead to p62 accumulation because of autophagy deficiency [29]. p62 is the positive upstream regulator of Nrf2. Various studies have shown that p62 is a substrate of autophagy and that autophagy impairment causes accumulation of this protein [40, 41]. Recent studies have demonstrated that p62 has a critical role in an oxidative stress response pathway by its direct binding to the ubiquitin ligase adaptor Kelch-like ECH-associated protein 1 (Keap1), an inhibitor of Nrf2. The binding between p62 and Keap1 makes Keap1 unavailable to inhibit Nrf2, leading to Nrf2 constitutive activation. Our studies have shown that in cadmium-transformed cells, a high level of p62 is accumulated due to autophagy deficiency, resulting in a decreased level of Keap1 and constitutive activation of Nrf2 [29]. It has been reported that Nrf2 is able to bind to the antioxidant response element (ARE) regions of p62 gene [30, 42]. Thus, p62 enhances Nrf2 activity through inactivation of Keap1 [43]. Nrf2, in turn, up-regulates p62 expression by binding of Nrf2 to ARE sites on the p62 promoter [30, 42]. Constitutively expressed Nrf2 and p62 form a positive feedback loop. This loop could increase several downstream functional proteins, including antioxidant enzymes, antiapoptotic proteins, inflammatory factors, and angiogenic molecules. The final consequences are reduced ROS accumulation and enhanced tumorigenesis.

Apoptosis Resistance

It is known that acquired resistance to apoptosis is a critical cellular event during carcinogenesis, and disruption of apoptosis has been shown to play a major role in tumor formation and malignant progression [44]. ROS are a key mechanism for toxic metal-induced apoptosis [21]. As discussed above, in metal-transformed cells, the basal levels of ROS are decreased due to up-regulation of antioxidant enzymes. The enhanced ROS scavenging system could be responsible for apoptosis resistance in transformed cells [25]. The apoptosis resistance due to low ROS levels may increase cell proliferation, providing a favorable environment for tumorigenesis of transformed cells. It has been shown that in arsenic-transformed cells, inhibition of catalase could increase ROS level and rescue the apoptosis capability [26]. Cadmium-transformed cells are observed to have apoptosis resistance as compared with parental non-transformed cells, which is correlated with low basal ROS levels in transformed cells [29]. Nrf2 has been reported to play a critical role in causing apoptosis resistance of metal-transformed cells [29]. Nrf2 is able to respond to further oxidative stress by enhancing endogenous antioxidant capacity that lowers oxidant stress and endows cell survival. Furthermore, constitutive activation of Nrf2 promotes

induction of Nrf2 target genes, such as protective enzymes and antiapoptotic proteins, resulting in resistance of tumor cells to oxidative stress, apoptosis, and anticancer agents [45]. It has been reported that Nrf2 is able to bind to ARE region of Bcl-2 gene, leading to up-regulation of Bcl-2 and increased cell survival [46, 47]. In concomitant with decreased ROS and increased Bcl-2, the transformed cells developed apoptosis resistance as indicated by decreases in cleaved poly ADP ribose polymerase (C-PARP) and caspase-9 (C-caspase) and by elevation of antiapoptotic protein Bcl-2 [30]. Nrf2-dependent increased antiapoptotic proteins along with decreased ROS generation might be responsible for apoptosis resistance in arsenic- and cadmium-transformed cells [29, 30], as well as chromium-transformed cells (unpublished data).

Metal-Induced Inflammation

Similar to ROS generation and Nrf2 activation, metal-induced inflammation is another event which showed difference between metal-transformed cells and their parent non-transformed ones. Carcinogenic metal exposure would induce inflammation in both types of the cells. Preliminary studies in our laboratory have shown that in non-transformed cells, the inflammatory proteins, such as COX-2, TNF-α, and NF-κB, are generated through metal-induced ROS reactions. In chromium-transformed cells, the constitutive activation of Nrf2 is responsible for the production of these inflammation regulatory proteins. Recent studies have shown that p62 is the positive upstream regulator of NF-κB [22]. Silencing of p62 led to significant inhibition of LPS-induced expression of TNF-α, IL-6, and cathelicidin/LL-37, as well as NF-κB reporter gene activity in HaCaT cells [22]. Dominant-negative mutants and the down-regulation of p62 markedly inhibited activation of NF-κB by TNF-α, suggesting a critical role for p62 in activation of NF-κB [48]. Our published and ongoing studies have shown that arsenic-transformed cells exhibited higher levels of COX-2, TNF-α, and NF-κB [15] in Nrf2-dependent mechanism, creating a chronic inflammatory microenvironment in these cells. The sustained chronic inflammatory microenvironment may contribute to tumor development through several mechanisms, including release of prostaglandin E_2 (PGE_2), which can suppress antitumor response [49] and facilitating tumor growth by inducing a range of angiogenic factors [50]. Taken together, metal-transformed cells create an inflammatory microenvironment and can also contribute to apoptosis resistance, tumor angiogenesis, and overall mechanism of metal carcinogenesis.

Angiogenesis

It is well known that HIF-1α is important in angiogenesis and in cancer development [51–53]. HIF-1α is a transcription factor regulator vascular endothelial growth factor (VEGF), which plays a key role in angiogenesis, tumorigenesis, and metastasis [54]. HIF-1α levels are elevated in more than half of human cancers and their metastases [55]. HIF-1α is activated in the blood of metal-exposed populations [56]. An increase of HIF-1α expression in the lung tumor tissue from a non-smoking worker occupationally exposed to chromium for 19 years has also been detected in our preliminary research. Our studies have shown epidermal growth factor receptor (EGFR)-dependent activations of HIF-1α and VEGF in chromium-transformed cells [57]. In arsenic- and chromium-transformed cells, HIF-1α expression is constitutive and its level is much higher than that in non-transformed cells (unpublished). These results suggest that in metal-transformed cells, HIF-1α is activated through signaling proteins, which could lead to angiogenesis. It is believed that Nrf2 is likely the transcription factor linking HIF-1α and EGFR. In chromium-transformed cells, Nrf2 is constitutively activated, the inhibition of EGFR reduces Nrf2 activation, and the inhibition of Nrf2 inhibits HIF-1α (unpublished). The high levels of COX-2, TNF-α, and NF-κB create an inflammatory microenvironment in arsenic-transformed cells, which could facilitate tumor growth by inducing a range of angiogenic factors, such as VEGF, endothelin-2, and urokinase-type plasminogen activator (uPA) [50, 58]. Both HIF-1α and Nrf2 are activated in animals exposed to arsenic or chromium via drinking water [59]. The increased expression of HIF-1α in arsenic- or chromium-exposed lung tissues suggests that angiogenesis is able to be activated and may contribute to metal carcinogenesis [59].

Tumorigenesis of Metal-Transformed Cell

Tumorigenesis is the late stage of metal carcinogenesis. It is widely accepted that tumorigenesis is a multistep process, the progression of which depends on the degree of malignancy within the transformed cells [60]. The most commonly used method to measure tumorigenicity is nude mouse tumor xenograft model [61]. We have investigated tumorigenesis of arsenic- [10], chromium- [28], and nickel-transformed cells (unpublished). Results have shown that transformed cells cause tumorigenesis, while the cells without metal exposure do not show any tumor formation, confirming that non-transformed cells are not malignant. In metal carcinogenesis studies, tumorigenesis studies are very useful to investigate the role of metal-induced autophagy deficiency, p62/Nrf2 accumulation, apoptosis resistance, chronic inflammatory microenvironment, and angiogenesis in tumor formation. In

metal-transformed cells, these disturbed cellular events work together to promote transformed cells to develop into tumor. In our preliminary studies, p62 is at the crossroads of oxidative stress, autophagy, apoptosis, and cancer. This protein is an emerging regulator of tumorigenesis. The roles of p62 in tumorigenesis of arsenic-transformed cells could be determined by tumor sizes under various treatments and control [30]. It has been demonstrated that increase of p62 level is critical for tumorigenesis, as overexpression of p62 in cells leads to increased tumor volume in mouse xenograft experiments [41]. Our unpublished results also show that suppression of p62 by shRNA decreases tumorigenesis of arsenic-transformed cells. Similar studies could be done to alter expression of key apoptosis regulatory proteins, such as Bcl-2, Mcl-1, and Bax, to change transformed cell apoptosis resistance. As discussed above, several important proteins are involved in metal carcinogenesis. Manipulated cells with altered expression of TFEB, COX-2, TNF-α, HIF-1α, VEGF, and Nrf2 are useful in investigating the roles of these autophagic, angiogenic, and inflammatory proteins in tumorigenesis of metal-transformed cells (unpublished). Taken together, the relationship of ROS, autophagy, apoptosis, inflammation, angiogenesis, and tumorigenesis in metal carcinogenesis is shown in Fig. 1.

Metal, ROS, and Genetic Instability

Genome instability is a hallmark of cancer [44]. Genomic instability is a high frequency of mutations, chromosomal rearrangements, or aneuploidy. It is believed that in the complicated process of carcinogenesis, genomic instability is the initial step of the processes that facilitates activation of oncogenes and inactivation of tumor suppressor and DNA damage repair genes. Most of carcinogenic metals have the ability to influence genome stability via different mechanisms. Arsenic, cadmium, chromium, and some other carcinogenic metals have been reported to initiate DNA damage, inhibit DNA repair, change epigenetic modification, and increase and alter telomere length and then followed by mutagenesis. ROS play an important role as mediators between carcinogenic metals and genome instability both directly and indirectly. As mentioned above, carcinogenic metals could induce ROS production, suggesting that these metals play a crucial role in inducing genome instability. ROS may interact with DNA, leading to modification and potentially mutant for the cell. Most carcinogenic metals, except of chromium, have been shown to have little mutagenic effect in bacterial systems or small mutagenic responses in mammalian cells [62], suggesting that metal-induced genome instability may be linked indirectly to DNA damage caused by ROS and oxidative damage.

Cell culture studies have shown that chromium picolinate is able to damage DNA directly [63], cause chromosomal aberrations [64], and induce mutations [65] at physiologically relevant doses. Cadmium is unable to induce direct DNA damage. Current evidences indicate that cells exposed to cadmium exhibit genomic instability, which refers to accumulation of alterations in the genome during the life cycle of cells, and this genomic instability is considered as a major driving force in carcinogenesis [66]. It is very interesting that cadmium showed a much higher activity in causing genomic instability in the human-hamster hybrid (A_L) cell system [67]. Arsenic-induced genomic instability is essentially multifactorial in nature and involves cross-talk among several cellular pathways and is modulated by a number of endogenous and exogenous factors. Arsenic and its metabolites generate oxidative stress, which, in turn, induces genomic instability through DNA damage, irreversible DNA repair, telomere dysfunction, mitotic arrest, and apoptosis. In addition to genetic alteration, epigenetic regulation through promoter methylation and microRNA (miRNA) expression alters gene expression profile, leading to a more vulnerable and unstable genome toward cancer risk. Our unpublished data using big blue transgenic mice show that arsenic is able to induce mutagenesis in mouse lung and liver. These in vivo results suggest that genome instability could contribute to overall mechanism of arsenic carcinogenesis.

Metal-Induced Cancer Stem Cells

The use of cancer stem cells is a relatively new approach in metal carcinogenesis studies [68]. To date, there is no report on low basal levels of ROS in metal-induced cancer stem cells. However, some studies reported low levels of ROS in different types of stem cells. It has been reported that hematopoietic stem cells [69] and central nervous system stem cells [70] exhibit lower levels of ROS compared to their more mature progeny. Our unpublished study shows that in nickel-induced cancer stem-like cells, superoxide dismutase (SOD) and catalase are up-regulated, leading to a reduced ROS level and contribution to stemness properties. This study also shows that overexpression of SOD1 could increase stemness properties. Pharmacological depletion of SOD1 functions markedly decreases the accumulation of cancer stem-like cells in nickel-induced transformed cells. The fact that cancer stem cells have lower ROS levels suggests that ROS scavenging systems are important to keep self-renewal functions and cancer-initiating capabilities of cancer stem cells. The lower ROS levels and enhanced ROS scavenging systems in cancer stem cells may be key factors which cause resistance to therapy [71].

Metal Exposure and Heritable Epigenetics

Epigenetics describes the study of mitotically and meiotically heritable changes in gene expression without changing the

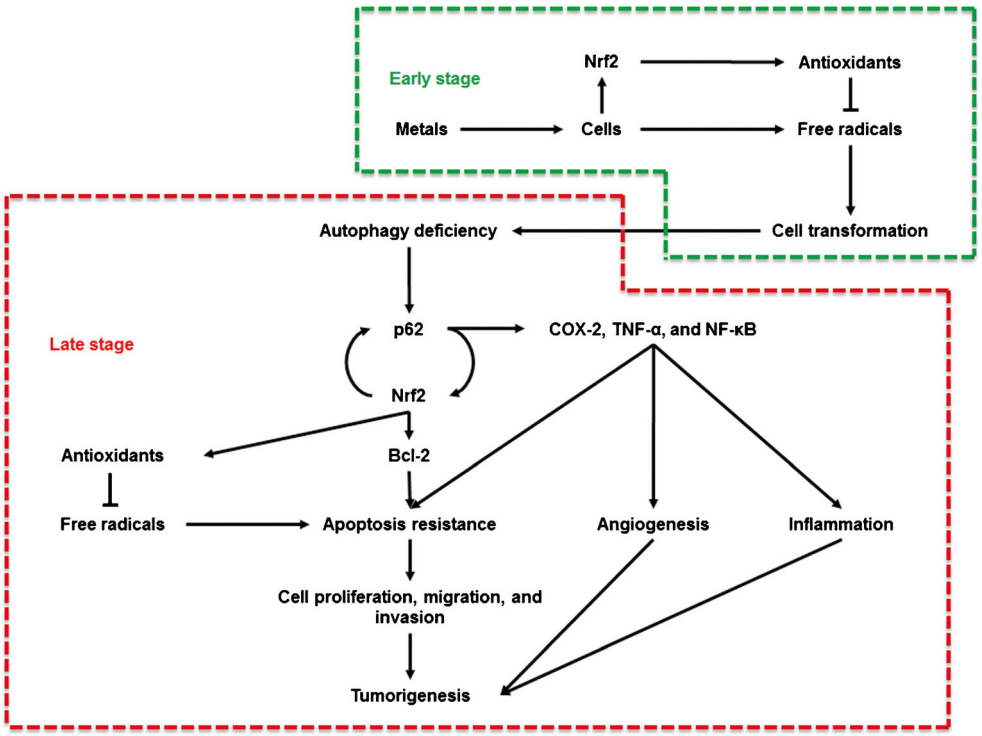

Fig. 1 The schematic diagram shows the relationship of free radical, autophagy, apoptosis, inflammation, angiogenesis, and tumorigenesis in metal carcinogenesis. Inducible Nrf2 is anticarcinogenic by inducing antioxidant enzymes and decreasing free radical (early stage). After transformation (late stage), the cells have the property of autophagy impairment and a constitutive Nrf2. The constitutive Nrf2 activation is oncogenic, which leads to tumorigenesis

DNA sequence [30]. Epigenetic regulation of gene expression can be influenced by a variety of environmental factors including carcinogenic metals, while dysregulations of epigenetic factors potentially contribute to carcinogenesis [72]. Inorganic arsenic compounds could affect DNA methylation status in the cells [73]. For example, it has been reported that arsenic could cause hypermethylation of the p53 gene [74]. In vivo, arsenic is able to be metabolized into a methylated form by using S-adenosyl methionine (SAM) as the methyl donor. This metabolism of arsenic may reduce intracellular methyl group storage, which could result in genome-wide hypomethylation [75]. Besides arsenic, many other carcinogenic metals have been reported to be able to alter DNA methylation. Histone modification also changed under exposure to carcinogenic metals [76–78]. Some in vitro studies focused on miRNAs for their role in promoting metal carcinogenesis. Studies have indicated that miRNAs play an important role in augmenting or suppressing the carcinogenic effects of arsenic [16], chromium [41], and cadmium [79].

Recently, studies on heritable epigenetics have obtained considerable attention. Heritable epigenetics or epigenetic inheritance occurs when phenotypic variations are transmitted to subsequent generations of cells or organisms without change in DNA base sequences [80]. A previous study showed that transgenerational effects of environmental toxins were transferred through the male germ line to nearly all males of all subsequent generations examined [81]. Carcinogenic metals have the potential to reprogram the germ line and to promote a transgenerational disease such as carcinogenesis in a heritable epigenetic way. One of our ongoing projects suggests that arsenic may induce heritable epigenetic modification. When exposed to arsenic in utero, the F1 and F2 generations of mice obtain different DNA methylations as well as histone protein modifications. If metal-induced heritable epigenetic alteration is real, it would have significant implications in the field of metal carcinogenesis.

Metabolic Reprogramming

Cancer cell metabolism is characterized by increased glycolysis and decreased oxidative metabolism, which is very different from normal cell metabolism [82]. Recently, metabolic reprogramming is recognized as a hallmark of cancer [44]. There are several singling pathways involved in metal-induced metabolic reprogramming, such as phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) pathway [83], HIF-1α pathway [84], and liver kinase B1 (LKB1)/AMP-activated protein kinase (AMPK) pathway [23]. Activated PI3K/Akt leads to enhanced glucose uptake and glycolysis [83]. It has been well documented that carcinogenic metals could induce PI3K/Akt and HIF-1α activation via ROS-dependent pathways [15, 26, 57]. Though none of these reports is focused on metabolic reprogramming, it is still very possible that carcinogenic metals could have influence on metabolic reprogramming, given the function of PI3K/AKT on cell metabolism. A recently proposed concept is that the primary functions of activated oncogenes and inactivated tumor

suppressors reprogram cellular metabolism. Altered metabolism may be a primary feature selected for tumorigenesis [85]. The works on fructose-1,6-bisphosphatase 1 (FBP1) may support this hypothesis. It has been reported that loss of FBP1 resulted in metabolic reprogramming synergizing with the loss of E-cadherin to sustain cancer stem cell-like properties during dissemination and metastasis [86]. Our unpublished data show that FBP1 is down-regulated in chromium-transformed cells, which suggests that chromium carcinogenesis may relate to metabolic reprogramming. Similar to FBP1, one of our ongoing projects shows that ATP citrate lyase (ACLY) is up-regulated by chromium, suggesting that chromium may involve in lipid metabolism reprogram. The studies on metabolic reprogramming make it possible to identify some classic metabolic enzymes as cancer markers or key regulators of carcinogenesis. The novel functions of these metabolic enzymes could improve our understanding of the mechanism of carcinogenesis induced by metal or other environmental factors.

Summary and Future Perspectives

For decades, chronic exposure to carcinogenic metals resulting in cancer has been the subject of extensive studies. The relationship between metal-induced ROS generation and carcinogenesis has been well established. Increasing evidences indicate that ROS generated by these metals mediate the carcinogenesis process, even for some non-redox active metals. Metal carcinogenesis is a very complicated process. As discussed in this review, different cellular responses are involved. These responses act in different carcinogenic stages. In the early stage, metal-induced ROS generation may lead to stress response, genetic instability, epigenetic alteration, and metabolic reprogramming. These aberrant processes work together to initialize malignant transformation. Once carcinogenesis progresses into late stage (cancer development of transformed cells), a series of oncogenic steps are initiated, including autophagy deficiency, oncogenic protein accumulation, lower ROS generation, apoptosis resistance, chronic inflammation, and angiogenesis. These oncogenic activities promote cancer development of these malignantly transformed cells.

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Compliance with Ethical Standards

Conflict of Interest The authors declare they have no actual or potential competing financial interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

1. Hughes MF. Arsenic toxicity and potential mechanisms of action. Toxicol Lett. 2002;133(1):1–16.
2. Yang M. A current global view of environmental and occupational cancers. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2011;29(3):223–49.
3. Yuan Y, Marshall G, Ferreccio C, Steinmaus C, Selvin S, Liaw J, et al. Acute myocardial infarction mortality in comparison with lung and bladder cancer mortality in arsenic-exposed region II of Chile from 1950 to 2000. Am J Epidemiol. 2007;166(12):1381–91.
4. Doll R, Morgan LG, Speizer FE. Cancers of the lung and nasal sinuses in nickel workers. Br J Cancer. 1970;24(4):623–32.
5. Hopenhayn-Rich C, Biggs ML, Smith AH. Lung and kidney cancer mortality associated with arsenic in drinking water in Cordoba, Argentina. Int J Epidemiol. 1998;27(4):561–9.
6. Katic J, Fucic A, Gamulin M. Prenatal, early life, and childhood exposure to genotoxicants in the living environment. Arh Hig Rada Toksikol. 2010;61(4):455–64.
7. Wild P, Bourgkard E, Paris C. Lung cancer and exposure to metals: the epidemiological evidence. Methods Mol Biol. 2009;472:139–67.
8. Galanis A, Karapetsas A, Sandaltzopoulos R. Metal-induced carcinogenesis, oxidative stress and hypoxia signalling. Mutat Res. 2009;674(1-2):31–5.
9. Haque R, Mazumder DN, Samanta S, et al. Arsenic in drinking water and skin lesions: dose-response data from West Bengal, India. Epidemiology. 2003;14:174–82.
10. Wang X, Son YO, Chang Q, Sun L, Hitron JA, Budhraja A, et al. NADPH oxidase activation is required in reactive oxygen species generation and cell transformation induced by hexavalent chromium. Toxicol Sci. 2009;123(2):399–410.
11. Tchounwou PB, Patlolla AK, Centeno JA. Carcinogenic and systemic health effects associated with arsenic exposure—a critical review. Toxicol Pathol. 2003;31(6):575–88.
12. Li B, Li X, Zhu B, Zhang X, Wang Y, Xu Y, et al. Sodium arsenite induced reactive oxygen species generation, nuclear factor (erythroid-2 related) factor 2 activation, heme oxygenase-1 expression, and glutathione elevation in Chang human hepatocytes. Environ Toxicol. 2013;28(7):401–10.
13. Kitchin KT, Conolly R. Arsenic-induced carcinogenesis—oxidative stress as a possible mode of action and future research needs for more biologically based risk assessment. Chem Res Toxicol. 2010;23(2):327–35.
14. Guzel S, Kiziler L, Aydemir B, Alici B, Ataus S, Aksu A, et al. Association of Pb, Cd, and Se concentrations and oxidative damage-related markers in different grades of prostate carcinoma. Biol Trace Elem Res. 2012;145(1):23–32.
15. Chang Q, Pan J, Wang X, Zhang Z, Chen F, Shi X. Reduced reactive oxygen species-generating capacity contributes to the enhanced cell growth of arsenic-transformed epithelial cells. Cancer Res. 2010;70(12):5127–35.
16. Wang L, Hitron JA, Wise JT, Son YO, Roy RV, Kim D, et al. Ethanol enhances arsenic-induced cyclooxygenase-2 expression via both NFAT and NF-kappaB signalings in colorectal cancer cells. Toxicol Appl Pharmacol. 2015;288(2):232–9.
17. Leonard SS, Harris GK, Shi X. Metal-induced oxidative stress and signal transduction. Free Radic Biol Med. 2004;37(12):1921–42.
18. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39(1):44–84.
19. Kwee JK. A paradoxical chemoresistance and tumor suppressive role of antioxidant in solid cancer cells: a strange case of Dr. Jekyll and Mr. Hyde. Biomed Res Int. 2014;2014:209845.

20. Wang S, Shi X. Molecular mechanisms of metal toxicity and carcinogenesis. Mol Cell Biochem. 2001;222(1-2):3–9.
21. Lee JC, Son YO, Pratheeshkumar P, Shi X. Oxidative stress and metal carcinogenesis. Free Radic Biol Med. 2012;53(4):742–57.
22. Li N, Alam J, Venkatesan MI, Eiguren-Fernandez A, Schmitz D, Di Stefano E, et al. Nrf2 is a key transcription factor that regulates antioxidant defense in macrophages and epithelial cells: protecting against the proinflammatory and oxidizing effects of diesel exhaust chemicals. J Immunol. 2004;173(5):3467–81.
23. Pooya S, Liu X, Kumar VB, Anderson J, Imai F, Zhang W, et al. The tumour suppressor LKB1 regulates myelination through mitochondrial metabolism. Nat Commun. 2014;5:4993.
24. Trachootham D, Alexandre J, Huang P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? Nat Rev Drug Discov. 2009;8(7):579–91.
25. Zhang Z, Pratheeshkumar P, Budhraja A, Son YO, Kim D, Shi X. Role of reactive oxygen species in arsenic-induced transformation of human lung bronchial epithelial (BEAS-2B) cells. Biochem Biophys Res Commun. 2015;456(2):643–8.
26. Wang L, Son YO, Ding S, Hitron JA, Budhraja A, Lee JC, et al. Ethanol enhances tumor angiogenesis in vitro induced by low-dose arsenic in colon cancer cells through hypoxia-inducible factor 1 alpha pathway. Toxicol Sci. 2012;130(2):269–80.
27. Degenhardt K, Mathew R, Beaudoin B, Bray K, Anderson D, Chen G, et al. Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. Cancer Cell. 2006;10(1):51–64.
28. Zhang T, Qi Y, Liao M, Xu M, Bower KA, Frank JA, et al. Autophagy is a cell self-protective mechanism against arsenic-induced cell transformation. Toxicol Sci. 2012;130(2):298–308.
29. Son YO, Pratheeshkumar P, Roy RV, Hitron JA, Wang L, Zhang Z, et al. Nrf2/p62 signaling in apoptosis resistance and its role in cadmium-induced carcinogenesis. J Biol Chem. 2014;289(41):28660–75.
30. Son YO, Pratheeshkumar P, Roy RV, Hitron JA, Wang L, Divya SP, et al. Antioncogenic and oncogenic properties of Nrf2 in arsenic-induced carcinogenesis. J Biol Chem. 2015;290(45):27090–100.
31. Sardiello M, Palmieri M, di Ronza A, Medina DL, Valenza M, Gennarino VA, et al. A gene network regulating lysosomal biogenesis and function. Science. 2009;325(5939):473–7.
32. Kobayashi M, Yamamoto M. Nrf2-Keap1 regulation of cellular defense mechanisms against electrophiles and reactive oxygen species. Adv Enzyme Regul. 2006;46:113–40.
33. Chan K, Han XD, Kan YW. An important function of Nrf2 in combating oxidative stress: detoxification of acetaminophen. Proc Natl Acad Sci U S A. 2001;98(8):4611–6.
34. Sporn MB, Liby KT. NRF2 and cancer: the good, the bad and the importance of context. Nat Rev Cancer. 2012;12(8):564–71.
35. Tew KD. NrF2 Keap1 as gatekeepers of redox homeostasis—do they prevent or cause cancer? Pigment Cell Melanoma Res. 2011;24(6):1078–9.
36. Wang XJ, Sun Z, Villeneuve NF, Zhang S, Zhao F, Li Y, et al. Nrf2 enhances resistance of cancer cells to chemotherapeutic drugs, the dark side of Nrf2. Carcinogenesis. 2008;29(6):1235–43.
37. Kansanen E, Kuosmanen SM, Leinonen H, Levonen AL. The Keap1-Nrf2 pathway: mechanisms of activation and dysregulation in cancer. Redox Biol. 2013;1:45–9.
38. Ohta T, Iijima K, Miyamoto M, Nakahara I, Tanaka H, Ohtsuji M, et al. Loss of Keap1 function activates Nrf2 and provides advantages for lung cancer cell growth. Cancer Res. 2008;68(5):1303–9.
39. Lau A, Whitman SA, Jaramillo MC, Zhang DD. Arsenic-mediated activation of the Nrf2-Keap1 antioxidant pathway. J Biochem Mol Toxicol. 2013;27(2):99–105.
40. Korolchuk VI, Mansilla A, Menzies FM, Rubinsztein DC. Autophagy inhibition compromises degradation of ubiquitin-proteasome pathway substrates. Mol Cell. 2009;33(4):517–27.
41. Mathew R, Karp CM, Beaudoin B, Vuong N, Chen G, Chen HY, et al. Autophagy suppresses tumorigenesis through elimination of p62. Cell. 2009;137(6):1062–75.
42. Jain A, Lamark T, Sjottem E, Larsen KB, Awuh JA, Øvervatn A, et al. p62/SQSTM1 is a target gene for transcription factor NRF2 and creates a positive feedback loop by inducing antioxidant response element-driven gene transcription. J Biol Chem. 2010;285(29):22576–91.
43. Komatsu M, Kurokawa H, Waguri S, Taguchi K, Kobayashi A, Ichimura Y, et al. The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. Nat Cell Biol. 2010;12(3):213–23.
44. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74.
45. Komatsu M. Potential role of p62 in tumor development. Autophagy. 2011;7(9):1088–90.
46. Niture SK, Jaiswal AK. Nrf2 protein up-regulates antiapoptotic protein Bcl-2 and prevents cellular apoptosis. J Biol Chem. 2012;287(13):9873–86.
47. Niture SK, Jaiswal AK. INrf2 (Keap1) targets Bcl-2 degradation and controls cellular apoptosis. Cell Death Differ. 2011;18(3):439–51.
48. Sanz L, Sanchez P, Lallena MJ, Diaz-Meco MT, Moscat J. The interaction of p62 with RIP links the atypical PKCs to NF-kappaB activation. EMBO J. 1999;18(11):3044–53.
49. Elgert KD, Alleva DG, Mullins DW. Tumor-induced immune dysfunction: the macrophage connection. J Leukoc Biol. 1998;64(3):275–90.
50. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer. 2004;4(1):71–8.
51. Mazure NM, Chen EY, Laderoute KR, Giaccia AJ. Induction of vascular endothelial growth factor by hypoxia is modulated by a phosphatidylinositol 3-kinase/Akt signaling pathway in Ha-ras-transformed cells through a hypoxia inducible factor-1 transcriptional element. Blood. 1997;90(9):3322–31.
52. Blancher C, Moore JW, Talks KL, Houlbrook S, Harris AL. Relationship of hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha expression to vascular endothelial growth factor induction and hypoxia survival in human breast cancer cell lines. Cancer Res. 2000;60(24):7106–13.
53. Choi KS, Bae MK, Jeong JW, Moon HE, Kim KW. Hypoxia-induced angiogenesis during carcinogenesis. J Biochem Mol Biol. 2003;36(1):120–7.
54. Bedogni B, Welford SM, Cassarino DS, Nickoloff BJ, Giaccia AJ, Powell MB. The hypoxic microenvironment of the skin contributes to Akt-mediated melanocyte transformation. Cancer Cell. 2005;8(6):443–54.
55. Huss WJ, Hanrahan CF, Barrios RJ, Simons JW, Greenberg NM. Angiogenesis and prostate cancer: identification of a molecular progression switch. Cancer Res. 2001;61(6):2736–43.
56. Fry RC, Navasumrit P, Valiathan C, Svensson JP, Hogan BJ, Luo M, et al. Activation of inflammation/NF-kappaB signaling in infants born to arsenic-exposed mothers. PLoS Genet. 2007;3(11):e207.
57. Kim D, Dai J, Fai LY, Yao H, Son YO, Wang L, et al. Constitutive activation of epidermal growth factor receptor promotes tumorigenesis of Cr(VI)-transformed cells through decreased reactive oxygen species and apoptosis resistance development. J Biol Chem. 2015;290(4):2213–24.
58. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001;357(9255):539–45.
59. Wang X, Mandal AK, Saito H, Pulliam JF, Lee EY, Ke ZJ, et al. Arsenic and chromium in drinking water promote tumorigenesis in a mouse colitis-associated colorectal cancer model and the potential mechanism is ROS-mediated Wnt/beta-catenin signaling pathway. Toxicol Appl Pharmacol. 2012;262(1):11–21.

60. Ashkenazi R, Gentry SN, Jackson TL. Pathways to tumorigenesis—modeling mutation acquisition in stem cells and their progeny. Neoplasia. 2008;10(11):1170–82.
61. Gatenby RA, Vincent TL. An evolutionary model of carcinogenesis. Cancer Res. 2003;63(19):6212–20.
62. Hartwig A. Metal interaction with redox regulation: an integrating concept in metal carcinogenesis? Free Radic Biol Med. 2013;55: 63–72.
63. Bagchi D, Stohs SJ, Downs BW, Bagchi M, Preuss HG. Cytotoxicity and oxidative mechanisms of different forms of chromium. Toxicology. 2002;180(1):5–22.
64. Stearns DM, Wise Sr JP, Patierno SR, Wetterhahn KE. Chromium(III) picolinate produces chromosome damage in Chinese hamster ovary cells. FASEB J. 1995;9(15):1643–8.
65. Coryell VH, Stearns DM. Molecular analysis of hprt mutations induced by chromium picolinate in CHO AA8 cells. Mutat Res. 2006;610(1-2):114–23.
66. Shen Z. Genomic instability and cancer: an introduction. J Mol Cell Biol. 2011;3(1):1–3.
67. Filipic M, Hei TK. Mutagenicity of cadmium in mammalian cells: implication of oxidative DNA damage. Mutat Res. 2004;546(1-2): 81–91.
68. Wang SH, Shih YL, Ko WC, Wei YH, Shih CM. Cadmium-induced autophagy and apoptosis are mediated by a calcium signaling pathway. Cell Mol Life Sci. 2008;65(22):3640–52.
69. Ito K, Hirao A, Arai F, Matsuoka S, Takubo K, Hamaguchi I, et al. Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. Nature. 2004;431(7011):997–1002.
70. Tsatmali M, Walcott EC, Crossin KL. Newborn neurons acquire high levels of reactive oxygen species and increased mitochondrial proteins upon differentiation from progenitors. Brain Res. 2005;1040(1-2):137–50.
71. Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, et al. Association of reactive oxygen species levels and radioresistance in cancer stem cells. Nature. 2009;458(7239):780–3.
72. Koturbash I, Beland FA, Pogribny IP. Role of epigenetic events in chemical carcinogenesis—a justification for incorporating epigenetic evaluations in cancer risk assessment. Toxicol Mech Methods. 2011;21(4):289–97.
73. Cheng TF, Choudhuri S, Muldoon-Jacobs K. Epigenetic targets of some toxicologically relevant metals: a review of the literature. J Appl Toxicol. 2012;32(9):643–53.
74. Chanda S, Dasgupta UB, Guhamazumder D, Gupta M, Chaudhuri U, Lahiri S, et al. DNA hypermethylation of promoter of gene p53 and p16 in arsenic-exposed people with and without malignancy. Toxicol Sci. 2006;89(2):431–7.
75. Sengupta S, McArthur JM, Sarkar A, Leng MJ, Ravenscroft P, Howarth RJ, et al. Do ponds cause arsenic-pollution of groundwater in the Bengal basin? An answer from West Bengal. Environ Sci Technol. 2008;42(14):5156–64.
76. Salnikow K, Zhitkovich A. Genetic and epigenetic mechanisms in metal carcinogenesis and cocarcinogenesis: nickel, arsenic, and chromium. Chem Res Toxicol. 2008;21(1):28–44.
77. Somji S, Garrett SH, Toni C, Zhou XD, Zheng Y, Ajjimaporn A, et al. Differences in the epigenetic regulation of MT-3 gene expression between parental and Cd+2 or As+3 transformed human urothelial cells. Cancer Cell Int. 2011;11(1):2.
78. Buning R, van Noort J. Single-pair FRET experiments on nucleosome conformational dynamics. Biochimie. 2010;92(12):1729–40.
79. Hassan F, Nuovo GJ, Crawford M, Boyaka PN, Kirkby S, Nana-Sinkam SP, et al. MiR-101 and miR-144 regulate the expression of the CFTR chloride channel in the lung. PLoS One. 2012;7(11), e50837.
80. Jablonka E, Raz G. Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. Q Rev Biol. 2009;84(2):131–76.
81. Anway MD, Cupp AS, Uzumcu M, Skinner MK. Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science. 2005;308(5727):1466–9.
82. Koppenol WH, Bounds PL, Dang CV. Otto Warburg's contributions to current concepts of cancer metabolism. Nat Rev Cancer. 2011;11(5):325–37.
83. Buzzai M, Bauer DE, Jones RG, Deberardinis RJ, Hatzivassiliou G, Elstrom RL, et al. The glucose dependence of Akt-transformed cells can be reversed by pharmacologic activation of fatty acid beta-oxidation. Oncogene. 2005;24(26):4165–73.
84. Semenza GL. HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. J Clin Invest. 2013;123(9): 3664–71.
85. Ward PS, Thompson CB. Metabolic reprogramming: a cancer hallmark even Warburg did not anticipate. Cancer Cell. 2012;21(3): 297–308.
86. Dong C, Yuan T, Wu Y, Wang Y, Fan TW, Miriyala S, et al. Loss of FBP1 by snail-mediated repression provides metabolic advantages in basal-like breast cancer. Cancer Cell. 2013;23(3):316–31.