[Frontiers in Bioscience, Scholar, 8, 312-320, June 1, 2016]

Role of mitochondria, ROS, and DNA damage in arsenic induced carcinogenesis

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1. ABSTRACT

The International Agency for Research on Cancer (IARC) declared arsenic a class I carcinogen. Arsenic exposure induces several forms of human cancers, including cancers of skin, lung, liver, and urinary bladder. The majority of the arsenic-induced cancers occur in skin. Among these, the most common is Bowen's disease, characterized by epidermal hyperplasia, full layer epidermal dysplasia, leading to intraepidermal carcinoma as well as apoptosis, and moderate dermal infiltrates, which require the participation of mitochondria. The exact mechanism underlying arsenic induced carcinogenesis remains unclear, although increased reactive oxidative stresses, leading to chromosome abnormalities and uncontrolled growth, and aberrant immune regulations might be involved. Here, we highlight how increased mitochondrial biogenesis and oxidative stress lead to mitochondrial DNA damage and mutation in arsenic induced cancers. We also provide therapeutic rationale for targeting mitochondria in the treatment of arsenic induced cancers.

2. INTRODUCTION TO THE HEALTH HAZARDS OF ARESNIC

Arsenic is a common element found in the Earth's crust. The name of arsenic is thought to come from 'arsenikon', the Greek name for the yellow pigment. Arsenic is a metalloid with both metallic and non-metallic chemical characteristics. In the element periodic table, arsenic belongs to the same family that includes nitrogen and phosphorus, both of which are important components in the biochemical process and integrative structure in living cells. This unique chemical characteristic of arsenic to interact with biological molecules might explain to its diverse and profound biological effects. Arsenic acts like a double-sided sword in human health. In contrast to its notoriously adverse health effects, arsenic has been used for treatment of lymphoma and leukemia and it still remains the drug of choice for acute promyelocytic leukemia, a special form of acute myeloblastic leukemia (1), due to, at least in part, the degradation of the aberrant PML-retinoic acid receptor α fusion protein (2). On the other hand, exposure to arsenic leads to several human cancers, including skin, lungs, urinary bladder, and liver (3). In addition to its associations with these cancers, arsenic exposure is also associated with the development of several vascular diseases, including stroke, ischemic heart diseases, and peripheral vascular diseases (4).

The major routes of human exposure to arsenic include drinking, inhalation, and skin contact. Drinking of water contaminated with arsenic remains the major route of human exposure. Hundreds of millions of people are exposed to arsenic by drinking contaminated water in many countries, including Bangladesh, West Bengal of India, Chile, Mexico, and China (5). In addition to environmental exposure, industrial exposure may also cause significant health effects. Arsenic has been used to produce paints, insecticides, wood preservatives, and

pesticides. For example, chromium copper arsenic is a wood preservative, although it is gradually being replaced. The important semiconductor alloy in the computer hardware and electronic chips includes allium arsenide, indium arsenide, and aluminum arsenide to modify their connectivity and plasticity. In 2014, it was estimated that the annual worldwide production of arsenic is around 45,000 tons, with more than half of which from China (6).

2.1. Biological conversion and metabolisms of arsenic

Based on the oxidative status, arsenic exists in two inorganic chemical forms. Arsenite (AsIII) exists in the trivalent form whereas arsenate (AsV) exists in the pentavalent form. Arsenite is about 2-10 times more toxic than arsenate. After gastro-enteric absorption, inorganic arsenic is obtained by erythrocytes and then distributed through bloodstream to multiple organs, including lungs, liver, and skin (7). Inside the cells, arsenic is methylated by the methyl group supplied by s-adenosylmethionine (SAM). Compared to the inorganic forms, the methylated metabolites are less genotoxic (8) and are excreted readily in urine (9, 10). In transit from blood to tissues, arsenate is reduced to arsenite. In the liver, arsenic is methylated into mono-methylarsenic acid (MMA V), which is further reduced to monomethyl arsonous acid (MMA III). A subsequent methylation reaction modifies MMA III to dimethylarsinic acid (DMA V) (11). In fact, the biological distribution and chemical conversion of arsenic necessitate the participation of mitochondria, which accounts for the production of ATP and reactive oxygen species (ROS).

2.2. Distinctive morphological and pathophysiological features of arsenic-induced cancers in humans

It has been estimated at around 10% of population exposed to arsenic develop skin abnormalities, including variegated hyper- and hypo-pigmentations, arsenic keratosis, Bowen's disease, and invasive skin cancers. However, only about 1% of exposed humans develop skin cancers (12). Long-term arsenic exposure results in the impairment of immunity in susceptible individuals, which may account for the development of cancers in these individuals. We previously reported that people with arsenical cancers exhibit an impaired contact hypersensitivity response (13), accompanied with a selective CD4+ apoptosis in tumor microenvironment (14) and an impaired activation of dendritic cells (15). Other investigators have also reported that early exposure to arsenic renders influenza infections (16).

Among arsenic-induced skin cancers, Bowen's disease is most common (17-19). Epidemiological studies have demonstrated a dose-response relationship between arsenic levels in the drinking water and the occurrence of skin cancers (18). Grossly, arsenic-induced Bowen's disease (As-BD) differs from classical (UV-induced) Bowen's disease by its multiplicity and its propensity in non-sun-exposed areas on skin (3, 20). Microscopically, As-BD is featured by marked keratinocyte proliferation, full-layered epidermal dysplasia, individual cell apoptosis, and moderate dermal infiltrates (17). Over time, As-BD can penetrate through the basement membrane, causing invasive squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) (21, 23). Patients with As-BD are more likely to develop cancers of lungs and urinary bladder (21, 24,25). It is estimated that As-BD starts within a decade, invasive skin cancer after 20 years (26), and lung cancers after 30 years following arsenic exposure (22).

In addition to the distinctive clinical features of Bowen's diseases, p53 protein expression is much higher in As-BD than it is in classical (UV-induced) BD (27, 28). Arsenic is able to induce mutant p53 accumulation via an ATM-dependent pathway (29, 30). The majority of the p53 mutation sites are located in the exon 5 and exon 8. Furthermore, the mutation sites and patterns of p53 gene in As-BD are different from those in classical (UV-induced) BD (31), indicating the pathogeneses of As-induced and UV-induced Bowen's disease are different. Although the direct link between p53 mutation and arsenic exposure is not clear, the effect of arsenic on p53-related intracellular pathways is well recognized. For example, it has been reported that arsenic leads to p53-mediated G2/M cell cycle arrest and DNA aneuploidy (32, 34).

As mentioned above, microscopically, there are coexisting hyperproliferative (epidermal hyperplasia) and individual apoptotic keratinocytes in As-BD lesions (35). In fact, this phenomenon is reflected *in vitro* as the biological effects of arsenic on keratinocytes are concentration dependent. At lower concentrations ($\leq$1 μM), arsenic induces keratinocyte proliferation and in parallel, enhances both NF-κB and AP-1 activity (35). On the other hand, at higher concentrations ($\leq$5 μM), arsenic induces keratinocyte apoptosis through the Fas/Fas ligand (FasL) axis. Because the promoter site of FasL contains AP-1 binding sites, arsenic-activated Fas/FasL signaling likely occurs through AP-1 activation (35-37).

2.3. The possible mechanisms in arsenic carcinogenesis

Although increased oxidative stress, chromosome abnormalities, altered growth factors, and aberrant immune regulations may contribute to arsenic carcinogenesis, the exact and direct mechanisms of arsenic carcinogenesis remains unclear (38).

Firstly, ROS production and oxidative DNA damages, such as 8-OHdG, have been found in the urine and skin tissue obtained from patients exposed to arsenic (39). One *In vitro* study showed that low concentrations of arsenic generates ROS, consequently increasing the transcription of NF-kB and

cell proliferation (40). The oxidative stress inside the cells originates mainly from mitochondria (41). Previous studies have illustrated this mechanism by showing that arsenic induces mitochondria oxidative stress, leading to mitochondria damages and mutations (42, 43).

Secondly, the increased oxidative DNA damages and mutations by arsenic affect DNA repair machinery, including nucleotide excision repair, DNA ligase, DNA base excision repair, and DNA strand break rejoining. In parallel, arsenic impairs the DNA repair process by inhibiting several key repair enzymes, rendering the damaged or mutated DNA irreparable (44, 45). In addition to its permanent DNA damages, arsenic also affects several epigenetic processes, where, for example, arsenic induces DNA hypomethylations, in most cases, through its inhibition of DNA methyltransferases (46, 47).

Thirdly, arsenic causes abnormalities in chromosome integrity. Arsenic induces chromosome abnormalities and abnormal sister chromatid exchanges (48). In fact, the chromosome abnormalities are highly correlated with oxidative stresses and DNA damage. The frequency of micronuclei is increased by arsenic. One xenograft cancer model by HaCaT cells showed that the tumorigenecity of HaCaT cells is correlated with increased numbers of micronuclei (49).

All the above plausible pathways leading to arsenic carcinogenesis necessitate the participation of mitochondria in the induction of apoptosis, cell proliferation, DNA damages, chromosomal abnormalities, and ROS generation. In this article, we will discuss in more detail and in greater depth the role of mitochondria in carcinogenesis, and more specifically, in chemical carcinogenesis.

2.4. Role of mitochondria in carcinogenesis

Traditionally, cancer cells were thought to have impaired mitochondrial function and to rely on aerobic glycolysis for metabolism, known as the Warburg Effect. The advantage of the preferential aerobic glycolysis in cancer cells is not exactly known. For example, apoptosis induced by Bax and Bak requires oxidative phosphorylation by disparate death stimuli, and thus the reliance of tumor cells on glycolysis may potentially be the way these cells evade apoptosis (50). In contrast to the Warburg Effect, however, there are several cancers that have increased mitochondrial oxidative phosphorylation (51). In addition, there is a metabolic heterogeneity within tumors, with some cells utilizing glycolysis while others using oxidative phosphorylation as the main energy source. Recently, studies of the tumor microenvironments revealed a Reverse Warburg Effect, where metabolic coupling exists between cancer-associated fibroblasts with high aerobic glycolysis and cancer with increased mitochondrial oxidative phosphorylation (52).

Although the metabolic abnormalities in Warburg effects have been implicated in the carcinogenesis, few studies have been performed to investigate how arsenic regulates Warburg effects in carcinogenesis. One of the important intracellular methyl group donors for arsenic metabolism, S-adenosylmethionine (SAM), has a high affinity to human mitochondrial SAM carrier (SAMC) (53). Through its cellular bindings and metabolisms, arsenic regulates the process of the Warburg effect as evidenced by increasing accumulation of intracellular and extracellular lactate, increasing extracellular acidification, and inhibition by the non-metabolized glucose analog, 2-deoxy-D-glucose (54).

In addition to the metabolic abnormalities, mitochondria might also contribute to carcinogenesis through ROS production, ATP production, energy consumption, and mitochondrial biogenesis. The multi-staged model in chemical carcinogenesis is introduced below for a better understanding of the role of mitochondria in different stages of chemical carcinogenesis.

2.5. Multi-staged model in chemical carcinogenesis

Chemical carcinogenesis is a special form of carcinogenesis in which a known chemical leads to tumor formation. The traditional sequential process in the chemical carcinogenesis involves initiation, promotion, and progression. Initiation, the first step in the multi-staged model, alters and damages cellular DNA, initiates irreversible genetic damages and mutations, allowing the daughter cells to carry the mutations upon next proliferation. Once cells have been mutated by an initiator, they are further susceptible to the effects of promoters, which enhances the abnormal cell proliferation and maintains the mutations and damages. Unlike initiators, promoters do not covalently bind to DNA or macromolecules within the cell. Many promotors bind to receptors on the cell surface in order to affect intracellular pathways that induce cell proliferation. The term progression refers to the malignant transformation of a benign tumor associated with aneuploidy (abnormal number of chromosomes).

2.6. Role of mitochondria in the initiation, promotion, and progressions of chemical carcinogenesis

Mitochondria may not contribute directly in the initiation process, though they may play a significant role on both the promotion and the progression. For example, the epidermal JB6 cells transformed with TPA (a common example of promotor) are less prone to form colonies in soft agar after TPA treatment when cells are blocked with mitochondrial coupling protein 2 (UCP2), suggesting that mitochondrial uncoupling may serve as an important regulator of p53 mitochondrial translocation and p53-mediated apoptosis during early tumor promotion (55). In the checkpoint for apoptosis, the regulation of bcl-2/bax

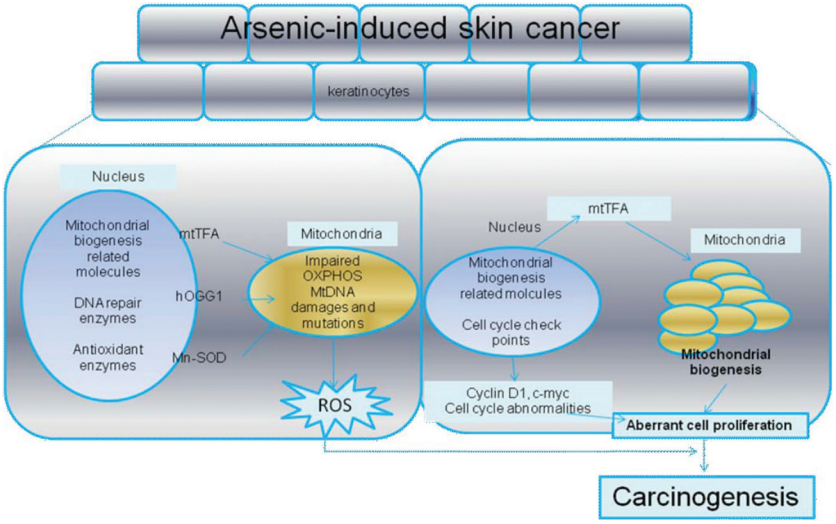

Figure 1. Schematic view of the role of mitochondria in the arsenic skin carcinogenesis. Arsenic induces mitochondrial biogenesis, through mtTFA upregulations, and eventually cell proliferations (Right). The aberrant cell proliferation contributes to the increased epidermal proliferation in the histopathology of arsenical cancers. In the context of increased mitochondrial biogenesis, arsenic also induced mitochondrial ROS production, leading to the upregulation of antioxidant and DNA repair enzymes, and eventually to the mitochondrial DNA damages and mutations (Left). This axis might contribute to the dysplasia, abnormal differentiation and apoptosis in the histopathology of arsenical skin cancers. The aberrant proliferation, under ROS stress and DNA damages, may contribute to the arsenic skin carcinogenesis in cooperation with oncogene, such as c-myc.

in the mitochondria determines the direction towards either to cell apoptosis or cell survival (56).

In tumor promotion, both proliferation and apoptosis requires the participation of mitochondria, at least in part. The proliferation requires energy while the apoptosis necessitates the involvement of mitochondria through altered transmitochondrial potential. In lymphoma, T cell tumorigenesis involves a simultaneous upregulation of mitochondrial biogenesis, mitochondrial respiration, and glycolytic activity. These processes allow cells to adapt to the stressful tumor environment by facilitating energy production and thereby promote tumor growth (57). Our previous study showed that arsenic induced mitochondrial biogenesis, ATP production, and oxygen consumption, suggesting that arsenic might act as a promotor in arsenic carcinogenesis (43).

In tumor progression, a typical human model of carcinogenesis is the malignant transformation from polyps in ulcerative colitis. In fact, mitochondrial loss precedes the development of dysplasia, but cancer cells restore mitochondria, suggesting that the mitochondrial biogenesis through PGC1α is needed for further proliferation (58). In addition to the role that mitochondrial biogenesis plays in tumor progression, mitochondria may play another role in the progression through their production of cellular ROS production. High ROS levels favor cancer cell mitochondrial metabolism and tumorigenesis. In breast cancer, metabolic synergy occurs in a nutrient-rich microenvironment to promote tumor growth through the generation of ROS and the induction of catabolism with autophagy, mitophagy and glycolysis (59). The ROS in stromal cells induces autophagy, causing cell death as compared to the ample mitochondrial volumes in the cancer cells (60). Our previous study of arsenic carcinogenesis showed that, although there is an increase in oxidative damage, expressions of DNA repair enzymes, and expressions of antioxidant enzymes, the arsenic-induced DNA damage and mutation are abolished when ROS is neutralized by antioxidants (42).

2.7. Mitochondria biogenesis, damages, and mutations in skin carcinogenesis

Regarding carcinogenesis in general, ROS, adaptation to ROS, and mitochondrial biogenesis compose a self-amplifying feedback loop in chronic lymphoid leukemia, which might be targeted therapeutically (61). In TPA-promoted keratinocytes, Stat3 translocates into mitochondria through the Stat3 phosphorylation and it binds mtDNA associated with mitochondrial transcription factor A (mtTFA) enhancing mitochondrial biogenesis (62). In a study with 1,815 patients with ovarian cancer, variants in mitochondrial biogenesis genes were found to possibly affect susceptibility to epithelial ovarian cancers (63). These studies indicate that mitochondrial biogenesis and its regulation play an important role in the epithelial carcinogenesis. With regard to arsenic carcinogenesis, we previously reported that low doses of arsenic induce cell proliferation through enhancement of mtTFA-mediated mitochondrial biogenesis (Figure 1) (43). It is important to note that arsenic induces expression of several oncogenes, including c-H-ras and c-myc (64). It has been found that the myc proto-oncogene, once

activated, robustly induces critical genes involved not only in cell-cycle progression but also in mitochondrial biogenesis and glucose oxidative metabolisms, further supporting the theory that mitochondrial biogenesis plays a critical role in arsenic skin carcinogenesis (65).

Not only does mitochondrial biogenesis only play an important role in arsenic-induced skin carcinogenesis, it is present and is regulated in several other cancers. Studies suggest that mitochondrial biogenesis, which promotes tumors by increasing metabolites and generating energy, is upregulated by the c-myc and downregulated by the p53 (66, 67). The role of mitochondrial biogenesis is controversial in that production of new healthy mitochondria may suppress tumor growth by stabilizing HIF-α (68). Whether mitochondrial biogenesis promotes or limits cancer may depend on the microenvironment, tissue type, and tumor stages. The effect of arsenic on the mitochondrial dysfunctions is not unique to skin keratinocytes. In BEAS-2B cells, the normal bronchial epithelial cells, arsenic generated a prolonged and steady increase in ROS levels, along with widespread up-regulation of genes associated with mitochondrial metabolism and increased ROS production and mitochondrial dysfunction (69).

In terms of mitochondrial oxidative phosphorylation in cancers, transgenic overexpression of mitochondrial uncoupling proteins (UCP1-3) with keratin-5 promotor in mice has been associated with a nearly complete resistance to chemically-mediated multistage skin carcinogenesis, suggesting that mitochondrial respiration may be a therapeutic target in the context of chemical carcinogenesis (70). Regarding to the role of mitochondrial biogenesis by arsenic, we have found that mitochondrial derived ROS contributes to mitochondrial DNA damages and mutations in arsenical skin cancers (Figure 1) (42). The arsenic-induced mitochondrial biogenesis and ROS production were reproduced in a study of prostate cancer, which showed that arsenic increased cell survival, DNA damage, and increased expression of mitochondrial transcription factor A (mtTFA) (71). Further evidence also supports this notion as mitochondria have been found to be the main target organelle for MMA-III-induced cytotoxicity (72).

3. CONCLUSIONS

The prototype of arsenical cancers is skin cancer, which is characterized microscopically by increased proliferation, individual cell apoptosis, and full-layered dysplasia, all of which necessitate the involvement of mitochondria which contribute to energy production, ROS development, cell proliferation, and DNA damages and mutations. Our study showed that arsenic induces mitochondrial biogenesis through mtTFA in keratinocytes. In the context of increased mitochondrial oxidative stress, arsenic contributes to increased oxidative damage and mutation to mtDNA in keratinocytes and in tumor tissues of patients with arsenical skin cancers. Arsenic may cause a "vicious cycle" of mitochondrial oxidative stress triggered by increased damage and mutation of mtDNA. Oxidative damage to mitochondria may drive the progression of carcinogenesis in arsenical cancers (Figure 1). Therefore, mitochondria might be an appealing therapeutic target in the treatment of arsenical cancers.

4. ACKNOWLEDGEMENT

This work is supported by grants from National Science Council (NSC 103-2314-B-182A-020, Dr. Lee; 102-2314-B-037-044, Dr. Yu), National Health Research Institute (NHRI-EO-096-PP-11 and NHRI CN-PD-9611P, Dr. Yu), and Chang Gung Medical Research Program (CMRPG8C0821, Dr. Lee).

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Key Words: Arsenic, Mitochondria, ROS, DNA Damages, Carcinogenesis, Review

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