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Molecular mechanisms of arsenic carcinogenesis

Chuanshu Huang,[1] Qingdong Ke,[1] Max Costa[1] and Xianglin Shi[2]

[1]*Nelson Institute of Environmental Medicine, New York University School of Medicine, Tuxedo, NY;* [2]*Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV, USA*

Abstract

Arsenic is a metalloid compound that is widely distributed in the environment. Human exposure of this compound has been associated with increased cancer incidence. Although the exact mechanisms remain to be investigated, numerous carcinogenic pathways have been proposed. Potential carcinogenic actions for arsenic include oxidative stress, genotoxic damage, DNA repair inhibition, epigenetic events, and activation of certain signal transduction pathways leading to abberrant gene expression. In this article, we summarize current knowledge on the molecular mechanisms of arsenic carcinogenesis with an emphasis on ROS and signal transduction pathways. (Mol Cell Biochem **255**: 57–66, 2004)

Key words: arsenic, carcinogenesis, gene expression, reactive oxygen species, genetic toxicity, signal transduction, kinase, methylation

Introduction

Although arsenic compounds can be used as a medicine to treat acute promyelocytic leukemia (APL) [1–3], they are also known as human carcinogens [4–5]. Inhalation exposure to arsenic occurs in industrial settings such as lead, copper and zinc smelting, from fossil fuel combustion in power plants, in the semiconductor industry, as well as in pesticide production. Non-occupational exposure to arsenic can take place through ingestion of contaminated food and water [4]. Contamination of well water with arsenic leached from underground sediments has occurred in large areas of India and Bangladesh, and hundreds of thousands of people have developed precancerous skin lesions due to arsenic ingestion [4, 5]. High concentrations of arsenic in drinking water are also found within the United States in the western and southwestern states and in Alaska. Exposure to high concentrations of arsenic is associated with skin, lung, liver and bladder cancers [4–9]. The mechanisms by which arsenic causes human cancers are not well understood. Human ingestion of arsenic is prevalent, and it was once used to treat syphilis and psoriasis. Arsenic is an atypical carcinogen because it is classified neither the initiator nor the promoter categories of carcinogenic agents [10, 11]. Thus, arsenic probably does not act as a classical carcinogen, but rather enhances the carcinogenic action of other carcinogens [12]. Furthermore, the challenge in elucidating the mechanism by which arsenic exerts its carcinogenic effect is the fact that no experimental animal model of arsenic-induced cancer has been developed, even though several types of animals, such as rodent, monkey, dog have been used [13–15]. In the absence of an acceptable animal model, normal human cells such as normal human keratinocytes, which represent a primary *in vivo* target of arsenite, provide a relevant and reasonable *in vitro* model to study the molecular mechanisms of arsenic carcinogenicity. Elucidation of the mechanism of arsenic-induced carcinogenesis is essential for risk assessment and establishment of exposure limits [16, 17].

In this review, we will first summarize the current knowledge of arsenic exposure and its metabolism. We will then emphasize the progress made in the last few years concerning generation of reactive oxygen species (ROS), genetic toxicology and signal transduction pathways leading to alteration of transcription factors and gene expression involved in arsenic-induced cancer development.

Address for offprints: C. Huang, Nelson Institute of Environmental Medicine, New York University School of Medicine, 57 Old Forge Road, Tuxedo, NY 10987, USA (E-mail: chuanshu@env.med.nyu.edu)

Arsenic exposure, metabolism, and toxicity

Arsenic is widely distributed in food, water, air and soil [18–21]. It is released into the environment from both natural and man-made sources [22]. Global natural emissions of arsenic and its compounds have been estimated to be 8000 tons each year, whereas emissions from man-made sources are about three times higher, 23,600 tons per year [22]. Arsenic combined with oxygen, chlorine, and sulfur is called inorganic arsenic, which represents the most common forms of either arsenate (As^{5+}) or arsenite (As^{3+}) in the environment [19–21]. Dimethylarsinic acid (DMA) is a major form of organic arsenic in the environment and has been used as a general herbicide or pesticide for many years [23]. It is also a major metabolite of inorganic arsenic in the urine of some mammals, including humans [24].

Exposure of arsenic is mainly through oral and inhalation routes [24]. Dermal exposure also occurs but to a lesser extent [24]. The oral route of exposure includes intake from water, food, drugs and tobacco products [24]. The US Environmental Protection Agency (EPA) has reduced the permissible levels of arsenic in drinking water from 50 to 10 μg/l [25]. In India, an estimated 200,000 people have developed pre-cancerous skin lesions due to contamination of drinking water with naturally-occurring arsenic. The magnitude of exposure translates into millions of people eventually developing basal or squamous cell carcinomas [26–30]. In epidemiologic studies in Taiwan, Chile, Argentina, Bangladesh and Mexico, arsenic ingestion is associated primarily with skin cancer [31, 32]. There is also increasing evidence that arsenic ingestion causes cancers of the liver, kidney, and bladder [20–22, 33–35]. However, the skin has been shown repeatedly to be the major target of arsenic toxicity [35–38]. Although present in the diet, both inorganic and organic arsenic can be absorbed and accumulated in human and animal skin [35, 36]. Many cases of skin cancer have been recorded among people exposed to arsenic through occupational or medicinal exposure [36, 39, 40]. The inhalation route is mainly related to occupational exposure of ore smelters, insecticide manufacturers, and sheep dip workers [24]. Inhalation of arsenic produces lung cancer, despite fact that inhaled arsenic is absorbed, distributed, metabolized, and excreted in a manner similar to that of ingested arsenic [41–45]. The toxicity of arsenite has been related to its high reactivity with vicinal sulfhydryl groups on macromolecules such as glutathione (GSH) and cysteine [46]. The reason that the skin is unusually sensitive to arsenic toxicity may be due to its richness in sulfhydryl-containing molecules, such as keratin [47, 48].

For occupational inhalation, humans can be exposed to slightly soluble arsenic trioxide, arsenic trisulfide, and calcium arsenate, whereas exposure from drinking water includes sodium arsenate and sodium arsenite. Previous results from both human and experimental animal studies indicate that ingested inorganic arsenic is quickly absorbed and rapidly enters the blood stream [49]. Arsenate is efficiently reduced to arsenite in blood through reactions with glutathione (GSH), which serves as an electron donor, and arsenite is subsequently transported to the liver [49]. No arsenate could be found in the blood of humans or mice exposed to arsenate orally but the reduced form of arsenite was present [49]. In the liver, mainly in the cytosol of hepatocytes, arsenite undergoes methylation to monomethylarsonic acid (MMAIII and MMAV) and subsequently to dimethylarsinic acid (DMAIII and DMAV) with S-adenosylmethionine (SAM) as the methyl donor [49].

The toxicity of arsenic is highly dependent on its oxidation state and chemical composition [50]. Arsenite is taken into cells by passive diffusion, while arsenate competes with phosphate for uptake. Arsenite is an extremely thiol-reactive. It can affect enzyme activities by binding to critical vicinal cysteinyl residues, such as those in the lipoamide of pyruvate-dehydrogenase, tyrosine phosphatases, and enzymes involved in protein ubiquination [51–53]. It is thought that arsenite is a sulfhydryl reagent having a high affinity mainly for vicinyl dithiols and also thiols located near hydroxyls. In contrast, arsenate is similar in structure to phosphate and may interfere with oxidative phosphorylation by forming an unstable arsenate ester [52]. Thus, arsenate affects phosphotransfer reactions, which are required for ATP generation. Furthermore, arsenate is excreted more rapidly than arsenite from the body [54]. Arsenite is therefore considerably more toxic and carcinogenic than arsenate [45]. It is also believed that inorganic arsenic was more toxic than organic arsenic and the methylation of inorganic arsenic was thought to be a detoxification process [56–59]. However, recent studies have shown that MMAIII and DMAIII, are actually more cytotoxic, more genotoxic and more potent inhibitors of the activities of some enzymes isolated from a variety of human and animal cell types [60]. The LD50 of MMAIII is lower than that of arsenite in hamster [61]. The reason that the methylation process was previously thought to be a possible detoxification mechanism might be that the methylated metabolites are less reactive toward tissue sulfhydryl groups and excreted faster than inorganic arsenic *in vivo* [57–59]. Additionally, DMAV, the primary excreted metabolite of inorganic arsenic, is less acutely toxic than inorganic arsenic [62]. Humans are more sensitive to arsenic than other species due to a lower efficiency of arsenic methylation in humans [63]. Human cells are also more sensitive to the cytotoxic effects of MMAIII than arsenite [60, 61]. Most of the absorbed arsenic is rapidly excreted in urine as a mixture of arsenate, arsenite, MMA, and DMA. DMA is the major form and accounts for 60–80% of total arsenic in urine. Approximately 55–80% of the absorbed arsenic is excreted in urine and 5% in feces [24].

Molecular mechanisms of arsenic carcinogenic effects

Cancer development is recognized as a stepwise phenomenon consisting of initiation, promotion and progression [64, 65]. In most cases, it is not known whether the metal compounds acts as tumor initiators or tumor promoters, or both. Because carcinogenesis is a complex process, it is likely that arsenic contributes to human carcinogenesis by multiple mechanisms. Although arsenic itself is not mutagenic, some deleterious effects on DNA have been reported, including potentiation of DNA damage by other agents, inhibition of DNA repair, sister chromatid exchange, and gene amplication [66–70]. It has also been reported that arsenic can promote the generation of reactive oxygen species (ROS), and induce signal transduction pathways. On the other hand, arsenic resembles many other classic carcinogens in inducing cell proliferation. The effect of arsenic on cell proliferation provides an insight into the mechanism of arsenic carcinogenicity. Below we have described a synopsis of the molecular mechanisms of arsenic-induced carcinogenesis:

(1) Involvement of reactive oxygen species (ROS) in arsenic response

Recent evidence has accumulated showing that ROS and nitrogen species are involved in arsenic-induced cell injury [71–73]. Arsenic generates ROS during its metabolism in cells [74]. Studies using electron paramagnetic resonance (EPR) spectroscopy have shown that arsenic rapidly increases both oxygen consumption and superoxide ($O_2^{\cdot -}$) formation [74]. Arsenic also increases extracellular accumulation of hydrogen peroxide (H_2O_2), measured as oxidation of homovanillic acid [74]. Recent investigations have revealed that arsenite activates NADH oxidase to produce $O_2^{\cdot -}$ [72, 75]. Moreover, arsenic treatment has been shown to enhance the expression of heme oxygenase and increase the fluorescence intensity of dichlorofluorescein in cells, indicating an elevated intracellular peroxide level [76]. It has been reported that arsenic treatment results in a 3-fold increase in intracellular oxyradical production as analyzed by confocal scanning microscopy using a fluorescent probe [77]. Co-treatment of cells with dimethyl sulfoxide (DMSO), an oxygen radical scavenger, reduced the fluorescent intensity to control levels [77]. The addition of superoxide radical dismutase (SOD) to culture medium was shown to inhibit arsenic-induced sister chromatid exchange in human cells [12]. Further evidence supporting the involvement of ROS in arsenic response is the finding that cells (XRS-5), which are deficient in catalase, are hypersensitive to arsenic cytoxicity and to arsenic-induced micronucleus formation [12]. In animal studies, it was also shown that exposure of rats to arsenite for 12 weeks resulted in a depletion of GSH, an increase in oxidized glutathione (GSSG), and an elevation of malondialdehyde in the liver and brain [78].

It is believed that ROS play a role in cancer development, both in the initiation and promotion stages of carcinogenesis [79–85]. At low levels, ROS can modulate gene expression by acting as second messengers, but at high levels they cause direct oxidative injuries leading to cell death [86]. ROS can damage cells by lipid peroxidation and by DNA and protein modifications. ROS can cause structural alternations in DNA, such as base-pair mutations, rearrangement, deletions, insertions, and sequence amplications, but not point mutations [87, 88]. Mutagenesis and DNA damage caused by ROS could contribute to the initiation of cancer. Among the ROS, neither $O_2^{\cdot -}$ nor H_2O_2 reacts with DNA bases, while hydroxyl ($OH^{\cdot}$) generates a multiplicity of products from all four DNA bases and this pattern appears to be a diagnostic 'finger-print' of $OH^{\cdot}$ attack [89]. If ROS causes damage to tumor suppressor genes or enhances the expression of proto-oncogenes, the cells may become tumor cells. In bovine aortic cells, arsenite causes DNA strand breaks. This effect could be decreased by nitric oxide synthase inhibitors, $O_2^{\cdot -}$ scavengers, and peroxynitrite scavengers. These observations indicate that arsenite induces generation of $O_2^{\cdot -}$ and nitric oxide (NO), and that these two species react to produce peroxynitrite [73].

ROS have been demonstrated to be involved in the signaling of the cell transformation response [90] and can affect cytoplasmic and nuclear signal transduction pathways that regulate gene expression [91, 92]. These effects are important in tumor promotion. ROS induced by low concentrations of arsenic (< 5 μM) have been shown to increase the transcription of the activator protein 1 (AP-1) and the nuclear factor kappa B (NFκB) [71, 93–95], which results in cell signaling, transcription factor binding to DNA, and subsequent stimulation of cell proliferation [96]. There were also increases in the expression of certain genes, including MDM2 protein [97], a key regulator of the tumor suppressor gene p53. In contrast, high concentrations of arsenic (> 10 μM) inhibited the activation of NFκB [98], which resulted in inhibition of cell proliferation and apoptosis [99]. As a form of programmed cell death, apoptosis is commonly used to eliminate harmful or unwanted cells from organisms during development. It is an important biologic mechanism contributing to the maintenance of the integrity of multicellular organisms, and the impairment of apoptosis has been associated with many forms of cancer [100–102]. It has been shown that arsenite induced apoptosis and inhibited cell proliferation in malignant lymphocytes [100, 101]. Apoptosis is triggered by the generation of H_2O_2 through the activation of flavoprotein-dependent superoxide-producing enzymes, for example, NADPH oxidase. The peroxide generating potential in the mitochondrial membrane is also involved in apoptosis

[103]. Arsenite was also shown to stimulate plasma membrane NADPH oxidase in vascular endothelial cells [104]. In addition, more and more data have shown that antioxidants may prevent or delay the development of some cancers [90–92]. Thus, ROS generation following arsenic exposure and interaction of arsenite with thiols may account for pleiotrophic activation or inhibition of a variety of cell functions.

(2) Genetic toxicology of arsenic compounds

Unlike many other carcinogens, arsenic fails to behave as a mutagen and cause point mutation in standard assays [12], but has been shown to induce large deletion mutations instead [75]. These multilocus deletions are incompatible with cell survival when mutations are measured at gene loci that are closely linked to essential genes. Therefore, due to their lethality, many types of mutations induced by arsenic are poorly recovered in bacterial and mammalian cell mutation assays [75, 88]. There are numerous reports showing that arsenic exposure induces chromosome aberration [105], aneuploidy and micronuclei formation [106, 107], DNA-protein cross-linking and sister chromatin exchange in animal and human cells [108]. Arsenite is reported to inhibit DNA repair by inhibiting DNA ligase [109]. In addition, it has also been reported that activation of NADH oxidase by arsenite may produce $O_2^{\cdot -}$ radical. Though $O_2^{\cdot -}$ and its secondary radicals, arsenite causes DNA strand breaks and large deletion mutations in mammalian cells [72, 75]. Furthermore, arsenic has been shown to enhance the amplification of dihydrofolate reductase gene [67]. Based on the fact that arsenic fails to behave as a point mutagen but it is indeed a carcinogen, and that oncogenes are amplified in several tumors, it has been proposed that the gene amplification induced by arsenic might play a role in the carcinogenic effect of arsenic [110].

Although arsenic may not be a classical mutagen (which can cause point mutations), it does affect the mutagenicity of other carcinogens [12]. A synergistic increase in the mutagenic activity of arsenic with UV light has been observed in mammalian cells and human cells, after exposing the UV-treated cells to arsenic [111, 112]. Consistently, the co-mutagenic effects of arsenic with chemicals, UV light or X-rays have also been shown *in vivo*. For example, mice exposed to arsenite in their drinking water together with UV radiation generated earlier, larger, and more invasive tumor with a 2.4-fold higher tumor incidence in the skin compared to those exposed to UV radiation alone [105]. Early studies have shown that animals exposed to arsenite and benzo(a)pyrene (B(a)P) developed more pulmonary lesions, such as adenomas, papillomas and adenomatoid lesions, than those treated with each chemical alone [113]. B(a)P is a carcinogenic aromatic hydrocarbon found in tobacco smoke. The populations from Japan and Taiwan exposed to arsenic in drinking water have been shown to be associated with a higher incidence of lung cancer in smokers than in non-smokers, indicating a synergy between these carcinogens [43, 45].

It has been found that DNA repair enzymes are inhibited in arsenic-exposed cells, resulting in a co-mutagenic response of arsenic with X-rays, UV light, or several chemicals [6, 114]. Arsenite inhibits the completion of DNA excision repair, perhaps via effects on DNA ligase, which is especially sensitive to arsenite in the cell [69]. DNA ligase activity in nuclear extracts has been shown to be decreased by arsenite [69]; whereas the enzyme level was not directly affected [69, 115], suggesting that arsenite may indirectly inhibit DNA ligase activity by altering cellular redox levels or by affecting signal transduction pathways and phosphorylation of proteins related to DNA ligase activity [115].

Exposure of liver epithelial cells to arsenic *in vitro* induces malignant cell transformation in parallel with DNA hypomethylation [116]. In contrast, a hypermethylation of a promoter region of the p53 gene was observed after exposure of human lung cells to arsenic for several weeks [117]. Altered DNA methylation status, both hypo- and hyper-methylation, could lead to changes in gene expression and consequently carcinogenesis [118]. How DNA hypo- or hypermethylation affects arsenic carcinogenesis remains to be clarified.

(3) Signal transduction pathways and gene expression induced by arsenic

A growing amount of evidence demonstrates that arsenic modulates gene expression by activating signal transduction pathways [55, 119]. Signal transduction is a process by which information from an extracellular signal is transmitted into the cell through the plasma membrane and along an intracellular chain of signaling molecules to stimulate a cellular response. The cellular response may be the activation of a gene transcription that occurs through various proteins known as transcription factors. A transcription factor consists of a regulatory protein that binds to specific DNA sequences in a gene. The net result of this binding is usually transcription of that gene and is referred to as transcriptional activation. Cell transformation is a complex process involving a variety of transcription factors and signaling pathways [120]. Transcription factors, such as AP-1, NFκB, and the classical mitogen-activated protein kinases (MAPKs) cascade play important roles in cell proliferation, differentiation and transformation [119, 120].

It has been reported that arsenite is a potent stimulator of proto-oncogene *c-fos* and *c-jun* expression and AP-1 transactivational activity [52]. A DNA binding protein composed of the *Jun* and *Fos* proteins, AP-1 regulates the transcription of various genes governing cellular processes, such as inflammation, proliferation and apoptosis. By using pre-

cision-cut rat lung slices, it has been shown that arsenite treatment resulted in an enhanced DNA-binding activity of AP-1 in both type-II epithelial cells and alveolar macrophages [121], revealing that arsenite is capable of activating signal transduction pathways in the lung [121]. The requirement of AP-1 for tumor promotion has been demonstrated in various cell models by various groups [119, 122, 123]. It was first considered to be a mediator of tumor promotion because of its ability to alter gene expression in response to tumor promoters, including epidermal growth factor (EGF), TPA and UV radiation. AP-1 activity was found to be progressively elevated in mouse epidermal JB6 cells representing various stages of tumor promotion, and the activation of AP-1 appears to be required for the preneoplastic-to-neoplastic progression [123]. Moreover, blocking of AP-1 activity by either pharmacological or molecular biological inhibitors could impair tumor development [124]. Because the skin is a major target of arsenic, we recently addressed the effects of arsenic on signal transduction pathways leading to the activation of transcription factors and kinases in the mouse epidermal cell line, JB6 cells, and in mouse skin [119, 125]. We have found that arsenic could induce activation of AP-1 both *in vitro* and *in vivo* [125]. The induction of AP-1 activity by arsenic appears to be mediated by activation of protein kinase C (PKC) and MAPKs family member [125]. The evidence to support this is that the induction of AP-1 could be dramatically blocked by either pretreatment of cells with PD98059, an inhibitor of ERKs pathway, or by the over-expression of a dominant negative protein kinase Cα (PKCα) [125]. Activation of PKC can result in activation of MAPKs pathways, including c-Jun N-terminal kinases (JNKs), extracellular signal-regulated protein kinases (ERKs), and P38 kinase [126]. Activated MAPKs phosphorylate the target transcription factor AP-1 and lead to induction of AP-1 transactivation and an increase in its DNA binding activity, resulting in enhanced AP-1 target gene expression [127–131].

MAPKs comprise a family of serine/threonine phosphorylating proteins that regulate the signal transduction pathways in response to a variety of extracellular stimulants and mediate the regulation of gene expression. ERKs typically transduce the signaling that leads to cell differentiation or proliferation and cell transformation, while JNKs and p38 kinase usually response to stress stimulation, which results in cell growth arrest and/or cell apoptosis. Arsenic has been found to induce activation of JNKs, p38 kinase and ERKs [119, 125, 132, 133] but differentially. Porter *et al*. reported that activation of JNKs by arsenic requires Rac, Rho, MEKK3, and MEKK4, but not Cdc42 or Ras in human embryonic kidney 293 cells [134]. We have demonstrated that low concentrations of arsenite induced ERKs activation, which is required for arsenite-induced cell transformation [119], while high concentrations of arsenite caused the activation of JNKs, which mediates cell apoptosis in mouse epidermal JB6 cells [132]. A conclusion reached from these findings is that ERKs activation may contribute to the carcinogenic effects of arsenic, whereas JNKs activation is associated with apoptosis and results in the anti-carcinogenic effects of arsenic [119, 132]. Although the mechanism by which JNKs mediates cell apoptosis in response to arsenite is not clear, recent several studies have shown that JNKs translocation to mitochondria is important for interactions with Bcl-x_L and Bcl-2 [135, 136]. JNKs are found to phosphorylate and inactivate Bcl-2, which plays a central role in regulation of apoptosis and the release of cytochrome *c* and apoptotic proteases [135, 136]. Very recently, it has been found that JNK perturbation occurred during arsenite-induced malignant transformation, which is resistant to apoptosis as compared with passage-matched control cells [137], suggesting that apoptotic control mechanisms are disrupted as cells become transformed through arsenic exposure. This apoptotic disruption may allow damaged cells to inappropriately escape apoptosis, and potentially proliferate, and further provide initiating events in carcinogenic development. Thus, this may lead to accumulation of genetically damaged cells that have a potential to become malignant.

NFκB is a collective term referring to dimeric transcription factors that belong to the Rel family [138]. These transcription factors are rapidly induced and intensify the transcription of various genes, including those for cytokines, growth factors, and acute response proteins [139–143]. NFκB activation is thought to be associated with initiation and acceleration of tumorigenesis [144]. The inhibition of NFκB was shown to block tumor promoter-induced cell transformation in JB6 cells [145]. Inhibitory kappa B (IκB), an inhibitory protein that binds to and the inactates NFκB in the cytosol, is phosphorylated and subsequently undergoes ubiquitin-dependent degradation, which results in its release from NFκB protein in responses to cell stimulation [142]. The free NFκB is then shuttled from the cytoplasm to the nucleus and regulates its target gene transcription by binding to the promoter region of its target genes [146, 147]. In response to arsenic, MAPKs signaling pathways influence not only AP-1 activation, but also NFκB activation. The results from our studies showed that both arsenite and arsenate markedly induced activation of NFκB in mouse epidermal JB6 Cl41 cells, but not in 30.7b cells, which had low levels of ERKs, suggesting that ERKs activation is involved in NFκB activation [133]. This notion was further supported by the data that the specific inhibition of arsenic-induced ERKs activation by PD98059 dramatically inhibited NFκB activation induced by arsenic [133]. Furthermore, we found that over-expression of a dominant-negative mutant JNK_1 also blocked arsenic-induced NFκB activation, revealing that JNKs activation is also required for NFκB induction by arsenic [133]. Because the blockade of activation of either ERKs or JNKs could lead to inhibition of arsenic-induced NFκB activation, we speculate that there

may be some cross-talk between ERKs and JNKs in JB6 cells in response to arsenic stimulation and this cross-talk is required for NFκB activation [133].

Consistent with the effects of arsenite on transcription factor activation, it has also been observed that arsenic treatment induced the growth arrest and DNA damage-inducible protein 153 and 45 (GADD153 and GADD45), which are responsible for the maintenance of the G2/M checkpoint that prevents improper mitosis [6, 148, 149]. In mammalian cells, the cell cycle arrest is often dependent upon the expression and functionality of these cell cycle inhibitory proteins. NFκB and JNK have been found to be reciprocally involved in the regulation of arsenite-induced GADD45 protein [150, 151]. While inhibition of NFκB caused increased and prolonged arsenite-induced GADD45, the induction of this protein was transient and less potent when NFκB was normal. Therefore, when the NFκB pathway is normal in the cells, arsenite may act as a carcinogen because the activation of NFκB may prevent the induction of cell cycle checkpoint proteins that maintain genomic stability. Inhibition of JNK, in contrast, caused decreased arsenite-induced GADD45 expression, suggesting that the activation of JNK may play a role in GADD45 induction and contribute to the anti-carcinogenic of arsenite [152]. It has been revealed that a low concentration of arsenite caused increased cyclin D1 expression in human fibroblasts [153]. Cyclin D1 is a key regulation protein in controlling cell proliferation and tumor formation [154]. Simeonova *et al*. found that arsenite is able to activate EGFR and ERKs in a human uroepithelial cell line, and that this EGFR phosphorylation by arsenic is ligand-independent and does not involve the major autophosphorylation site Tyr^{1173} [155]. They also found that c-Src activation by arsenite is required for its activation of EGFR and ERKs [155].

Additionally, it has been demonstrated that arsenic stimulated transforming growth factor-alpha (TGF-α) and granulocyte macrophage-colony stimulating factor (GM-CSF) expression in skin of humans exposed to arsenic from drinking water and in human keratinocyte cultures [6]. TGF-α is secreted by human cancer cells and retrovirus-transformed fibroblasts. It acts alongside other growth factors, such as type-beta transforming growth factor (TGF-β), to activate phenotypic cellular changes in certain cell lines. It shares about 30% sequence identity with EGF and competes with EGF for the same membrane-bound receptor sites. High amounts of TGF-α/EGF receptor complexes have been noticed in some human cancers. GM-CSF can participate in inflammatory reactions and can be induced in keratinocytes by a number of cytokines [156, 157]. It is also a growth factor for keratinocytes and has been implicated in tumor promotion via mediating inflammatory cell influx and increasing dark cell numbers in mouse skin [158]. It is actively transcribed during the tumor promotion and is involved leukocyte migration and activation [159]. Over-expression of TGF-α and GM-CSF has been associated with neoplastic transformation in the skin. TGF-α transgenic mice exhibit keratinocyte hyper-proliferation and tumors in the epithelium [6], revealing that the over-expression of TGF-α may complete both tumor initiation and promotion.

Summary

It has been known for decades that arsenic is a human carcinogen. Extensive studies have shown that ROS, genetic changes and signal transduction are involved in arsenic carcinogenesis. The metabolism of arsenic may also play a role in this process. Species differences in arsenic metabolism and toxicity, for example, may make arsenic research complicated. As mentioned above, humans are more sensitive to arsenic toxicity than several other species because arsenic methylation in humans is thought to be less efficient. Mice, rabbits, and hamsters excrete less MMA and more DMA than humans [160, 161]. Rats also excrete less inorganic and methylated arsenic than humans. Guinea pigs, marmosets and chimpanzees are unable to methylate arsenic [160, 161]. Studies with cultured human and rat hepatocytes have also identified interindividual and interstrain differences in the rate of formation of MMA and DMA after *in vitro* exposure to arsenite [115].

Furthermore, differences in individual susceptibility of humans to arsenic should be considered in conducting research on arsenic. The individual susceptibility may result from differences in genes controlling metabolism of xenobiotics, DNA repair, cell transport, immune response, antioxidant defenses and cell cycle control. The ability to repair DNA strongly influences predisposition to cancers. Genetic susceptibility to arsenic toxicity and/or carcinogenicity may reflect the sensitivity of the DNA repair apparatus to inhibition by arsenic.

In addition, previous studies have also generated some controversy regulating carcinogenic actions of arsenic. For instance, conflicting results show that arsenic has no effect on p53-dependent transcription [132], or it was reported to decrease p53 expression [162] or induce p53 phosphorylation and accumulation [163–166]. Since p53 protein is a crucial tumor suppress gene, it is important to understand the relationship of p53 and arsenic in carcinogenesis.

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