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Focused Review

New perspectives in arsenic-induced cell signal transduction

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Abstract

Although the carcinogenicity of arsenic has been well established, the underlying molecular mechanisms have not yet been fully identified. Accumulating evidence indicates that the alteration of cellular signal transduction is directly related to the carcinogenesis of arsenic. This review focuses on recent advances in arsenic-induced signal transduction, including reactive oxygen species (ROS) production, tyrosine phosphorylation, MAPK signaling, NF-κB activation, cell cycle arrest, and apoptosis.

Keywords: Metal; Arsenic; Tyrosine phosphorylation; Reactive oxygen species; Cell cycle; Apoptosis; MAPK; NF-κB

1. Introduction

Metals are trace elements that are essential for the human body. However, certain metals and their derivates are toxic and have a wide variety of adverse effects, including carcinogenicity, neurotoxicity, and immunotoxicity [1]. Among these adverse effects, the carcinogenic potential of certain metals may have the greatest biological significance. The molecular mechanisms for metal carcinogens are still poorly understood. Since many metals are essential to life and the human body has evolved to their existence, many toxic metals and their derivates are able to evade the natural protective barrier of the human body and utilize existing normal cellular physiological systems to affect specific tissues or cell constituents, forming the basis for the intricate mechanisms that leads to metal-induced carcinogenesis. There are two major mechanisms by which toxic metals may damage cells. They may directly bind to cellular molecules to either induce conformational changes or displace physiological (essential) metals from their natural binding sites, thus disrupting normal cell function. Secondly, they may act as a catalytic center for the redox reactions that produce reactive oxygen species (ROS) capable of damaging a wide variety of cellular macromolecules [2]. Ultimately, both mechanisms may alter cellular signal transduction, leading to cancer development.

Arsenic has long been considered a toxic metal. There is substantial evidence indicating that exposure of humans to arsenic via contaminated air and drinking water causes cancer [3]. There are four main forms of arsenic found in mammals, including humans, namely, arsenate (As(V)), arsenite (As(III)), MMA (monomethylarsonic acid), and DMA (dimethylarsenic acid) [4]. Arsenite (As(III)) is a reduced form of arsenate (As(V)). The reduction reaction involves either arsenate reductase or glutathione. Following the reduction, arsenite can be methylated to form MMA and DMA via methyltransferase enzymes in vivo [5]. There remains controversy as to which form of arsenic poses the greatest carcinogenic risk to human life. Different chemical forms of arsenic exhibit varying potencies in carcinogenesis studies. Furthermore, arsenic forms can be interconverted and the rates of interconversion vary. Therefore, this review will not specify individual chemical forms of arsenic compounds in a particular cell signaling event. Increasing evidence indicates that the reduction of arsenic both in vivo and in vitro is associated with the formation of ROS. The formation of hydrogen peroxide, superoxide anion, singlet oxygen, and hydroxyl radical has occurred in many cells in response to arsenic stimulation. Therefore, arsenic-induced ROS generation is thought to play an important role in arsenic-induced carcinogenesis [4,6].

This review will summarize the recent advances in the

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understanding of arsenic-induced cell signal transduction. Arsenic has been shown to induce carcinogenesis in vivo by inducing a wide spectrum of changes in cell signal transduction, including the alterations in cell differentiation, proliferation and cell apoptosis [3,7]. Given the intricate nature of the interactions of arsenic with the human body and the complexity of cell signaling pathways, it is not possible to cover all signal transduction pathways affected by arsenic in the present short report. Therefore, this review will cover only arsenic-induced ROS generation, tyrosine phosphorylation, MAPK activation, NFκB activation, cell cycle arrest, and apoptosis.

2. Reactive oxygen species (ROS)

ROS is a collective term for the intermediates formed during oxidative metabolism, encompassing both oxygen radicals and non-radical reactive oxygen derivatives. These include the superoxide anion ($O_2^{\cdot -}$), the hydroxyl radicals ($^{\cdot}OH$), and hydrogen peroxide (H_2O_2). Cells can normally adapt to low physiological concentration of ROS with antioxidant defense systems. However, high concentration of ROS can cause oxidative damage to cellular macromolecules, including DNA, proteins and lipids.

Arsenic has been demonstrated to induce the formation of ROS in a wide variety of cells, including human vascular smooth muscle cells [8], human–hamster hybrid cells [9], vascular endothelial cells [10], murine keratinocytes [11], acute promyelocytic leukemia cells [12], Chinese hamster ovary cells [13], chronic lymphocytic leukemia cells [14], and peripheral human lymphocytes [15]. However, the underlying mechanisms for ROS regulation have not yet been identified. Recently, it has been proposed that arsenic may induce the formation of ROS through: (1) the ubiquinone site of the respiratory chain [11], (2) a decrease in cellular mitochondrial membrane potential [16], (3) alteration of glutathione (GSH) concentration [17], (4) activation of NADH oxidase [8], and (5) the oxidation of arsenite to arsenate [18].

Arsenic-induced ROS has been demonstrated to cause DNA damage, lipid peroxidation, and protein modification, as well as alterations of antioxidant defenses. Among the effects of arsenic-induced ROS, the DNA damage may have the most biological significance. Hei and colleagues found that treatment of A_L hybrid cells with arsenic induced both intragenic and multilocus deletion mutations of DNA in a dose-dependent manner. Cotreatment of the cells with an ROS scavenger, dimethyl sulfoxide (DMSO), reduced these mutations, indicating that arsenic-induced ROS formation might be involved in DNA damage [19]. Later, they demonstrated that treatment of the cells with arsenic increased intracellular oxyradical production by three-fold within 5 min. These oxyradicals were measured using a fluorescent probe (5′,6′-chloromethyl-2′7′-dichlorodihydrofluorescein). The increase in intracellular oxyradical production could be inhibited by co-incubation with a radical scavenger DMSO. Using ESR spectroscopy with TEMPOL-H (4-hydroxy-2,2,6,6-tetramethyl-1-hydroxypiperidine) as a probe and superoxide dismutase and catalase as ROS scavengers, this group observed an increase in the generation of superoxide-derived hydroxyl radicals as well as hydrogen peroxide in arsenic-treated A_L cells. Furthermore, depletion of intracellular nonprotein sulfhydryls (mainly glutathione) increased arsenic-induced mutation up to five-fold. Taken together, their results indicated that arsenic might induce the DNA damage through the following cascade: arsenic→superoxide anions→hydrogen peroxide→hydroxyl radicals→DNA mutations [9]. Recently, they reported that removal of ROS by addition of either superoxide dismutase or catalase inhibited arsenic-induced mutagenicity by two- to three-fold. They further demonstrated that the DNA lesions were associated with 8-hydroxy-2′-deoxyguanosine (8-OhdG), a major DNA damage adduct. Their results suggest that ROS played an important role in arsenic-induced genotoxicity in mammalian cells [6].

3. Tyrosine phosphorylation

Tyrosine phosphorylation plays an important role in the regulation of cell proliferation, differentiation, cell-cycle regulation, and cell signal transduction [20]. The induction of tyrosine phosphorylation is mediated by the activation of protein tyrosine kinases and/or the inhibition of protein tyrosine phosphatases. There are two major types of protein tyrosine kinases: receptor tyrosine kinases (RTKs) and nonreceptor tyrosine kinases (NTKs). RTKs are a protein family that has a transmembrane-spanning receptor and an intrinsic protein tyrosine kinase activity. They are activated upon either ligand binding or receptor cross-linking. Upon stimulation, RTKs undergo autophosphorylation on the tyrosine residues located in their own carboxy terminus and induce conformational changes. This enhances kinase activities and creates binding sites for cellular substrates through SH2 domain interactions. Most of RTKs are protein growth factor receptors, including EGF receptor (EGFR), PDGF receptor (PDGFR) and VEGF receptor (VEGFR). NTKs, which include Src family members [21], are the cellular substrates of RTKs. Normally, they are under tight control by an intramolecular interaction between their own SH2 domains and carboxy terminal phosphorylated tyrosine. They are activated upon disruption of the intramolecular interaction via either binding to the activated RTKs or dephosphorylation by protein tyrosine phosphatases. Following activation, the NTKs phosphorylate cellular substrates that affect a wide variety of cellular activities.

Deregulation of tyrosine phosphorylation, mainly constitutively elevated tyrosine phosphorylation, is directly related to aberrant cell signaling, uncontrolled cell growth

in culture and cancer development in vivo. It has been documented that tyrosine phosphorylation is closely linked to malignancies, such as leukemia, lymphoma, multiple endocrine neoplasia type 2, small lung cancer, breast cancer, and colon cancer [22–24].

Previous studies found that exposure of the cells to arsenic increased total cellular tyrosine phosphorylation in a dose-dependent manner. Western blot analysis showed that the sizes of tyrosine phosphorylated proteins were 110–120, 90, 70, 56, and 40 kDa [25]. Later studies identified that EGFR was tyrosine-phosphorylated and activated upon arsenic stimulation [26]. It is not known how arsenic induces the activation of EGFR. One proposed mechanism is that arsenic may directly interact with EGFR molecules to induce either the conformational changes or the dimerization of EGFR, which results in the activation of EGFR [27]. It was also proposed that arsenic might activate EGFR through the generation of ROS that, in turn, triggered the conformational changes in the receptor [28]. Following the activation of EGFR in response to arsenic stimulation, EGFR recruits Shc and phosphorylates its tyrosine residues, which results in the enhancement of the interactions between Shc and Grb2. Signals are then relayed to downstream signaling proteins [26]. Consistent with these observations, Wu and colleagues demonstrated that arsenic-induced tyrosine phosphorylation of EGFR was directly responsible for the activation of the MAPK family in human airway epithelial cells. Their results showed that inhibition of EGFR kinase with a specific inhibitor, PD-153035, blocked arsenic-induced activation of MAPKs [27]. Recently, they further demonstrated that the Ras protein mediated signal transduction from the tyrosine phosphorylated EGFR to the activation of MAPKs in response to arsenic stimulation in human airway epithelial cell lines [29]. Their results showed that arsenic treatment induced the activation of both EGFR and Ras. Inhibition of the activation of EGFR abolished arsenic-induced activation of Ras and MAPKs, while inhibition of Ras was only able to block the activation of MAPKs, indicating that Ras is an intermediate protein in arsenic-induced EGFR signaling [29].

Arsenic-induced EGFR signaling may involve the non-receptor tyrosine kinase, Src, in human epithelial cell lines. It was found that arsenic induced the activation of EGFR and MAPKs, as well as Src in several cell lines [30,31]. Consistent with the results of cell culture, exposure of mice to arsenic induced the activation of EGFR, MAPKs and Src [31]. Inhibition of Src abolished arsenic-induced activation of EGFR and MAPKs. In contrast, inhibition of EGFR had no effects on Src activation in response to arsenic stimulation, demonstrating that arsenic-induced activation of EGFR and MAPKs is dependent on Src. It is not clear how the non-receptor tyrosine kinase, Src, acts as an upstream protein in activating the receptor tyrosine kinase, EGFR, in response to arsenic stimulation. It has been speculated that arsenic may activate Src through: (1) direct reactions with the vicinal sulfhydryl groups of the Src molecule, (2) direct interactions with extracellular matrix proteins to induce integrin rearrangements, or (3) the generation of ROS [31]. It has been demonstrated that Src has the capability to activate EGFR. Biscardi and colleagues found that Src was able to phosphorylate EGFR at two unique tyrosine residues, distinct from the auto-phosphorylation sites, to activate EGFR, in association with the activation of other cell signaling proteins [32,33]. Indeed, further studies found that the induction of Src by arsenic was able to activate MAPKs either in the presence or in the absence of EGFR. This implies that Src, in response to arsenic stimulation, activates downstream proteins, e.g. MAPKs, through two parallel pathways, one EGFR-dependent and the other EGFR-independent [31].

4. Mitogen-activated protein kinases (MAPKs)

MAPKs are a family of protein serine–threonine kinases that transmit extracellular signals to induce the expression of a variety of genes that mediate cell apoptosis, differentiation, proliferation, and transformation. The extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNK) and p38 are the three major classes of MAPKs [34]. ERKs are mainly involved in growth factor-induced cell differentiation, proliferation and transformation signaling, while JNK and p38 mediate cytokine and numerous stress-induced cell responses, cell growth arrest and apoptosis [5].

An increasing amount of evidence indicates that arsenic can activate all three classes of MAPKs in a variety of human cell lines in a time-dependent manner [5]. Arsenic can activate MAPKs in concentrations ranging from 0.1 to 500 μM [5]. The activation of individual MAPKs by arsenic involves several different signaling pathways. The activation of ERKs and p38 appears to be mediated by the Ras/Raf/Mek pathway [29,35], whereas the activation of JNKs has proven to involve the Rac, Rho and MEKK3-4 [36].

Recent studies indicate that arsenic differentially activates ERKs, p38 and JNKs to mediate opposing effects on carcinogenesis, which are dependent on time, dose, and oxidative form of arsenic, and the type of target cell. The activation of ERKs by arsenic can induce cell proliferation and transformation leading to carcinogenesis, whereas the activation of JNKs and p38 can induce cell growth arrest and apoptosis that leads to the inhibition of carcinogenesis [5]. Indeed, recent studies have found that arsenic induces dual effects on the individual MAPK pathways in rat liver TRL1215 cells previously transformed by arsenic [37]. Qu and colleagues first produced a malignant transformed rat liver TRL1215 cell line by chronic treatment with arsenic. Then, they challenged the transformed cells with arsenic to examine changes in apoptosis. In comparison with the passage-matched TRL1215 control cells, the transformed

cells were resistant to arsenic-induced apoptosis. This resistance was substantially removed upon the treatment with a JNK specific activator, Ro318220, indicating that arsenic suppressed JNK activities in the transformed cells [37]. Further studies demonstrated that arsenic treatment inhibited JNK activity and, interestingly, increased both ERK and p38 activities in these transformed cells. Probing for JNK-induced apoptotic regulatory proteins revealed that the expression of Bcl-x_L and Bcl-2, two anti-apoptotic proteins, were substantially increased, whereas the expression of Bax, a pro-apoptotic protein, was significantly decreased. This indicated that arsenic treatment disrupted the activity of the JNK signaling pathway and led to the inhibition of apoptosis in the transformed cells [37]. Additionally, an independent study demonstrated that arsenic inhibited JNK activity through interactions with a JNK-inactivating dual-specificity threonine–tyrosine phosphatase M3/6 [38]. These results demonstrate that arsenic stimulation differentially affects the individual MAPK pathways to produce opposing effects on cell growth and differentiation.

5. Nuclear factor-kappa B (NF-κB)

NF-κB is a transcription factor that mediates a variety of cellular processes, including cell-to-cell interaction, intracellular communication, cell recruitment or transmigration, amplification of primary pathogenic signals, and initiation or acceleration of carcinogenesis [39]. NF-κB is a heterodimer composed of p50 and p65 (RelA) and is inactive in the cytoplasm through the association with one of its inhibitors, such as IκB, p105, or p100 molecules. Upon stimulation, NF-κB inhibitors undergo either protein tyrosine or serine phosphorylation and the subsequent degradation. After dissociation with its inhibitor, NF-κB is able to translocate to the nucleus to activate the transcription. IκB is normally regulated by its upstream kinase of the IKK family, which are, in turn, activated by several protein kinases, such as MEKK1, Akt, NIK, NAK, and PKC. A wide variety of stimuli have been demonstrated to activate NF-κB, including cytokines, mitogens, environmental and occupational particles, toxic metals, intracellular stresses, and UV light [39].

Arsenic-induced NF-κB activation is time-, dose- and cell type-dependent. Low and noncytotoxic concentrations of arsenic (1–10 μM) usually activate NF-κB, while high concentrations of arsenic (>10 μM) generally inhibit this transcription factor [5,39]. The same dose of arsenic that induces NF-κB activation in one type of cell does not necessarily have the same effect in other cell types. The molecular mechanisms by which arsenic affects the activity of NF-κB have not been fully identified. It was found that the generation of ROS is involved in the arsenic-induced activation of NF-κB in endothelial cells [40]. There is no convincing evidence to show that the changes in the phosphorylation and subsequent degradation of IκB play a major role in the induction of NF-κB activity in response to arsenic stimulation. Instead, arsenic was found to activate NF-κB independently of the degradation of IκB and the subsequent translocation of NF-κB in airway epithelial cells, indicating the existence of an alternative mechanism for the arsenic-induced activation of NF-κB [41]. Indeed, several groups found that exposure to arsenic induced an enhancement of basal NF-κB DNA-binding activity without an increase in IκB phosphorylation nor an increase in the translocation of NF-κB in lung epithelial cells and alveolar macrophages [42,43].

The role of MAPKs in arsenic-induced NF-κB activation is complicated. Recently, Chen and Shi found that arsenic activated NF-κB through the SEK1-JNK pathway in wild-type and sek1 [stress-activated protein kinase (SAPK)/ERK kinase] gene knockout mouse embryo stem cells independently of the activation of ERK and p38 [39]. In contrast, the activation of ERK was required for arsenic-induced NF-κB activation in mouse skin epidermal JB6 cells, which was supported by the observations that inhibition of ERK activities with either a specific inhibitor PD98059 or knock-out of the ERK gene blocked arsenic-induced activation of NF-κB [44].

Interestingly, arsenic inhibits the activity of NF-κB through different mechanisms at different concentrations. It was found that arsenic at the concentrations between 12.5 and 100 μM inhibited TNF-α-induced activation of IKK and NF-κB via direct binding to Cys-179 in the activation loop of the IKK catalytic subunits to inhibit IKK activities, and overexpression of IKKβ abolished arsenic-induced inhibition of NF-κB [45], indicating that IKK is a critical protein in arsenic-induced NF-κB inhibition. Consistent with these observations, two groups demonstrated that arsenic inhibited the phosphorylation and the degradation of IκBα and the translocation of NF-κB in both human bronchial epithelial (BEAS 2B) cells and human T-cell leukemia virus type 1 (HTLV-1) cells [46,47]. However, arsenic at superphysiological concentrations (100–500 μM) was found to inhibit NF-κB activities through interfering with DNA binding of NF-κB [39].

6. Cell cycle

The cell cycle is the process by which cells reproduce. This involves duplication of cellular contents and subsequent division into two cells. For most eukaryotic cells, the cell cycle is composed of four arbitrary phases: M, G_1, S, and G_2 phases. During the M phase, cells are divided into two daughter cells. The S phase is the time that cells replicate their DNA. The interval between the completion of mitosis and the beginning of DNA synthesis is called the G_1 phase. The G_2 phase is the interval between the end of DNA synthesis and the beginning of mitosis. Cells in G_1 can be forced out of the cell cycle, and pause in a

quiescent state for a variable amount of time before reentering the cell cycle. This quiescent state is referred to as G_0 phase.

The cell cycle is mainly controlled by a series of regulatory proteins called cyclins and cyclin-dependent kinase (CDKs). The cyclins exert their regulatory roles through binding to CDKs to form the cyclin/CDK complexes, which are further controlled by a series of kinases and phosphatases. The activated CDKs phosphorylate downstream proteins to regulate the cell cycle. The control of the cell cycle is mainly achieved through checkpoints in the G_1, G_2 and M phases. The activation of G_1 checkpoint is predominantly through p53-regulated signal pathways, which involve p21, Cyclin E/CDK2 and Cyclin D/CDK4/6. The control of G2 checkpoint is mostly through ATM-regulated signal pathways, which involve GADD45, CDC25 and CDC2/Cyclin B. The M phase checkpoint is regulated by microtubules [48,49].

Cells maintain the proper growth and tissue homeostasis through the control of the cell cycle. Incomplete DNA replication and DNA damage have been proven to arrest cell growth at the checkpoints. DNA damage may be repaired during cell cycle arrest, allowing cells to re-enter into the cell cycle. If cells are unable to correct DNA damage during the cell cycle arrest, they will undergo either apoptosis, i.e. programmed cell death, or transformation. Thus, the investigation of cell cycle arrest and apoptosis is an important part of our overall understanding of the mechanisms of metal-induced carcinogenesis.

Arsenic has been observed to damage DNA and induces the cell arrest at G_1 or at G_2-M phase [48]. Unfortunately, the molecular mechanisms for arsenic's effects have not yet been fully elucidated. The limited numbers of studies indicate that arsenic trioxide is able to reduce the steady-state levels of CDK/cyclin complexes, such as CDK2, CDK6, as well as cyclins A, D1 and E, in a variety of human cancer cell lines [50,51]. In addition, treatment with arsenic trioxide increases both the expression level and the binding activity of CDK inhibitory protein p21 [50]. Furthermore, it has been found that arsenic trioxide arrests the cell cycle via the inhibition of cdc2-associated kinase and CDK2/6-associated kinases in consort with the reduction of cdc25B/C phosphatases and the hypophosphorylation of Rb protein, respectively [50,51].

Accumulating evidence indicates that the p53 signaling pathway plays a critical role in arsenic-induced cell cycle arrest. Yih and Lee [52] found that exposure of human fibroblast (HFW) cells to arsenic caused DNA strand breaks and subsequently induced a significant accumulation of p53. Following the activation of p53, the expression of both p21 and the human homolog of mdm-2 (murine double minute-2), two protein downstream of p53, was increased significantly and the cell cycle arrest occurred at the G_2-M phase. Taken together, these data indicate that p53 is involved in arsenic-induced G_2-M phase arrest. ATM, a member of PI3-kinase-related protein kinase, was also observed to be involved in activation of p53 in response to arsenic stimulation. Pretreatment of the cells with wortmannin, an inhibitor of PI3-kinase, abolished arsenic-induced p53 accumulation. A mapping of p53 phosphorylation revealed that serine 15, a site specifically phosphorylated by ATM, was phosphorylated in response to arsenic stimulation. Furthermore, deletion of ATM abolished arsenic-induced activation of p53, as well as G_2-M phase arrest. These results demonstrate that arsenic induce cell cycle arrest at the G_2-M phase through an ATM→p53→p21 signaling pathway [52]. Another group found that p53 signaling was necessary for arsenic-induced G_1 phase arrest in human glioblastoma cell lines [53]. They stimulated two human glioblastoma cell lines, which differed in p53 status (U87MG-wt and T98G-mutated), with arsenic to evaluate the changes in the cell cycle. After arsenic treatment, the p53-wt U87MG cells displayed a reduced expression of cyclin D1 and cell cycle arrest at the G_1 phase. The p53-mutated T98G cells did not undergo cell cycle arrest, demonstrating that the activation of the p53 signaling pathway is involved in arsenic-induced G_1 arrest.

Interestingly, our group recently demonstrated that arsenic induced G_2/M phase arrest is through the induction of GADD45, a cell cycle G_2/M checkpoint protein, which functions independently of p53 activation in human bronchial epithelial cells [54]. In these studies, we found that exposure of the cells to arsenic induced a transient and less potent expression of GADD45 in association with G_2/M phase arrest, which was enhanced by the inhibition of NF-κB activation in a p53-independent manner. We also found that the inhibition of NF-κB enhanced arsenic-induced JNK and ERK activities. These results indicate a negative regulatory role of NF-κB in the induction of GADD45 and MAPKs in response to arsenic stimulation. Furthermore, inhibition of JNK activity abolished arsenic-induced GADD45 production in NF-κB mutant cell lines, indicating a positive regulatory role of JNK in the induction of GADD45. Taken together, these results demonstrated that NF-κB and JNK exert opposite effects on arsenic-induced, p53-independent induction of GADD45 in association with the G_2/M phase arrest [54].

7. Apoptosis

Apoptosis, or programmed cell death, is a physiological process whereby cells 'commit suicide', which is controlled by cell signal transduction. Apoptosis is a critical cellular response to maintain normal cell development and the proper function of multicellular organisms [55]. The dysregulation of apoptosis is directly associated with cancer development. The characteristics of apoptotic morphological changes have been well-documented. They include plasma membrane blebbing, cytoplasmic shrinkage, and chromosomal DNA condensation and breakdown

[48,55]. The main apoptotic regulatory proteins are the caspase family, the tumor necrosis factor receptor (TNFR) family, adaptor proteins, and the Bcl-2 family. Caspase family proteins are a group of cysteine proteases that normally exist as inactive zymogens, which are activated by specific proteins upon stimulation. There are two types of caspases: initiators and effectors. The initiator caspases (such as caspases 8 and 9) cleave and activate the effector caspases (such as caspase 3) in the process of apoptosis. The TNFR family members, also referred to as death receptors, trigger apoptosis through binding to death-inducing ligands. The linkage between the death receptors and caspases are called adaptor proteins that usually contain a death domain (DD), a death effector domain (DED) and a caspase recruitment domain (CARD). Bcl-2 family proteins are a group of proteins that are either anti-apoptotic (e.g. Bcl-2 and Bcl-x_L), or pro-apoptotic (e.g. Bax and Bak) upon activation [55].

There are two general kinds of apoptosis: receptor-mediated and mitochondria-mediated apoptosis. Receptor-mediated apoptosis is triggered by ligand binding to death receptors and is involved in the recruitment of the apoptotic adaptor proteins, and the subsequent activation of caspase 8. Mitochondria-mediated apoptosis is triggered by a variety of stimuli that are involved in the release of cytochrome C from damaged mitochondria, followed by the activation of caspase 9. Both kinds of apoptotic pathways cause the cells to undergo apoptosis through caspase 3. Multiple cellular signaling proteins are involved in regulating apoptosis, including NF-κB, JNK and p53 [48,55].

Arsenic has been shown to induce apoptosis in a variety of cell lines. There are several common characteristics that are associated with arsenic-induced apoptosis, including increased oxidative stress, activation of caspase pathways, decreased mitochondrial membrane potential, increased pro-apoptotic protein expression and decreased anti-apoptotic protein expression [5]. However, the underlying molecular mechanisms remain largely unknown. It has been shown that arsenic directly condenses the mitochondrial matrix and decreases mitochondrial transmembrane potential to trigger apoptosis via the release of cytochrome C from mitochondria and the subsequent activation of the caspase proteins [12,56]. Recent studies have found that arsenic promotes histone H3 phosphoacetylation at the chromatin of CASPASE-10 locus. This results in the expression and activation of caspase 10 in acute promyelocytic leukemia cells, which subsequently activates caspases 8 and 3 and induces apoptosis [57].

Increased oxidative stress has been found to be directly related to arsenic-induced apoptosis. One group found that the induction of ROS upon arsenic stimulation was associated with the inhibition of Akt-mediated cell survival pathways in acute promyelocytic leukemia U937 cells, and that inhibition of Akt contributed to the activation of caspase 3 [58]. Interestingly, recent studies show that exposure of human androgen-independent prostate cancer cell lines (PC-3) to arsenic induces the formation of ROS, and activates p38, JNK and caspase 3, concomitant with the occurrence of apoptosis. Removal of ROS abolished arsenic-induced apoptosis in PC-3 cells, whereas inhibition of p38, JNK and caspase 3 had no effects on apoptosis. This indicates that ROS may be able to directly induce apoptosis independently of MAPK pathways and caspase pathways in PC-3 cells in response to arsenic stimulation [59]. Consistent with the oxidative stress model, the sensitivity of a variety of tumor cells to arsenic-induced apoptosis is inversely related to the level of their intracellular glutathione (GSH), an important cellular antioxidant, and the induction of GSH synthesis in response to arsenic stimulation [5,60].

The involvement of TNFR in arsenic-induced apoptosis is controversial. One group found that disruption of TNFR with a blocking antibody was unable to inhibit arsenic-induced apoptosis in HTLV-1 infected T-cell lines [61]. This indicates that TNFR is not involved in arsenic-induced apoptosis. Conversely, TNFR was observed to be essential for arsenic-induced apoptosis in human acute myeloid leukemia cells. Furthermore, their results implied that TNFR mediated arsenic-induced apoptosis through the autocrine action of TNF-α [62].

Although p53 has been demonstrated to play a major role in the process of apoptosis in general, the involvement of p53 in arsenic-induced apoptosis has not yet been clarified. Previous reports showed that the activation of p53 was not involved in arsenic-induced apoptosis [63,64]. However, two recent reports showed contradictory results. Arsenic induced apoptosis in association with the activation of p53 in both human gastric cancer cell lines and human glioblastoma cell lines. The inhibition of p53, using a p53 anti-sense oligo-nucleotide, suppressed arsenic-induced caspase expression and apoptosis in human gastric cancer cell lines, demonstrating the involvement of p53 in arsenic-induced apoptosis [53,65].

Numerous reports showed that arsenic-induced apoptosis is associated with the cell cycle arrest, especially at the G_2/M phase [50,51,53,66–68]. The underlying molecular mechanisms are still unclear. One group found that arsenic treatment caused cell cycle arrest in human non-small-cell lung cancer cell lines during mitosis in association with an increase in tubulin polymerization. Immunocytochemical and EM studies revealed that arsenic treatment induced changes in the cellular microtubule network and microtubular polymerization. Further studies showed that arsenic treatment induced the up-regulation of cyclin B1, the activation of the cdc2/cyclin B1 kinase, and the phosphorylation of Bcl-2, concomitant with the activation of caspases 3 and 7 in arsenic-induced mitotic cells [68]. These results indicate that arsenic-induced apoptosis may result from the onset of cell cycle arrest.

8. Perspectives

Over the past decade, the toxicology sub-discipline of molecular toxicology and carcinogenesis has been developed. New techniques are available to unravel the mechanisms of arsenic toxicity and carcinogenesis in precise molecular terms so that intricate biological interrelations can be elucidated. Significant efforts are being focused on: (1) understanding perturbations of arsenic at the molecular, cellular, tissue and organ levels; (2) exploring basic integrative links between various organ systems as they pertain to human health effects of arsenic exposures; and (3) understanding the biological components that influence the outcome of arsenic exposure.

Given that arsenic induces a global change in gene expression and that cell signal transduction pathways vary among different types of cells, the challenge of dissecting the individual signaling transduction pathways involved in the response to arsenic stimulation is tremendous. Advance in the molecular toxicology techniques, genomics and proteomics, will enable researchers to elucidate how arsenic affects the macromolecules globally. Such information can define cellular networks of response genes, identify target molecules of toxicity and carcinogenicity, provide future biomarkers and alternative testing procedures, and identify individuals with increased susceptibility to arsenic. Ultimately, scientists will find the specific signaling pathways for arsenic and develop preventative strategies against arsenic toxicology and carcinogenesis.

9. Abbreviations

As(V)	Arsenate
As(III)	Arsenite
CARD	Caspase recruitment domain
CDK	Cyclin-dependent kinase
DD	Death domain
DED	Death effector domain
DMA	Dimethylarsenic acid
EGFR	EGF receptor
ERK	The extracellular signal-regulated kinase
GSH	Glutathione
H_2O_2	Hydrogen peroxide
JNK	c-Jun N-terminal kinase
MAPK	Mitogen-activated protein kinases
MMA	Monomethylarsonic acid
NF-κB	Nuclear factor-kappa B
NTK	Nonreceptor tyrosine kinase
$^{\cdot}OH$	Hydroxyl radicals
8-OhdG	8-hydroxy-2′-deoxyguanosine
$O_2^{\cdot}$	Superoxide anion
PDGFR	PDGF receptor
ROS	Reactive oxygen species
RTK	Receptor tyrosine kinase
SOD	Superoxide dismutase
TEMPOL-H	4-hydroxy-2,2,6,6-tetramethyl-1-hydroxy-piperidine
TNFR	Tumor necrosis factor receptor
VEGFR	VEGF receptor

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