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Disruption of Mitotic Progression by Arsenic

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Abstract

Arsenic is an enigmatic xenobiotic that causes a multitude of chronic diseases including cancer and also is a therapeutic with promise in cancer treatment. Arsenic causes mitotic delay and induces aneuploidy in diploid human cells. In contrast, arsenic causes mitotic arrest followed by an apoptotic death in a multitude of virally transformed cells and cancer cells. We have explored the hypothesis that these differential effects of arsenic exposure are related by arsenic disruption of mitosis and are differentiated by the target cell's ability to regulate or modify cell cycle checkpoints. Functional p53/CDKN1A axis has been shown to mitigate the mitotic block and to be essential to induction of aneuploidy. More recent preliminary data suggest that microRNA modulation of chromatid cohesion also may play a role in escape from mitotic block and in generation of chromosomal instability. Other recent studies suggest that arsenic may be useful in treatment of solid tumors when used in combination with other cytotoxic agents such as cisplatin.

Arsenic is an enigmatic xenobiotic that on one hand causes a variety of chronic diseases and on the other has therapeutic uses. Chronic arsenic exposure, most commonly as arsenite or arsenate contaminating drinking water, is a worldwide problem affecting 140 million people [1, 2]. Chronic arsenic exposure causes cancer, cardiovascular disease, neurological disorders, reproductive problems, chronic pulmonary disease, ocular disorders, pigmentary changes, and diabetes [3–8]. Arsenicals have been used in Chinese traditional medicines for centuries [9], are still used to treat infectious tropical diseases [10], and arsenic trioxide (ATO, As₂O₃) is approved by USFDA to treat acute promyelocytic leukemia (APL) [11]. This review focuses on the role that arsenic-induced mitotic disruption plays in carcinogenesis and potentially in chemotherapy of solid tumors.

Arsenic is a group I human carcinogen, and chronic inorganic arsenic exposure causes skin, lung, and bladder cancer and likely causes prostate, liver, and kidney cancer in humans [12]. Transplacental inorganic arsenic exposure causes lung, liver, ovary, and adrenal tumors in mice [13, 14]. Proposed modes of action in arsenic carcinogenesis include DNA repair inhibition, co-mutagenicity, altered DNA methylation, oxidative stress, cell proliferation, and aneuploidy, including both structural and numerical chromosomal abnormalities. There are ample data supporting each of these proposed modes, but aneuploidy was deemed the most plausible mechanism or mode of action for arsenic carcinogenicity [15, 16]. Since these NRC reports were published, much research has been performed in pursuit of modes

other than aneuploidy. However, appreciation of the role of aneuploidy, or chromosomal instability, as a major driver of carcinogenesis is regaining attention [17–19]. Thus, this review is focused on mechanisms related to aneuploidy. The disruption of mitosis by arsenicals is central to the induction of aneuploidy.

Aneuploidy classically refers to chromosomal numerical abnormalities, e.g., trisomy 21 or a loss of heterozygosity event. Numerical chromosomal abnormalities arise from chromatid mis-segregation events resulting in uneven distribution of chromatids in daughter cells [19]. Structural chromosomal abnormalities arise from clastogenic events. These structural abnormalities include translocations, partial loss of chromatids, gene amplifications, and copy number variations, all common events in carcinogenesis [19].

ATO is an effective chemotherapeutic in combination with all trans retinoic acid (ATRA) for treating acute APL [20–22]. The mechanism of action for ATO in treating APL appears to be by enhancing SUMOylation and subsequent degradation of the PML-RAR fusion protein (result of a translocation) that drives this malignancy [23]. There has been interest in the potential use of ATO in treatment of solid tumors, but clinical trials of ATO as a single agent have failed. However, clinical trials of ATO in combination chemotherapies have not been performed.

Many investigators have used sodium arsenite rather than ATO for in vitro studies. Chemically, these agents are both converted to arsenous acid and exist as arsenous acid $[\text{As}(\text{OH})_3]$ in neutral solution [24]. Indeed, Trisenox®, the pharmacological formulation of ATO used in the clinic to treat APL, is prepared by dissolution of ATO in 30 mM NaOH followed by titration with HCl to near neutral pH (<http://www.trisenox.com/hcp/trisenox-prescribing-information.pdf>). ATO and sodium arsenite induce apoptosis in vitro in many types of cancer cells derived from solid tumors including melanoma [25], lung cancer [26], neuroblastoma [27], ovarian cancer [28, 29], transitional cell carcinoma [30], and prostate cancer [29]. Arsenite has long been known to disrupt mitosis, but normal diploid cells can survive this disruption albeit with an increase in aneuploidy. Normal diploid fibroblasts incubated with 5 μM sodium arsenite exhibited a prolonged mitotic delay from which the cells recovered with an increased incidence of aneuploidy [31]. The toxic effect of arsenic clearly is related to the effects on mitotic progression. Confluent telomerase immortalized diploid human fibroblasts, which retain most characteristics of their non-immortalized parental cells [32], tolerate up to 30 μM NaAsO_2 for at least 6 days with no overt signs of toxicity whereas growing cells are dying by 48 h [33]. Incubation of phytohemagglutinin-stimulated peripheral blood lymphocytes with sodium arsenite in vitro induced mitotic arrest and aneuploidy with arsenite concentrations as low as 100 fM. [34]. Mitotic index and aneuploidy showed concentration response in these lymphocytes from 100 fM. to 10 nM arsenite. Aneuploidy also is induced in telomerase-immortalized diploid human fibroblasts exposed to modest concentrations of arsenite [35]. SV40-transformed human fibroblasts arrest in mitosis and undergo an apoptotic death when exposed to 1–5 μM sodium arsenite [33]. Thus, the induction of aneuploidy is dependent on the ability to escape the arsenite-induced inhibition of mitotic progression.

Cell cycle control mechanisms play an important role in cell survival after arsenic exposure. We showed that SV40-transformed human skin fibroblasts were sensitive to arsenic-induced mitotic arrest and underwent apoptotic death [33]. SV40 transformation results in sequestration of p53 and retinoblastoma (Rb) proteins by the SV40 T-antigen. This sequestration abrogates the ability of p53 and Rb to perform their cell cycle arrest functions. The role of p53 in escaping arsenic-induced mitotic arrest and avoiding the ensuing mitotic death was demonstrated in TR9–7 cells, a human fibroblast cell line with a tetracycline-regulated p53 expression cassette [36]. The induction of cyclin-dependent kinase inhibitor 1A (CDKN1A, *aka* p21^{CIP1/WAF1}) was shown to mediate the p53 effect [37]. Suppression of CDKN1A induction by transfected small interfering RNA (siRNA) directed to CDKN1A messenger RNAs (mRNAs) caused arsenite-treated p53 expressing cells to undergo mitotic death at high levels similar to cells not expressing p53. Thus, consistent with roles for p53 and CDKN1A in escape from arsenic-induced mitotic arrest, these proteins are also necessary for the induction of aneuploidy in telomerase-immortalized diploid human fibroblasts [35]. Arsenic exposure has long been known to induce micronuclei which can contain either whole chromosomes or fragments of chromosomes [38–42]. Micronuclei containing whole chromosomes are demonstrated by in situ hybridization with a centromeric probe and indicate a classic aneuploidogenic event giving rise to chromosomal numerical abnormality. Centromere negative micronuclei indicate a chromosomal fragment from a clastogenic event that gave rise to this chromosomal structural abnormality. Suppression of p53 or CDKN1A by transfection of siRNAs targeting p53 or CDKN1A mRNAs caused a shift from a predominance of centromere positive to negative micronuclei indicating a shift from aneuploidogenesis to clastogenesis [35]. Thus, these proteins that have central roles in mediating cell cycle checkpoint responses are essential to an imperfect escape from mitotic arrest resulting in aneuploidy.

A wide variety of cancer cell lines including melanoma, HeLa, glioma, and lung, ovarian and breast cancer cells undergo an apoptotic death after mitotic arrest induced by ATO or sodium arsenite exposure [43–47]. This apoptotic death follows centrosome fragmentation [48, 49] and can be prevented by induction of the cyclin-dependent kinase inhibitor CDKN1A (*aka* p21^{CIP1/WAF1}) in p53 expressing cells [37]. It is likely that induction of CDKN1A serves to inhibit cyclin-dependent kinase 1 (CDK1), thus releasing the cells from the CDK1-dependent mitotic arrest [25]. This conclusion is supported by the observation that roscovitine, a CDK inhibitor, also releases cells from arsenite-induced mitotic arrest [25]. The centrosome fragmentation can be augmented by heat shock or inhibition of the heat shock system [50, 49]. Thus, the ability to induce mitotic arrest and apoptosis also may play a role in chemotherapy of solid tumors. However, recent studies suggest that arsenicals may have greater potential acting in combination with other chemotherapeutics such as cisplatin [51–59]. Detailed studies in ovarian cancer models suggest that arsenite enhances sensitivity to cisplatin but only in cells expressing p53 [57]. Both in cells in vitro [57] and in xenotransplant tumors in mice [58], arsenite co-treatment prevents the responses of DNA repair systems associated with cisplatin resistance. Induction of nucleotide excision repair genes and suppression of mismatch repair genes by cisplatin are inhibited by the arsenite co-treatment. Further investigation of effects on cell cycle revealed that arsenite co-treatment resulted in abrogation of the G2 checkpoint normally activated by cisplatin; the cells then

passed mitosis without dividing and entered into a pseudo-G1 state where they underwent apoptosis [59]. These results point to a complex effect of arsenite on mitotic progression that is not yet completely understood.

How arsenic, which inhibits mitosis, can induce cancer which is a disease of uncontrolled cell replication appears paradoxical on its surface. The mechanisms of arsenic disruption of mitosis likely play a role both in carcinogenesis and in induction of mitotic death. The *in vitro* studies discussed above implicate p53 and CDKN1A in escape from arsenic-induced mitotic arrest and subsequent aneuploidy. Failure to escape the mitotic arrest can lead to mitotic death, also referred to as mitotic catastrophe in earlier publications. This induction of mitotic death, consequent to failed cytokinesis, is a major mechanism of apoptotic death induced by arsenicals in many types of cancer cells. Thus, we believe that the response to arsenical-induced mitotic arrest is the common link between arsenical-induced carcinogenesis in diploid cells and arsenical-induced apoptosis in cancer cells (Fig. 1).

The mitotic catastrophe we observed in arsenite-treated cells included degradation of the chromosomes. The appearance of these catastrophic chromosome spreads was very similar to the chromosome fragmentation observed by others in cancer cells treated with a variety of stressors [60–62]. Chromosome fragmentation can lead to genome chaos [63], a severe form of aneuploidy, and contribute to carcinogenesis. Arsenite clearly induces cellular stress and can induce centrosome fragmentation [49] which can contribute to chromosome fragmentation [60]. However, in our studies, the end result of the mitotic disruption was cell death with activated caspases [44, 25, 37] clearly indicating that an apoptotic death pathway is activated. Indeed, the catastrophic mitotic figures are absent, and the morphologically distinct mitotic figures are more abundant when caspases are inhibited [25, 37]. Thus, at least at concentrations relevant to those achieved in chemotherapy, it is unlikely that the arsenite-induced mitotic death will contribute to genome chaos. The impact of low, environmental concentrations remains to be investigated.

Progression through mitosis is a complicated process and involves four morphologically distinct phases. Cell cycle transitions are governed by CDKs. Most transitions require induction of cyclin expression, but progression from mitosis (M phase) to G1 phase is different. Loss of cyclin B is required for this transition. Cyclin B is the cognate cyclin for CDK1, the master kinase governing entry into and progression through the early stages of mitosis. Thus, normal expression of cyclin B in G2 and early M activates CDK1 which is essential for progression through G2 and prophase to meta-phase. Cells are held at the metaphase/anaphase junction by the spindle assembly checkpoint until all chromosomes are attached to the spindle. A functional spindle assembly checkpoint is necessary for arsenite-induced mitotic arrest [25]. In order to activate the mitotic exit process and to progress through anaphase to telophase, cyclin B normally is ubiquitinated by the anaphase promoting complex/cyclosome (APC/C) and degraded by the proteasome to inactivate CDK1 (Fig. 2) [64]. Cyclin B is stabilized in arsenite-exposed cells [37, 25] suggesting inhibition of APC/C. Inhibition of CDK1 by CDKN1A [37] or roscovitine [25] allows mitotic exit in arsenite-arrested cells indicating that persistent CDK1 activity is preventing mitotic exit. The p53 induction of CDKN1A in arsenite-exposed cells, as discussed above, appears to be a mechanism for overcoming this phase of the mitotic block in diploid cells.

Anaphase is the period in which the chromatids separate and move toward the spindle poles. The chromatids are held together in the chromosomes by a protein complex of cohesins. In order to separate the chromatids, the cohesins must be cleaved by the protease separase. Separase is held in an inactive state by binding of securin until mitotic exit is activated [65]. Then, securin is ubiquitinated by the APC/C and degraded by the proteasome, thus releasing separase to cleave the cohesins in the metaphase to anaphase transition (Fig. 2) [64]. Securin also is stabilized by arsenite [25], also consistent with inhibition of the APC/C. Until recently, it was not clear how cells might overcome the stabilization of securin. MicroRNA expression was compared in biopsies of hyperkeratoses and squamous cell carcinomas obtained from West Bengali patients exposed to high levels of arsenic in their drinking water. The hyperkeratoses are premalignant lesions that give rise to both basal and squamous cell carcinomas and are pathognomonic for arsenicosis. Total RNA was purified from keratinocytes laser captured microdissected from the skin lesions, and microRNA expression was profiled on qRT-PCR arrays. The microRNA most elevated in squamous cell carcinoma relative to hyperkeratosis is hsa-miR-186 (unpublished data). Securin (*aka* PTTG1) is a validated target of this microRNA [66]. Thus, we propose that elevated expression of hsa-miR-186 in keratinocytes chronically exposed to arsenic provides the suppression of securin needed to overcome the arsenic exposure-induced mitotic disruption and to progress to a malignant state. In addition, overexpression of hsa-miR-186 would likely induce a state of chromosomal instability due to premature separation of chromatids further contributing to carcinogenesis. We are currently testing this hypothesis.

Summary

The studies discussed clearly indicate that arsenite at physiological levels (10 fM–10 nM) and therapeutic levels (1–10 μ M) disrupt normal mitotic progression. The consequences of this disruption depend upon the ability of the cell to regulate cell cycle checkpoints. Clearly, cyclin B and securin are stabilized by arsenite. The cyclin B stabilization results in persistent CDK1 activity which prevents mitotic exit. Cells with an intact p53/CDKN1A axis induce CDKN1A which, in turn, inhibits CDK1, thus activating mitotic exit. However, securin also is stabilized in arsenite-exposed cells. Recent preliminary results suggest that miR-186 is induced by chronic arsenic exposure and this microRNA suppresses securin expression. This suppression would lead not only to facilitated progression from metaphase through anaphase but also to chromosomal instability. The stabilization of cyclin B and securin suggests that arsenite is inhibiting the APC/C. Inhibition of the APC/C combined with microRNA suppression of securin is a likely mode of action for arsenic carcinogenesis by induction of aneuploidy. In cells lacking adequate cell cycle checkpoint control, such as most cancer cells, arsenite induces mitotic arrest leading to centrosome fragmentation and mitotic death. In cells co-treated with cytotoxic drugs like cisplatin, the mitotic disruption causes entry into a pseudo-G1 state where the cells undergo apoptosis. These findings suggest that ATO has potential use in combination chemotherapy for cancer.

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Fig. 1.

Cell checkpoint control mechanisms mediate the carcinogenic (aneuploidogenic) vs chemotherapeutic (apoptotic) response to arsenic exposure. Lack of p53 is associated with failed cytokinesis and induction of mitotic death. Functional p53/CDKN1A (p21) axis relieves mitotic block, and miR-186 overexpression allows anaphase progression and contributes to aneuploidy by promoting premature chromatid separation

Fig. 2.

Arsenic impact on mitotic progression. Progression from prophase through telophase to aneuploid progeny is diagrammed across the middle of the figure. Key events in the transitions are driven by cyclin B/CDK1 activity (prophase to metaphase progression) or inactivation (mitotic exit: metaphase to anaphase transition) and by separase activation and cleavage of cohesins to allow chromatid separation in anaphase. Cyclin B and securin are ubiquitinated by the anaphase promoting complex/cyclosome (*APC/C*) and degraded by the proteasome. Cyclin B and securin are stabilized by arsenic (*As*) exposure. Arsenic induces p53 expression which, in turn, induces CKDN1A that then inhibits CDK1 activity and allows mitotic exit. miR-186 is overexpressed in arsenic-induced squamous cell carcinoma and is known to suppress securin expression. It is hypothesized that the lower levels of securin would allow separase activation in the presence of arsenic and also would contribute to aneuploidy by inducing premature chromatid separation.