Chapter 5
Regulatory Effects of Arsenic on Cellular Signaling Pathways: Biological Effects and Therapeutic Implications

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Abstract Arsenic compounds exert important biological effects and arsenic trioxide has been approved by the Food and Drug Administration (FDA) for the treatment of patients with acute promyelocytic leukemia (APL). Much of arsenic's actions in cells reflect its ability to bind thiol groups in cellular proteins or to affect the production of reactive oxygen species (ROS), leading to the engagement and regulation of several cellular signaling pathways. Arsenic has been also shown to degrade abnormal fusion proteins found in myeloid leukemias. It has also been shown to effect NFκB, MAPK, mTOR and Hedgehog pathways which can modulate the viability of cancer cells. Many clinical trials have been performed to examine the clinical efficacy of arsenic trioxide alone or in combination with other agents in the treatment of various hematological malignancies. The continuous advances in basic and translational research and the better understanding of the mechanisms of action of arsenic should lead to more effective combinations with other agents that could result in better clinical outcomes.

Keywords Arsenic • Leukemia • Cancer • Cell signaling

5.1 Clinical Uses of Arsenic Trioxide

Arsenic has been used empirically for centuries, for the treatment of countless diseases, including syphilis, cancer, malaria, and ulcers [1]. It was first described as a drug to treat leukemia in 1878 [2]. In the modern medical era, one of the compounds of arsenic, arsenic trioxide, has shown significant clinical activity in certain malignant diseases, as discussed below.

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5.1.1 Acute Promyelocytic Leukemia (APL)

Over the last two decades there has been extensive evidence accumulated indicating that arsenic trioxide (ATO) has major clinical activity in the treatment of one form of acute myeloid leukemia (AML), acute promyelocytic leukemia (APL) and ATO was approved for the treatment of relapsed APL by the Food and Drug Administration (FDA) of the United States in 2001 [3]. This relatively rare variant of AML is associated with the reciprocal chromosomal translocation t(15;17) that brings together the promyelocytic leukemia (PML) gene on chromosome 15 and the retinoic acid receptor (RAR)α gene on chromosome 17 [4]. The resultant chimeric protein (PML–RARα) causes a maturation block of myeloid cells at the promyelocytic stage, resulting in the accumulation of abnormal promyelocytes in the bone marrow [4]. Historically, APL has been associated with a severe bleeding dysfunction associated with disseminated intravascular coagulation (DIC) and a fatal course of only weeks [5]. With the implementation of chemotherapy, a complete remission (CR) rate of 75–80 % in newly diagnosed patients was achieved, however the median duration of remission ranged from 11 to 25 months, with only 35–45 % of the patients being cured [4]. The introduction of a regimen consistent of *all-trans* retinoic acid (ATRA), which targets the RAR moiety of the fusion transcript, together with anthracycline-based chemotherapy dramatically raised the remission rate up to 90–95 % and the 5-year disease free survival (DFS) to 74 % [6]. Since the early 1990s, ATO was introduced for the treatment of relapsed APL, and has shown major clinical activity [7]. Since ATO is less toxic than chemotherapy, its role as a single agent in newly diagnosed patients is currently being researched with the aim to minimize the use of cytotoxic chemotherapy in this condition, especially for those with a compromised cardiac function and/or for older patients [8, 9].

5.1.2 Clinical Trials of ATO in Multiple Myeloma

In vitro studies have shown that ATO induces apoptosis in myeloma cells [10–13], therefore investigators have evaluated its potential in the treatment of refractory and relapsed multiple myeloma (MM) [14]. Some clinical activity was seen in a phase II study performed in 14 patients with refractory or relapsed MM [15]. In another trial using a higher but not as frequent dose of ATO, reduction of M-protein in serum of more than 25 % was obtained in eight patients (33 %), while six patients had stable disease, with a median duration response time of 130 days [16]. Investigators have also developed combination studies using ATO together other agents previously known to be useful for the treatment of this condition. Berenson et al. administered a combination of melphalan, ATO and ascorbic acid to 65 patients with MM who had failed more than two previous regimens [17]. This combination (also known as MAC regimen) produced objective responses in 31 patients (48 %), ranging from CR in two patients to minor responses in 14 of them [17]. More recently, the combination of MAC regimen plus bortezomib was evaluated in a different randomized trial and was found to be safe and well tolerated by patients [18]. Other combination

regimens including ATO have also demonstrated efficacy in patients with relapsed or refractory MM [19].

5.1.3 Myelodysplastic Syndromes

There has been also evidence for some clinical activity of ATO in the treatment of myelodysplastic syndromes (MDS). Hematologic improvement was obtained in MDS with the use of single agent ATO in two different trials [20, 21]. In other studies, thalidomide was used in combination with ATO in 28 patients with transfusion dependent MDS, accomplishing a response in 25 % of them, including one CR and responses in three of five patients with high baseline levels of EVI1, which is a known poor prognostic marker [22]. More recently, the combination of thalidomide, ATO, dexamethasone, and ascorbic acid (TADA regimen) was used in patients with myelodysplastic/myeloproliferative neoplasms (MDS/MPN) or primary myelofibrosis (PMF), achieving a response in 29 % of patients [23].

5.2 Effects of Arsenic on Cellular Signaling Pathways in Malignant Cells

5.2.1 Arsenic Compounds

Arsenic is found is two different oxidative states, As (III) or trivalent arsenic and As(V) or pentavalent arsenic. Pentavalent arsenic can substitute for phosphate and cause hydrolysis of compounds such as ATP [24]. Trivalent arsenic can bind to thiol groups in the cysteines of proteins in cells and alter their structure resulting in the modulation of protein stability, folding, and function, thus affecting cellular signaling pathways [24–26]. For instance, arsenic can bind to tubulin and other cytoskeletal proteins and affect polymerization and mitosis [27–30]. Arsenic can also affect signaling pathways through the production of reactive oxygen species (ROS) and there is evidence that it increases ROS in cells in two ways. First, arsenic can inhibit the activity of enzymes, such as thioredoxin reductase by its ability to bind via cysteine groups, which are involved in regulating the cellular redox state [31]. Second, methylation of arsenic during its cellular metabolism also leads to the production of ROS [32, 33].

5.2.2 Effects on Fusion Proteins in Leukemia

Arsenic trioxide has been shown to cause the degradation of multiple fusion proteins found in leukemia by various mechanisms. ATO's proposed mechanism of action in acute promeylocytic leukemia is via degradation of the PML-RAR fusion

protein [34]. In APL, the fusion protein alters the localization of PML from nuclear bodies, which contributes to aberrant cell growth [35, 36]. Arsenic trioxide targets both PML and PML-RAR to nuclear bodies in APL cells and leads to its subsequent degradation [37]. Targeting PML protein expression with arsenic has also been shown in quiescent leukemia initiating stem cells in CML [38]. A recent publication demonstrated that arsenic specifically binds to the PML zinc finger domain at cysteine residues displacing the zinc and causing a shift in secondary structure as well as aggregation that leads to increased sumolyation and degradation [39, 40]. Another recent publication showed that autophagy induction by ATO and ATRA also contributes to the degradation of the PML-RAR fusion protein [41].

Besides APL, arsenic has shown cytotoxicity in chronic myleogenous leukemia (CML), as well. It is of particular interest that historically, arsenic was used to treat CML in the nineteenth and twentieth centuries [1]. Imatinib combined with arsenic sulfide showed enhanced anti-leukemic effects over either agent alone in a mouse model of CML [42]. Recent evidence has shown that arsenic is cytotoxic in Ph + leukemia cells by degradation of the BCR-ABL fusion protein by the autophagic machinery, where p62 binds to BCR-ABL in the autophagosome [43]. Arsenic trioxide has been also shown to degrade another fusion protein, AML1/MDS1/EVI1, via targeting of the MDS1/EVI1 portion of the fusion protein [44]. The EVI1 portion contains two zinc finger DNA binding domains therefore similar to PML, arsenic could be binding to the cysteine residues in zinc finger domains in EVI1 and lead to the degradation of the fusion protein [40, 44].

5.2.3 mTOR Pathway

Arsenic has been shown to activate the mTOR pathway although the precise mechanism of such engagement is unknown (Fig. 5.1) [45]. Treatment with rapamycin or the dual PI3K/mTOR inhibitor, PI-103, was shown to enhance the antileukemic effects of arsenic, indicating that activation of mTOR occurs in a negative feedback manner in order to suppress the cytotoxic effects of arsenic [45, 46]. Therefore combining arsenic with mTOR pathway inhibitors could conceivably enhance its antileukemic effects in vivo and this needs to be examined in future work.

5.2.4 MAPK Pathways

Arsenic has been shown to affect the various MAPK pathways such as p38 MAPK, JNK and ERK. JNK activation has been shown to be important for the anti-leukemic effects of arsenic (Fig. 5.1) [47–49]. ATO-resistant APL cell lines showed little activation of JNK due to upregulation of glutathione (GSH) [47]. Treating cells with compounds that deplete GSH in cells enhance ATO's cytotoxic effects [47, 50]. Increased GSH levels in leukemia cells has been correlated with a decrease in

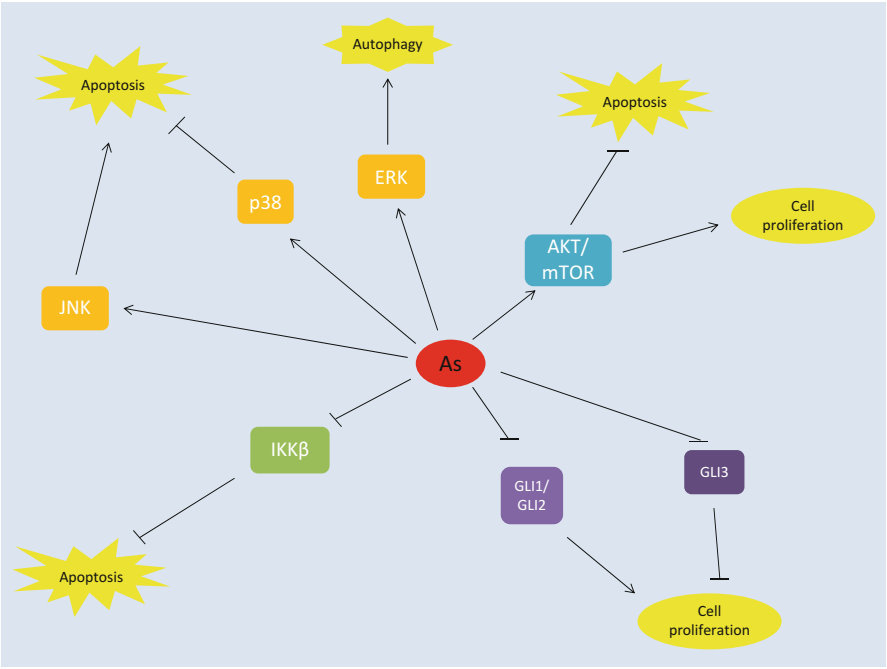

Fig. 5.1 Arsenic's positive and negative effects on cell viability and proliferation. Arsenic can affect MAPK pathways, by activating the MEK/ERK branch leading to the induction of autophagy. At the same time it can either activate p38 or JNK leading to the inhibition, or induction of apoptosis. Additionally, arsenic can activate the PI3K/mTOR pathway by activation of AKT signaling or mTOR signaling which leads to the inhibition of apoptosis and increase in cellular proliferation. Arsenic can inhibit GLI1 and GLI2 which leads to an inhibition of cellular proliferation. Arsenic's inhibition of GLI3, however, can lead to activation of cellular proliferation in some cellular contexts

sensitivity to arsenic, which could affect sensitivity by either GSH decreasing the amount of ROS in cells directly, or binding arsenic leading to its metabolism and subsequent excretion [51–53]. Ascorbic acid has been shown to synergize with arsenic in multiple myeloma and myeloid leukemia cells by decreasing GSH levels and increasing ROS levels [52, 54, 55]. In chronic lymphocytic leukemia (CLL), JNK activation was an early event leading to the upregulation of PTEN, which results in PI3K, AKT, NFκB inhibition, and an increase in ROS production [56]. In addition, combining arsenic with PI3K inhibitors was shown to enhance arsenic's cytotoxic effects on CLL cells [56].

Other studies have shown that arsenic modulates ERK activity. The induction of autophagy by arsenic trioxide was shown to be important for its antileukemic properties and the ERK pathway is required for induction of the autophagic state in this context [57]. ATO-dependent ERK2-mediated phosphorylation of PML has also been shown to lead to increased sumoylation/degradation of the PML protein and ultimately resulting in induction of apoptosis [58].

Arsenic trioxide also activates p38 MAPK in several leukemia cell types [59]. However, inhibition of p38 MAPK or its downstream effectors MNK or MSK1 attenuated the cytotoxic effects of ATO and/or increased JNK activation in leukemia cells [60–62]. This indicates that p38 MAPK is activated as a negative feedback loop in leukemia cells, which limits arsenic's cytotoxicity. Co-treatment of breast cancer or leukemia cells with ATO and MEK inhibitors leads to a greater induction of apoptosis, suggesting a possible therapeutic approach to enhance arsenic's cytotoxic effects [63, 64].

5.2.5 Effects on the NFKB Pathway

The canonical NFκB pathway has been shown to be inhibited by arsenic. When the canonical NFκB pathway is not active, the negative regulator IκB binds to the NFκB dimer and prevents it from translocating to the nucleus [65]. Activation of this pathway in response to TNFα or other stimuli leads to activation of the IKK complex [65]. IKK phosphorylates IκB leading to its degradation, which allows NFκB to translocate to the nucleus and activate pro-tumorigenic genes that help lead to the evasion of apoptosis [66]. In multiple myeloma cells, arsenic trioxide was shown to prevent NFκB activation by TNFα [10]. Arsenic can directly bind to IKKβ at cysteine residue 179 in the activation loop of the catalytic subunit of IKKβ and inhibit its activity, to engage the NFκB canonical signaling (Fig. 5.1) [67]. Since IKKβ can have effects independently of NFκB such as by regulating MAPK and mTOR pathways [66], the inhibition of IKKβ by arsenic can also conceivably effect those pathways in addition to NFκB.

5.2.6 Hedgehog Pathway

Recent work has shown that arsenic can inhibit the hedgehog pathway by inhibiting GLI1/2 (Fig. 5.1) [68, 69]. Such inhibition was shown to be at the level of GLI1/2 because ATO was found to inhibit hedgehog signaling when GLI1/2 was overexpressed or in $SUFU^{-/-}$ MEFs, in contrast to upstream pathway inhibitors that cannot inhibit Hh signaling in this context [68, 69]. Notably, some tumors activate the pathway by overexpression of ligand, patched inactivation or mutations that activate Smoothened [70–76]. Other cancers, however, can activate the pathway at the level of GLI, independent of Smoothened or Patched, either by mutations in negative regulators SUFU or REN, chromosomal amplification of GLI, chromosomal translocations that involve GLI, an increase in GLI protein stability or activation via non-canonical mechanisms involving other pathways [77–89]. Arsenic is able to inhibit the growth of both upstream activated medulloblastoma cancer cell lines as well as Ewing sarcoma cells lines which have activation of GLI1 independently of SMO [68].

The exact mechanisms by which arsenic inhibits GLI1/2 still need further investigation. Since one of the studies demonstrated that arsenic can directly bind to GLI1 [68] and given prior evidence of arsenic's ability to bind to cysteines in the zinc finger domain of PML, it is highly plausible that ATO binds to the zinc finger domains in GLI1. However, this remains to be directly addressed in future studies and the overall mechanisms by which arsenic affects GLI function necessitates further investigation.

Another study showed that arsenic activates Hedgehog signaling [90]. The authors of that study found that arsenic activated GLI1/Hedgehog signaling in these cells by inhibiting the GLI3 repressor. However, in this study sodium arsenite was used, whereas the other two studies used arsenic trioxide. It is possible that sodium arsenite has preferential binding to GLI3 over GLI1 and GLI2 and thus activates signaling instead of repressing it. Notably, sodium arsenite has been previously shown to have opposing effects to the ones of arsenic trioxide in other cancer models. For instance, arsenic trioxide promotes apoptosis in breast cancer cell lines [91, 92], while sodium arsenite binds to the estrogen receptor-α (ER-α) and increases the proliferation of MCF-7 cells [93]. Arsenic trioxide and sodium arsenite have been also shown to exhibit differential effects when combined with radiation [94].

The precise mechanisms of how arsenic induces autophagy are not known, other than the requirement for MEK/ERK signaling [54]. Recent evidence suggests that the hedgehog pathway antagonizes autophagy through inhibition of autophagosome synthesis most likely through repression of genes required for autophagy [95]. Thus, the inhibition of the hedgehog pathway by arsenic could mechanistically contribute to its ability to induce autophagy and this hypothesis remains to be examined in future studies.

5.2.7 Effects on Nuclear Receptor Pathways

Arsenic has been shown to alter multiple nuclear receptor pathways. Notably, it has been shown to directly bind and inhibit the glucocorticoid receptor [96]. Nuclear receptor function has been shown to be inhibited by arsenic trioxide though JNK activation and phosphorylation of the retinoid X receptor (RXR) [97]. Arsenic's effects on the estrogen receptor are controversial as multiple groups have shown differential effects. As mentioned previously, sodium arsenite can bind to the ligand pocket of ER-α and activate it, leading to proliferation of MCF-7 cells [93]. Arsenic trioxide was shown to lead to a decrease in expression of ER-α in ER-positive breast cancer cell lines, resulting in suppression of cellular proliferation [98, 99]. More recently arsenic trioxide treatment was found to result in increased expression of ER-α in ER-negative breast cancer cells by promoting demethylation of the promoter, leading to re-sensitization to endocrine therapy [100, 101]. The differences in effects may be due to differences in cell contexts (ER-positive vs. ER-negative cells) as well as mentioned previously the differential effects of sodium arsenite and arsenic trioxide.

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