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PI3K/Akt/mTOR Signaling Pathway and the Biphasic effect of Arsenic in Carcinogenesis

Qiao Yi Chen and Max Costa

Department of Environmental Medicine, New York University School of Medicine,

Tuxedo NY, USA

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Running title: Arsenic carcinogenesis: PI3K/Akt/mTOR Signaling Pathway

Corresponding Author:

Professor Max Costa

Department of Environmental Medicine

New York University School of Medicine

57 Old Forge, Tuxedo NY, 10987

Telephone: 845-731-3515

Fax: 845-351-2118

Email: Max.Costa@nyumc.org

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Abbreviations:

ACAP1	Arf-GAP with coiled-coil
AKT	Protein kinase B
APL	Acute promeylocytic leukemia
AP-1	Activator protein 1
ATO	Arsenic trioxide
As2S2	Arsenic disulfide

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BAX	Bcl-2-associated X protein
B-CLL	B-cell chronic lymphocytic leukemia
BEAS-2B	Human bronchial epithelial cells
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
FOXO	Forkhead box O
HBEC	Human bronchial epithelial cells
HCS-2/8	Human chondrocyte cell
HIF-1	Hypoxia-inducible factor 1
HIF-1a	Hypoxia-inducible factor 1-alpha
HOS	Human osteosarcoma cell
HT-29	Human colorectal adenocarcinoma cell
HUC1	Normal human bladder cells
H3K9ac	Histone H3 Lysine K9 acetylation
H4K16ac	Histone H4 Lysine K16 acetylation
IARC	International Agency for Research on Cancer
IGF-1	Insulin-like growth factor-1
IKK	IkB kinase
IL-6	Interleukin 6
JB6Cl41	Mouse epidermis-derived cells
JNK	c-Jun N-terminal kinase
MG-63	Human osteosarcoma cell
mTOR	Mammalian target of rapamycin
mTORC1	mTOR complex 1
NFkB	Nuclear factor kappa-light-chain-enhancer of activated B cells
OUMS-27	Human chondrosarcoma cell
Pak1	P21/Cdc42/Rac1-Activated Kinase 1
PDK1	Phosphoinositide-dependent kinase
PHLPP	Pleckstrin homology domain leucine-rich repeat protein phosphatase
PIP2	Phosphatidylinositol-3,4,5-triphosphate
PIP3	Phosphatidylinositol-4,5-bisphosphate

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Abstract

Arsenic is a naturally occurring ubiquitous metalloid found in the Earth's crust. In its inorganic form, Arsenic is highly toxic and carcinogenic, and is widely found across the globe and through out the environment. As an International Agency for Research on Cancer (IARC) defined Class I human carcinogen, arsenic has been found to cause multiple human cancers including liver, lung, urinary bladder, skin, kidney, and prostate. Mechanisms of arsenic-induced carcinogenesis remain elusive and this review specifically focuses on the role of PI3K/AKT/mTOR pathway in promoting cancer development. In addition to exerting potent carcinogenic responses, arsenic is also known for its therapeutic effects against acute promyelocytic leukemia. Current literature suggests that arsenic is capable of achieving both therapeutic as well as carcinogenic effects, and this review serves to examine the paradoxical effects of arsenic, specifically through the PI3K/AKT/mTOR pathway. Furthermore, A comprehensive review of current literature reveals an imperative need for future studies to establish and pinpoint the exact conditions for which arsenic can, and through what mechanisms it is able to differentially regulate the PI3K/AKT/mTOR pathway in order to maximize the therapeutic and minimize the carcinogenic properties of arsenic.

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Introduction

Arsenic is a naturally occurring ubiquitous metalloid found in the Earth's crust. In its inorganic form, Arsenic is highly toxic and carcinogenic, and is widely found across the globe and through out the environment. Along with natural corrosion of rocks and minerals, anthropogenic sources of contamination such as burning of fossil fuels, mining, and application of arsenical pesticides further aggravate arsenic-elicited public health issues, which first gained recognition beginning in the 1990s. Today nearly 200 million people are exposed to arsenic at levels above the World Health Organization's recommended limit of 10 parts per billion (ppb). Startling levels of arsenic have been found around various regions around the world, including the French Mediterranean costal areas, Bangladesh, China, United States, Mexico, etc. (Chen & Costa 2017). In the United States, excessive amounts of arsenic in drinking water have been found in the New England, Western, and Midwestern regions (Carpenter and Jiang 2013). In fact, more than 70 countries documenting arsenic contamination reported levels up to 5000 ppb (Shankar et al. 2014). Arsenic is known to pollute the water system, but the route of exposure is not limited to drinking water. Adding to the extensity of arsenic's prevalence, toxic dust of this metalloid can be found in common occupational settings such as copper smelters and areas neighboring the smelter (Axelson et al. 1978; Enterline et al. 1987; Carpenter and Jiang 2013).

High concentrations of arsenic can elicit rapid toxic effects resulting in death. As a matter of fact, arsenic is infamously known as the "Poison of the Kings." On the other hand, non-lethal doses of chronic arsenic exposure can elicit potent carcinogenic effects. As an International Agency for Research on Cancer (IARC) defined Class I human

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carcinogen, arsenic has been found to cause multiple human cancers including liver, lung, urinary bladder, skin, kidney, and prostate (Naujokas et al., 2013). Chen et al. 2011 reported that at levels between 10-300 ppb, serious conditions such as cardiovascular and neurological diseases, skin lesions, hepatic and renal dysfunctions have been reported. An estimated 100 million people are chronically exposed to more than 50 ppb of arsenic via drinking water (Moon et al. 2012). And in regards to the wide-range health effects of arsenic exposure, tremendous amounts of scientific efforts have been invested in elucidating the mechanisms of arsenic-induced toxicity and carcinogenicity. While current studies suggest the mechanisms are manifold, this review will specifically focus on the role of the PI3K/AKT/mTOR, a prominent cellular signaling pathway, in arsenic-induced carcinogenesis.

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Potential Mechanisms of Arsenic-induced Carcinogenicity

The processes of cancer initiation, promotion, and progression are overwhelmingly complex, and arsenic-induced carcinogenesis may occur through multiple mechanisms. Current evidence suggests that arsenic works through both genotoxic and cytotoxic, as well as epigenetics pathways, which further complicates the quest to mitigate arsenic-associated diseases and cancers. Research suggests that reactive oxygen species (ROS) generated through arsenic metabolism can contribute to cancer initiation and promotion (Barchowsky et al. 1996; Liu and Jan,2000; Lynn et al. 2000; Huang et al. 2004). Reactive oxygen species are effective at modifying the DNA by inducing base-pair mutations, insertions, deletions, etc. (Rossman et al. 1980; Huang et al. 2004). DNA damage of essential tumor suppressor genes, can give rise to carcinogenesis. ROS have also been suggested to disturb important cytoplasmic and nuclear signal transduction pathways, both of which are vital for controlling gene expression (Sen and Packer 1996; Lander 1997; Li et al. 1998; Huang et al. 2004).

Cellular response to activation or inhibition of specific gene transcription are stimulated through extracellular signals which are transmitted through the cell plasma membrane, passed along a chain of intracellular signaling molecules to regulatory transcription factors, which consist of proteins that recognize specific DNA sequences and initiate transcription (Huang et al. 2004). Note, that basal transcription as well as endogenous stressor-induced transcription can also occur. In the case of malignant cell transformation, a variety of signaling pathways and transcription factors such as AP-1, NFκB, regulate the expression of genes that carry out cell proliferation, differentiation, and transformation (Huang et al., 1999; Huang et al., 2004; Wang et al. 1996;). Current

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evidence also suggests that arsenic can exert its carcinogenic effects through disrupting important signal transduction pathways (Huang et al. 1999; Huang et al. 2004; Snow, 1992). One prominent example is the PI3K/AKT/mTOR pathway. Due to the many important roles of PI3K, AKT, and mTOR in cell survival, cellular physiology, and pathologic alterations; perturbations of this pathway have been shown to elicit cancers of the breast, colon, neck, ovary, and lung (Levine 2007; Bartholomeusz and Gonzalez-Angulo 2012; Kandoth et al. 2013; Li and Wang 2014; Guimaraes et al. 2015; Tai et al 2017;). Alterations in the PI3K/AKT/mTOR pathway can be caused by various factors including genetic mutations, Hepatitis C virus, chemical toxicants such as heavy metals, and physiological products including free fatty acids and interleukin (IL)-6 (Li and Wang 2014). Chemical toxicants such as arsenic, mercury, cadmium, vanadate, and nicotine have been shown to induce malignant cell transformation through the PI3K/AKT/mTOR pathway (Gao et al. 2002; West et al. 2003; Gao et al. 2004; Wang et al. 2009; Jing et al. 2012; Rauch et al. 2012; Li and Wang 2014; Roy et al. 2014). Multiple studies demonstrate that this signaling cascade is often found disrupted after chronic arsenic exposure (Jiang and Liu et al., 2009; Carpenter et al. 2011; Carpenter and Jiang 2013). This review provides a comprehensive perspective on arsenic-induced carcinogenesis focusing on the PI3K/AKT/mTOR pathway.

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Phosphoinositide 3-kinase

Phosphoinositide 3-kinase (PI3K) activity can be activated by various growth factor receptors and oncogenes. In fact, elevated PI3K signaling is regarded as a distinct hallmark of cancer (Fruman et al., 2017). PI3K family members consist of heterodymeric enzymes, and are divided based on structure, function, and substrate specificity, into three major classes. In almost all tissue types studied to date, PI3K signaling has been found to have important implications in a variety of physiological processes. And notably, PI3K family members are also involved in an extensive range of cellular regulatory processes such as cell proliferation, migration, and metabolism (Fruman et al., 2017). Consequently, alterations in the PI3K signaling pathway can lead to a variety of human diseases including cardiovascular, diabetes, as well as neurological and immunological disorders (Fruman et al., 2017). Furthermore, as demonstrated using cancer genomic analyses, PI3K gene mutations are frequently found in human tumors (Samuels et al., 2004).

Class I PI3Ks have the unique ability to catalyze the phosphorylation of phosphatidylinositol-4,5-bisphosphate (PIP2) into phosphatidylinositol-3,4,5-triphosphate (PIP3), a secondary messenger and mediator of PI3K activity, specific for the recruitment of cytoplasmic proteins to the endo- or plasma membranes (Czech, 2000; Rameh and Cantley, 1999). Studies suggest that PIP3 is critical to PI3K related oncogenicity, elevated levels of PIP3 are frequently found in cancer cells, thus class I, rather than II and III, are specifically linked to cancer development (Fruman et al., 2017; Zhao and Vogt 2008). Within Class I, there are four catalytic isoforms: p110a, b, g, and d, encoded by PIK3CA, PIK3CB, PIK3CG, and PIK3CD, respectively. In general, Class I PI3Ks are

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stimulated by various upstream activators such as receptor-coupled tyrosine kinase (RTK), heterotrimeric G proteins, and small Ras-related GTPases (Fruman et al., 2017). In the event of RTK activation, two PI3K subunits, PI3KR1 (PI3K p85) and PI3KCA (PI3K p110) are recruited to form an active PI3K complex, which will then proceed to phosphorylate PIP2 to form PIP3 (Manning and Cantley, 2007). PI3K signaling is negatively regulated by lipid phosphatases such as tumor suppressor PTEN, which can rapidly remove the 30-phosphate on PIP3 and terminate PI3K signaling. Many human tumors are associated with loss of PTEN function as well as elevated levels of PIP3, which are now two of the most frequently altered gene functions in human cancers (Fruman et al., 2017; Lawrence et al., 2014).

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Protein Kinase B (AKT)

Members of the Protein Kinase B (AKT), serine/threonine kinase family, exist in three main isoforms: Akt1, Akt2, and Akt3, and are common downstream effectors of PI3K signaling pathway (Fresno et al., 2004). AKT is a master regulator of tumor cell invasion, migration, and metastasis, capable of phosphorylating a number of regulatory proteins such as ACAP1, POSH, Pak1, Girdin, etc. (Manning and Cantley 2007; Jiang and Liu 2009; Dillon and Muller 2010; Xue et al. 2012; Li et al. 2015). As a proto-oncoprotein, AKT can inhibit apoptosis by binding to Bcl-2-associated X protein (BAX) and hinder its ability to form openings in the mitochondrial outer membrane. Evidence reveals that in prostate cancer cells, activation of AKT corresponds to increased resistance to apoptosis (Patrucco et al. 2004; Guimaraes et al. 2015). Upon PIP3 formation, AKT and its upstream activating kinase, phosphoinositide-dependent kinase-1 (PDK-1), will translocate from the cytoplasm to the plasma membrane. The constitutively active PDK-1 will then induce the phosphorylation of AKT kinase domain at Thr308 and Ser473 in the carboxyl-terminal position to initiate the complete activation of AKT (Carpenter and Jiang 2013). AKT is capable of phosphorylating various substrates associated with cell metabolism, proliferation, survival, and motility (Liu et al., 2014). For example, AKT activity can positively regulate cell survival through activation of IkB kinase (IKK), a regulator of NfkB (Guimaraes et al. 2015). Mutations in its PH domain have been frequently found in cancers, which further supports the notion that AKT is an important effector for PI3K-associated oncogenic signaling (Fruman et al., 2017; Manning and Toker, 2017). AKT activation is also reversible as protein phosphatase 2A (PP2A) and pleckstrin homology domain leucine-rich repeat protein phosphatase (PHLPP)

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are capable of dephosphorylating pAKT and convert it back to inactive AKT (Wang 2013; Li and Wang 2014).

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Mechanistic Target of Rapamycin (mTOR)

As an essential protein highly conserved through evolution, mechanistic target of rapamycin (mTOR) is known to regulate downstream signaling cascades by integrating both intra- and extra-cellular signals (Meng et al., 2018). Functions of mTOR are carried out by two cellular complexes, mTORC1 and mTORC2, each with its own distinct subunit composition and substrate selectivity (Saxton and Sabatini, 2017). mTORC1 consists of five components: mTOR, Raptor, mLST8, PRAS40, and DEPTOR (Hara et al., 2002; Meng et al., 2018; Kim et al., 2002; Kim et al., 2003; Peterson et al., 2009; Sancak et al., 2007; Vander Haar et al., 2007; Wang et al., 2007;). mTORC2 is composed of six components; mTOR, Rctor, mLST8,DEPTOR, mSin1, and Proctor 1/2 (Gaullier et al. 1998; Dou et al. 2013; Guimaraes et al. 2015; Meng et al., 2018; Jacinto et al., 2004; Sarbassoy et al., 2004; Kim et al., 2003; Peterson et al., 2009; Jacinto et al., 2006; Schroder et al., 2007; Yang et al., 2006; Pearce et al., 2007). As a key signaling node, mTORC1 regulates important cellular processes including autophagy, protein and lipid synthesis, and growth factor signaling (Jewell and Guan, 2013; Meng et al., 2018; Saxton and Sabatini, 2017). mTORC1 is able to stimulate protein synthesis and cell proliferation through the phosphorylation of S6 kinase-1 (S6K1) and 4EBP1, which elevates glycolysis and protein biosynthesis to initiate a “carcinogenic” metabolic reprogramming, and regulates translation initiation, respectively (Fruman et al., 2017; Dibble and Cantley, 2015, Ma and Blenis, 2009; Magnuson et al., 2012). On the other hand, mTORC2 is primarily responsible for cell survival, growth, proliferation, and cytoskeletal remodeling through phosphorylation of protein kinases A, G, and C family members (Ebner et al., 2017; Gan et al., 2012; Li and Gao 2014; Sarbassoy et al., 2004; Thomanetz et al., 2013).

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The PI3K/AKT signaling pathway is known to stimulate mTORC activity by inactivating tuberous sclerosis complex (TSC) 1/2 phosphorylation (Carpenter and Jiang 2013; Manning et al., 2002; Rad et al., 2017). TSC consists of three components, TSC1, TSC2, and TBC1D7. The TSC complex primarily functions to inhibit the mTORC1 activator, Ras homolog enriched in the brain (Rheb) (Inoki et al., 2002; Manning et al., 2002).

Current studies suggest that mTOR is involved in a broad spectrum of functions including lipid generation, biosynthesis of nucleotide precursors, metabolic transformation, metastasis, etc. (Ben-Sahra et al., 2013; Rad et al., 2017; Valvezan et al., 2017; Yecies et al., 2011). Activated mTOR will also enhance the production of de novo proteins, mainly through increased number of ribosomes and accelerated protein translation (Iadevaia et al., 2014). In addition, mTOR can speed up G1 to S transition, which will drive more rapid cell proliferation and growth (Fingar et al., 2004). Examples of proteins regulated by mTOR include cyclin D1, HIF, and VEGF, which are required for the survival of many tumors (Mu et al. 1995; Stenmark et al. 1996; Guimaraes et al. 2015). mTOR/ribosomal protein S6 kinase beta-1 (p70S6K1) can also up-regulate AP-1 as well as other pro-angiogenic factors in promoting carcinogenic effects. Given the various cancerous characteristics promoted by mTOR activation, it is no surprising to find mTOR activation in approximately 70% of human cancers and this activation is often correlated with resistance to cancer therapy and overall poor survival rate for the patient (Forbes et al., 2011; Jiang & Liu, 2008; Rad et al., 2017).

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Arsenic and the PI3K/AKT/mTOR pathway

The PI3K/AKT/ mTOR pathway has been regarded as a key regulator for many physiological processes including cell proliferation, growth, metabolism, and macromolecular synthesis (Li and Wang 2014; Guimaraes et al. 2015). Although PI3K/AKT and mTOR are two separate pathways, they are often considered as a single unique pathway due to functional interconnectedness. PI3K is evolutionarily conserved to respond to external growth signals. In mammals, activation of the pathway begins as a cellular response to various extracellular stimuli including epidermal growth factor receptor (EGFR), PDGF receptor (PDGFR), insulin-like growth factor receptor (IGFR), and insulin receptor (INSR) (Li and Wang 2014; Fruman et al., 2017). In consideration to its many important physiological roles, a homeostatically balanced PI3K/AKT/mTOR network is fundamental in maintaining normal cellular growth. On the other hand, aberrant activation of the signaling cascade will lead to considerable interruptions in cell proliferation, which can result in angiogenesis, metastatic competence, and potential therapy resistance (Porta et al., 2014). In other words, the PI3K/AKT/mTOR pathway exists as one of the most attractive targets for cancer development. Evidence suggests that chronic arsenic exposure-induced cell proliferation, migration, invasion, and anchorage-independent growth are strongly correlated with PI3K and AKT activation (Carpenter and Jiang 2013). A likely mechanism of arsenic-induced pathway activation is through stimulating upstream signals such as EGFR, which has been shown to induce aberrant epithelial cell proliferation (Gao et al. 2004; Hennessy et al. 2005; Jiang and Liu, 2008; Jiang and Liu 2009; Lee et al. 2010; Simeonova et al. 2002; Andrew et al. 2009; Wen et al. 2010; Carpenter and Jiang 2013). EGFR is a member of the ErbB family of receptor

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tyrosine kinases and is commonly over-expressed in human malignancies such as cancers of the lung, breast, esophageal, etc, (Hirsch et al. 2003; Suzuki et al. 2005; Carpenter and Jiang 2013; Seshacharyulu et al., 2012). Conformational changes due to ligand-binding-induced EGFR dimerization allows for auto-phosphorylation at the C-terminal section of the receptor. Activated EGFR can interact with the p85 regulatory subunit of PI3K, which will stimulate the catalytic activity of p110 and initiate the activation of the PI3K/AKT pathway (Carpenter and Jiang 2013: 148-150). Subsequently, AKT can indirectly inhibit TSC2, thereby activate mTOR signaling (Carnero, 2010; Jiang and Carpenter, 2013). In fact, studies have shown that cells treated with arsenic along with PI3K inhibitor LY294002 will result in reduced p70S6K phosphorylation and subsequent mTOR activation, indicating that mTOR activity is regulated by upstream PI3K activation (Altman et al. 2007; Wang and Proud 1997; Jung et al 2003; Skinner et al. 2004; Castorina et al. 2008; Lee et al. 2010; Carpenter et al. 2011; Wu et al. 2011; Carpenter and Jiang 2013; Yoon et al. 2006). EGFR and activated PI3K/AKT, and mTOR pathways have all been shown to stimulate cell transformation and angiogenesis, see Figure 1. Effectors of the signaling cascade include HIF-1, AP-1, FOXO, NF-kB, which are important factors in promoting cancer formation (Carpenter and Jiang 2013; Hu et al. 2005; Xia et al. 2006; Fang et al. 2007; Jiang and Liu 2009).

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Arsenic can differentially regulate PI3K/AKT/mTOR in different cell types

Cancer promoting

As a potent human carcinogen, chronic arsenic exposure has been long been known to induce cancers of the lung. In multiple studies, human bronchial epithelial (BEAS-2B) cells illustrated activated AKT and mTOR activity after arsenic exposure (Carpenter et al., 2011; Liu et al., 2012; Beezhold et al., 2011; Zhang et al., 2006). Despite high variability in dosage (0-20uM), type of compound ($NaAsO_2$, $AsCl_3$), and length of exposure (4 hours to 26 weeks), all of the treatment conditions resulted in increased pathway activity when compared to the control groups, see Table 1. Along with activated AKT and mTOR activity, arsenic-exposed cells also demonstrated higher rate of proliferation, survival, and anchorage-independent growth, all of which are unique hallmarks of malignant cell transformation. Similar results were also found in primary human bronchial epithelial (HBEC) cells (Wang et al., 2012). In addition to bronchial epithelial cells, mouse epidermis-derived JB6 Cl41, as well as normal human bladder SV-HUC-1 and A375 cells all demonstrated activated PI3K and AKT activity after arsenic exposure (Ouyang et al., 2005; Li et al., 2015; Wang et al., 2013). In vivo experiment treating Wistar rats and C57BL/6 mice with 1.0 umol/L of roxarsone revealed activated PI3K and AKT phosphorylation in the vascular endothelial cells (Wang et al., 2016). In fact, both acute and chronic arsenic treatments have been found to promote PI3K/AKT phosphorylation. In one study, after treating SV-HUC-1 cells with 1uM of arsenic trioxide for eight to 10 months, results from western blotting analysis showed significant elevation in the expression of mTOR protein as well as phosphorylation of PI3K and AKT (Michailidi et al. 2015).

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Cancer suppressing

While evidence suggests arsenic is capable of activating the PI3K/AKT/mTOR pathway and stimulating cell proliferation in normal cells such as BEAS2B and SV-HUC-1, the opposite phenomenon seems to occur in cancerous cells. Specifically, arsenic induces apoptosis to varying degrees in different types of cancer cells such as HT-29 colon, neuroblastoma, prostate, B-cell leukemic, and gastrointestinal cancer cells (Akao et al. 1999; Cha et al. 2006; Ma et al. 2014; You et al. 2015; Stevens et al. 2016). In most of these studies, arsenic trioxide is used instead of sodium arsenite or arsenic trichloride, which are typically used for treatment of non-carcinoma cell studies, see Tables 1 and 2. Arsenic trioxide has been used as a key ingredient in traditional Chinese medicine for over 5000 years (Au 2011). Over time, the demand and appeal for arsenic diminished due to its intrinsic toxicity. Nevertheless, in the 1970s, researchers from China discovered the therapeutic effects of arsenic trioxide against acute promeylocytic leukemia, and the FDA later approved its usage in 2000 (Antman 2001). Today, arsenic trioxide is still being used as an effective drug against acute promyelocytic leukemia, mainly through inducing cancer cell death (Baysan et al. 2007; Li and Wang, 2014). However, tolerable doses of arsenic trioxide has been found to be most effective in inducing apoptosis in acute APL, but not as effective in other malignant cells (Koren et al. 2008). Furthermore, arsenic may preferentially target cancer cells rather than normal cells. For instance, arsenic trioxide (ATO) has been found to limit PI3K/AKT induced cellular proliferation and increase the amount of apoptosis in B-CLL cells, but not in normal peripheral blood lymphocytes (Redondo-Muñoz et al. 2010).

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In both human Burkitt's lymphoma and human leukemia cell lines, high exposure to arsenic trioxide, above 5uM for over 24 hrs, has been shown to induce cell apoptosis (Chen et al. 1996; Baysan et al. 2007; Li and Broome 1999; Choi et al. 2012; Li and Wang 2014). A likely explanation for this phenomenon is the induction of reactive oxygen species through arsenic toxicity, which in turn decreases AKT activity and promote pro-apoptotic features. In human gastric cancer (SGC-7901) cell lines, arsenic trioxide exposure for up to 72 hrs led to decreased phosphorylation at two AKT sites, Ser 473 and Thr308, although the protein levels stayed relatively same, indicating that arsenic is capable of reducing activation of AKT rather than the total protein (Gao et al. 2014). In another study led by Wang et al. 2017, As_2S_2 has been found to inhibit the AKT/mTOR signaling pathway and induce autophagy and apoptosis in several osteosarcoma cell lines. Specifically, human osteosarcoma 143B, MG-63, U-2OS, and HOS cells were subjected to either 24h or 48hr treatment. Dosages of arsenic ranged from 3.02uM to 13.06uM. Western blotting data suggests that As_2S_2 led to a reduction in both the protein amount as well as activity/phosphorylation of mTOR and AKT (Wang et al. 2017). In a separate study based on chondrosarcoma cells, researchers were able to find similar therapeutic benefits of arsenic in suppressing cancer cell growth (Jiao et al. 2015). Human chondrosarcoma cells, HCS-2/8, OUMS-27, and SW1353, were subject to 12, 24, or 48hr of arsenic trioxide exposure, concentrations ranging from 1-20uM. MTT assays after the treatment illustrated decreased cell viability and high levels of apoptosis. Western blot analyses demonstrated a dose-dependent decrease in AKT and mTOR phosphorylation due to arsenic exposure, which led the researchers to conclude that arsenic trioxide may induce apoptosis via inhibition of the AKT/mTOR pathway.

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On the other hand, ATO may not always be effective when acting alone. In one study, HL60 cells were treated with both ATO and 2-Deoxy-D-Glucose, an anti-glycotic drug, resulting in dephosphorylated AKT. However, ATO alone lead to insignificant changes to the phosphorylation levels (Estan et al., 2012). Similarly, Lonidamine, an anti-tumor drug, has also been shown to inactivate PI3K/AKT when used in combination with ATO (Calvino et al., 2011). In addition, dosage and length of ATO exposure also seems to control the PI3K/AKT pathway. In a study using both NB4 and gastric cancer cells, after the initial 4 hour ATO treatment, AKT phosphorylation heightened. But after decreased again after 16-24hrs of treatment (Li et al., 2009). The controversial biological effect of arsenic, hence its biphasic effect on carcinogenesis, may depend on the type of compound and strength of exposure (Wang et al., 2016).

Challenges in targeting PI3K/AKT/mTOR pathway for cancer therapy

In consideration to the many important roles in multiple physiological functions, cell growth and metabolism, the PI3K/AKT and mTOR pathways are crucial targets for cancer therapy. However, efforts using target inhibitors have proven to be inefficient mainly due to three reasons. First, activation of the pathways can be initiated by various receptors due to cancer cells' high plasticity in amplifying the upstream targets to stimulate compensatory pathways and maintaining persistent signal flow (Fruman et al., 2017). Second, persistent administration of the inhibitors can induce resistance due to mutations of regulatory genes responsible for the pathways. In fact, the PI3K and mTOR pathways are important targets for resistance to cancer immunotherapy (Fruman et al., 2017). Third, studies suggest that significant reductions in PI3K and AKT phosphorylation is needed to generate optimal therapeutic outcome in most cancer

patients (Fruman et al., 2017). For instance, in order to induce hyperglycemic response in mice, inhibition of hepatic PI3K/AKT signaling needs to reach more than 90% efficiency (Fruman et al., 2017; Taniguchi et al., 2006). However, inhibitors with the greatest potentials for effective therapeutic activity, such as pan-pI3K and pan-specific PI3K/mTOR inhibitors, also exert high toxicity burdens. So while high doses may be effective in targeting tumor tissues, the resulting adverse effects such as hyperglycaemia and liver damage releasing transaminases, are limiting factors for administrating most effective dosages.

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Discussion

More than 200 million people around the world are exposed to arsenic at levels above the maximum contaminant level of 10 ppb, either through drinking water, inhalation, and or diet. As a class I human carcinogen, exposure to arsenic has been documented to cause neurodevelopmental deficits, cardiovascular disease, various human cancers including that of the lung, bladder, kidney, liver etc. Various mechanisms of arsenic-induced carcinogenesis have been proposed, and this review specifically focused on the PI3K/AKT/mTOR pathway in an effort to better understand arsenic's ability to induce the intrinsic characteristics of cancer development: cell proliferation and anti-apoptosis. As a critical pathway for a multitude of biological and physiological functions such as cell survival, proliferation, apoptosis, and metabolism. Multiple studies have demonstrated a link between arsenic and the activity of the PI3K/AKT/mTOR pathway in stimulating malignant cell transformation through uncontrolled cell proliferation. In addition, it is interesting to note that mTOR acts as a major negative regulator of autophagy, a process in which old and damaged cells are degraded (Heras-Sandoval et al., 2014). The activation of PI3K/AKT/mTOR signaling pathway not only stimulates growth, but can also halt autophagy, which may promote the extended survival of damaged cells and subsequent replication. In fact, previous studies have illustrated that arsenic is capable of inducing prolonged activation of Nrf2 through autophagic dysfunction as a way of promoting cancer development (Lau et al., 2013). The link between arsenic and PI3K/AKT/mTOR pathway-induced autophagy may be an interesting area for future studies. On the other hand, arsenic trioxide has been successfully used to treat acute promyelocytic leukemia through inducing cell death in the cancer cells. The specificity of

this successful treatment is likely due to the narrow mutations of specific pro-apoptotic proteins that drive PML. Current studies suggest that arsenic can manipulate the PI3K/AKT/mTOR pathway to induce cell proliferation as well as apoptosis, two conflicting mechanisms. The ability for arsenic to achieve opposite cellular responses may be related to the exposure conditions (dose and time) as well as the type of cells used. It is imperative for future studies to establish and pinpoint the exact conditions for arsenic to differentially regulate the PI3K/AKT/mTOR pathway in order to maximize the therapeutic and minimize the carcinogenic effects of arsenic.

Author Contributions

Both authors contributed equally to this work.

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Figure legend

Figure 1. Graphical representation illustrating the role of the PI3K/AKT/mTOR pathway in promoting cellular growth.

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Table 1. Studies showing arsenic activating PI3K/AKT/mTOR pathway

Arsenic compound	Treatment	Cell Type	Technique platform	Pathway Effect	Tissue type	Reference
Arsenite (As 3+)	1.25 uM for 72 hours	JB6 Cl41	Cell proliferation, cell cycle, gene reporter, PI-3 kinase assay, western blotting, cyclin D1 expression assay,	Activated PI3K and AKT	Epidermis	Ouyang et al. 2005
$NaAsO_2$	0,0.25,1, 5 uM for 26 weeks	BEAS-2B	Cell proliferation, soft agar, immunoblotting, ROS detection,	Activated AKT and mTOR	Lung	Carpenter et al. 2011
$AsCl_3$	10uM 0-16 hr	BEAS-2B	Western blotting, ELISA, cell migration	Activated AKT	Lung	Liu et al. 2012;
$AsCl_3$	0-20uM 6-12 hr	BEAS-2B	Western blotting, rt-pcr, reporter assay, cell proliferation, transformation assay, enzyme-linked immunosorbant assay	Activated AKT	Lung	Beezhold et al. 2011
$AsCl_3$	0-20uM of arsenic chloride for up to 20	BEAS-2B	RT-PCR, qPCR, RNA immunoprecipitation, immunofluorescence	Activated AKT	Lung	Zhang et al. 2006
$NaAsO_2$	2.5uM for 6 weeks	HBEC	Cell migration, invasion, western blotting, immunofluorescence	Activated AKT	Lung	Wang et al. 2012
$NaAsO_2$	0-20 uM for 6hrs	A375	Western blotting,	Activated AKT	Skin	Li et al., 2015
$C_6AsNH_6O_6$	1.0 μmol/L	Vascular endothelial cells male Wistar rats and C57BL/6 mice	MTT, cell proliferation, migration, tube formation, xenograft, immunohistochemical staining, westernblotting	Activated PI3K and AKT	Thoracic aorta	Wang et al., 2016
$NaAsO_2$	0,1,2,4,8,10 uM	SV-HUC-1	RT-PCR, western blotting, elisa,	Activated PI3K and AKT	Ureter	Wang et al., 2013

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Table 2. Studies showing arsenic inhibiting PI3K/AKT/mTOR pathway

Arsenic compound	Treatment	Cell Type	Technique platform	Pathway Effect	Tissue type	Reference
$NaAsO_2$	0-2 uM for 7 days	3T3-L1	qRT-PCR, western blotting, immunofluorescence,	Inactivation of AKT	Adipocytes	Xue et al. 2011
As_2O_3	0-10uM for 48 hrs or 5uM for up to 72 hrs	SW1353	MTT, Western blotting, cell viability, clone formation, apoptosis, immunofluorescence,	Inactivation of AKT and mTOR	Chondrosarcoma	Jiao et al. 2015
As_4O_6	1uM for 48 hrs	SW620	Cell viability, ROS generation, cell cycle, nuclear staining, western blotting, inhibitors assay	Decreased PI3K/AKT	Colorectal cancer	Nagappan et al., 2017
As_2O_3	0-16 uM for 24 hrs	SGC-7901	Cell viability, mitochondrial membrane potential, apoptosis, western blotting	Inactivation of AKT	Gastric cancer	Gao et al. 2014
As_2O_3	3mg/kg foe 7 days	Male Wistar rats	Histological analysis, western blotting	Inactivation of PI3K and AKT	Liver	Zhang et al., 2017
NA	NA	U937	NA	Inactivation of AKT	Myeloid cancer	Choi et al. 2012
As_2O_3	1 uM for NB4 and 4uM for THP1 cells for 4 hrs	NB4, THP1	Flow cytometry, cell proliferation, viability, apoptosis, necrosis, ROS and GSH level, immunoblotting	ATO alone has no effect on AKT, only with 2-DG (2-deoxy-d-glucose)	Myeloid cancer	Estan et al., 2012
As_2O_3	1uM for 24 hrs in NB4 cells and 10uM for 16 hurs in MGC803 cells	NB4 and MGC803	Cell viability, cell cycle, western blotting	Inactivation of PI3K and AKT	Gastric and myeloid cancer	Li et al., 2009

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As_2S_2	3.02uM to 13.06uM for 24h or 48hr treatment	143B, MG-63, U-2OS, and HOS	Cell viability, clone formation, cell cycle, apoptosis, histopathology, immunohistochemistry, human osteosarcoma xenograft, western blotting, ROS generation	Inactivation of AKT and mTOR	Osteosarcoma	Wang et al. 2017
As_2O_3	3uM for 48 hrs	B-CLL	Cell apoptosis, ROS, western blotting	Inactivation of PI3K and AKT	Peripheral blood	Redondo-Muñoz et al. 2010

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Figure 1. Overview of arsenic-elicited PI3K/AKT/mTOR pathway activation and subsequent increase in cell growth