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Roles of EGFR, PI3K, AKT, and mTOR in Heavy Metal-Induced Cancer

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Abstract: Humans are exposed to heavy metals through a variety of occupational and non-occupational means. Growing evidence has accumulated that prolonged exposure to these heavy metals is associated with cancer occurrence at various body sites including lung, liver, bladder, colon, and skin. Much research effort has been placed on discovering the mechanisms by which heavy metals induce different kinds of cancers. Results from these mechanistic studies have varied for different metals, but increased activation of signaling pathways is often observed. This review will focus on the signaling molecules including epidermal growth factor receptor (EGFR), phosphatidyl inositol 3-kinase (PI3K), AKT, and mammalian target of rapamycin (mTOR) in carcinogenesis and cancer progression; and how these molecules are affected by the exposure to heavy metals: arsenic, chromium, nickel, and cadmium. Furthermore, drug targets for the prevention and therapy of cancers induced by heavy metals will be discussed with a focus on drugs that are currently in clinical trials for these targets.

Keywords: AKT, Arsenic, cadmium, cancer, chromium, EGFR, mTOR, nickel, PI3K, p70S6K1.

INTRODUCTION

Humans are exposed to several different heavy metals that exist in different forms in the natural course of living. These heavy metals are naturally occurring elements in part of the earth's crust. Heavy metals exist all over the world in soil, water, and the atmosphere. Human interactions with the earth have increased heavy metal levels in water and the atmosphere from activities such as mining. Concurrently, the estimated number of new cancer cases for 2010 is greater than 1.5 million cases, which is increased from 2009 [1, 2]. The estimated number of cancer deaths for 2010 is greater than that in 2009 [1, 2]. Many heavy metals are known to induce cancer including arsenic, chromium, nickel, and cadmium with enough evidence to deem all four of these metals as human carcinogens [3-5]. However, a clearly-defined mechanism of carcinogenesis induced by these heavy metals remains to be defined. A consistent factor found with these metals is an increased flux through cell growth and proliferative signaling pathways and increased tumor angiogenesis. Mechanisms of metal-induced carcinogenesis have been previously reviewed [6-9]. The purpose of this review is to discuss evidence for these heavy metals as carcinogens through inducing prominent growth and proliferative signaling pathways in epidermal growth factor receptor (EGFR), phosphatidyl inositol 3-kinase (PI3K), AKT, mammalian target of rapamycin (mTOR), and p70S6K1.

HEAVY METALS AND CANCER

Arsenic

Like many heavy metals, arsenic can have toxic effects at high concentrations but also has a strong association with the formation of several cancers with long exposure to low arsenic concentrations. The carcinogenic effects of arsenic are thought to be primarily attributable to the form arsenite (As^{+3}) rather than arsenate (As^{+5}) because arsenite exists greater amount in well water for drinking, and has a higher cellular uptake, which is reduced to arsenite when it enters the reducing environment of cells [8, 10]. Exposure to arsenic is strongly associated with development of cancers of lung, bladder, and skin. In an exhaustive review, the International Agency for Research on Cancer (IARC) found sufficient evidence for carcinogenicity of arsenic in humans [3]. Arsenic is highly associated with cancers of the lung, bladder, and skin. Exposure to carcinogenic levels of arsenic occurs primarily through arsenic in drinking water, occupational exposure in ore mining and smelting, and cigarette smoking (Table **1**) [3]. Drinking water contamination with arsenic is a global public health issue with large populations exposed in the Gulf of Bengal, South America, and Taiwan [11]. There is an estimated 25 million people in Bangladesh and 6 million people in India who consume water with arsenic levels greatly exceeding the current maximum contaminant level (MCL) set forth by the U.S. Environmental Protection Agency (EPA) [12, 13]. Arsenic in drinking water is also considered a public health issue in the Western, Midwestern, and New England areas of the United States [12, 14, 15]. Classic epidemiological evidence comes from Taiwan where elevated risks for cancers of the lung, bladder, skin, kidney, liver, and colon are strongly associated with frequencies of black-foot disease, an arsenic-induced disease [16, 17]. In addition to drinking water, there is increased risk of death due to respiratory cancer in populations working in or living near copper smelters where there are increased levels of arsenic dusts, throughout the United States and other countries [18-29]. Arsenic is carcinogenic regardless of exposure to atmospheric or drinking water contaminated with arsenic.

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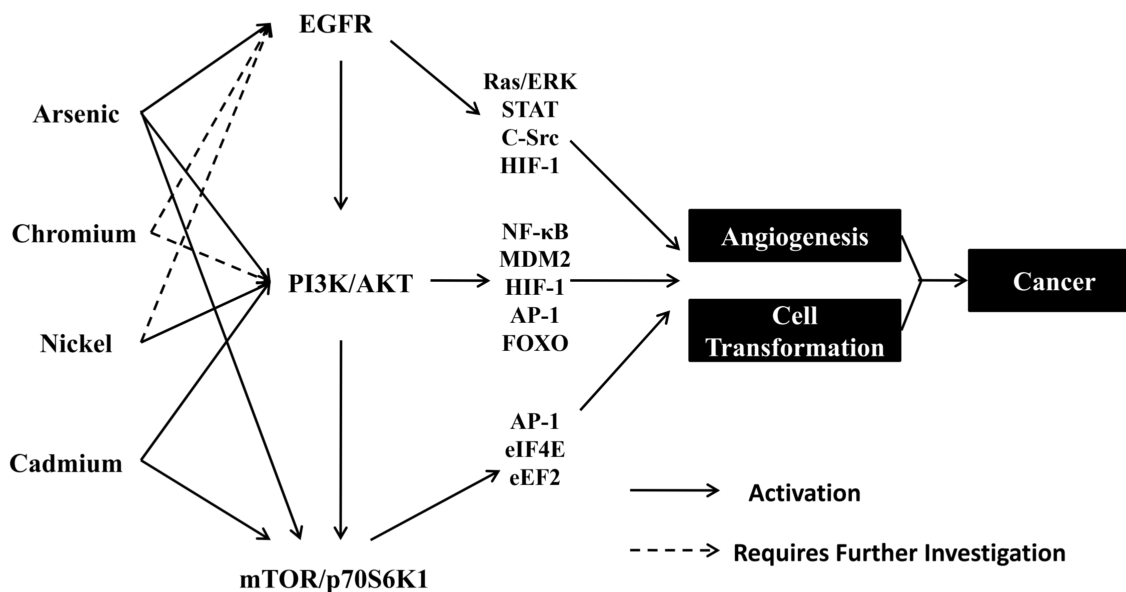

Fig. (1). Signaling Mechanisms in Heavy Metal Carcinogenesis. EGFR, PI3K/AKT, and mTOR/p70S6K1 all can promote cell transformation or angiogenesis. EGFR uses mediators such as PI3K, Ras/ERK, STAT, c-SRC, and HIF-1 signaling to promote cellular behaviors for cell survival and proliferation in addition to promoting release of pro-angiogenic factors. PI3K/AKT can activate mTOR, NF-κB, HIF-1, AP-1, FOXO, enhance MDM2 inhibition of p53, and induce release or pro-angiogenic factors to promote pro-carcinogenic effects. mTOR/p70S6K1 can activate AP-1, protein synthesis factors, and pro-angiogenic factors to induce cancer formation. Evidence suggests that arsenic can activate EGFR, PI3K/AKT, and mTOR/p70S6K1 to promote cell transformation or angiogenesis. Less is known regarding chromium but current evidence suggests chromium can activate PI3K/AKT and possibly induce amplification of EGFR but further studies are needed to establish these mechanisms. Nickel has shown to activate PI3K, and induce amplification of EGFR that increases its sensitivity to ligands in addition to its well established effects of stabilizing HIF-1 signaling *via* inhibition of prolyl-hydroxylase enzymes. Cadmium is the least studied of the metals currently discussed but evidence shows cadmium can activate PI3K/AKT and mTOR/p70S6K1 signaling but direct evidence is yet to be established regarding the involvement of these pathways in cadmium carcinogenesis. EGFR amplification is an often-seen phenomenon in cancer cells and appears as a likely result of exposure to several heavy metals that may increase sensitivity to growth factors. EGFR can also activate PI3K/AKT, Ras/ERK, and mTOR/p70S6K1 signaling, all of which are oncogenic pathways. The PI3K/AKT pathway is a potent cell survival/proliferation pathway with oncogenic potential. mTOR/p70S6K1 signaling strongly promotes protein synthesis and cell growth required by proliferating cancer cells. The potential activation of these pathways and their interactions should be fruitful areas of future investigations.

Chromium

Chromium exists in several forms [30], and most human exposure is occupational. Chromium exists as chromite in minerals; chromium metal is used in steel and other alloys; and chromium chemicals are used for chrome plating, dyes and pigments, and other applications. There is increased risk for lung cancer in chromium platers [31] and welders of stainless steel exposed to chromium (Table **1**) [32]. Nasal cancers have also been identified in workers exposed to chromium [33, 34]. Chromium exposure is associated with elevated risks for lung cancer [31, 32, 35, 36] with the highest risk coming from workers in the "wet" end of chromium chemical production process [35]. The IARC found sufficient evidence for chromium hexavalent compounds as "carcinogenic to humans" with the most common site for cancer development being the lung [5, 37]. This comprehensive review did not find enough data to make any conclusions regarding trivalent chromium but several studies identify hexavalent chromium as being more toxic [38, 39], which may be due to the ability of hexavalent chromium to pass through cell membranes. Most epidemiology studies published show an association of lung cancer with chromium exposure [36, 40-52]. Chromium is most carcinogenic in its hexavalent form and shows strong associations with the formation of cancers, especially of the lung.

Nickel

There is clear evidence for cancer development in the nasal cavity, lung, and the larynx upon exposure to nickel. The International Committee on Nickel Carcinogenesis in Man concluded that several forms of nickel give rise to lung and nasal cancer [53]. A comprehensive review by the IARC in the same year also concluded that nickel is carcinogenic to humans [5]. Exposure is commonly occupational with high associations in workers employed in roasting, smelting, and electrolysis settings, nickel alloy plants, and welders (Table **1**) [37]. Approximately 3-fold increased risk for lung cancer and 50-fold increased risk for nasal cancer were found among workers where oxidic and sulfidic nickel are primary exposures [53]. Risk appears to be associated more strongly with estimated cumulative exposure to soluble nickel rather than nickel oxide [54]. Soluble nickel appears to be the most consistent form to act as a carcinogen, consistent with several animal studies [5, 55-57]. There may also be a possible interaction between nickel exposure and tobacco making workers in the nickel industry who smoke

Table 1. Sources of metal exposure and associated cancers.

Cancer Location	Metal Source	Citations
Arsenic		
Lung, Pharynx	Drinking Water, Copper Smelter, Tobacco, Tin Mining, Metal Refinery	[12, 14, 16-25, 27-29]
Skin	Drinking Water	[12, 14, 16-17]
Bladder	Drinking Water	[12, 14, 16-17]
Kidney	Drinking Water	[16, 21]
Liver	Drinking Water	[16-17]
Colon, Rectum	Drinking Water, Metal Refinery	[16, 21, 28]
Chromium		
Lung, Nasal	Chromeplating, Chromium Pigment Production, Chromium Chemical Production; Chromate Production, Gas Compression (Chromium Additive); Chrome Leather Tannery	[31, 33-36, 40-45, 47-48, 50-52]
Nickel		
Lung, Nasal	Tobacco, Nickel Refining; Nickel Smelting	[54, 58-61, 76, 86]
Stomach	Nickel Smelting	[59, 87]
Cadmium		
Lung, Nasal	Tobacco, Ni-Cd Battery Factory, Cadmium Factory; Cadmium Recovery Plant; Area Near Smelting Factory	[66, 69, 76-80, 84-86]
Kidney	Occupational	[70-72, 74]
Breast	Tobacco, Pesticide*, Hazardous Waste*	[67, 73, 88]
Endometrial	Dietary Sources	[89-90]
Pancreas	Soil, Water	[75, 91]
Prostate	Cadmium Factory	[68-69]

particularly susceptible to a high risk for cancer development [54]. Several epidemiological studies observe an association between nickel exposure and lung cancer [54, 58-61]. Nickel has been deemed a carcinogen by several organizations and committees with data showing special attention should be paid to soluble forms of nickel.

Cadmium

Like many other metals, cadmium exposure is often occupational and has lasting effects in humans. Cadmium is commonly associated with ores and minerals and is produced primarily as a byproduct of extracting zinc and other metals followed by usage in nickel-cadmium batteries, pigments, metal coatings, and alloys [4]. Human exposure to cadmium occurs by mining ores and minerals associated with cadmium, industrial contamination of topsoil and edible plants, battery production, production of cadmium alloys, and cigarette smoking, among several others (Table **1**). Human ingestion of cadmium is doubled in smokers who smoke one pack per day compared to ingestion from dietary sources alone [62] as the tobacco plant takes in cadmium from soil [63]. Cadmium oxide fumes generated at high temperatures are readily absorbed *via* the lung while inhalation of dusts depends on particle size [64]. The IARC found sufficient evidence for carcinogenicity of cadmium compounds in humans [4]. Cadmium has low excretion rates causing it to have a long half-life in the body of 15-20 years [65]. This long half-life leads to accumulation, especially in the kidneys and liver [4], and danger for carcinogenicity long after exposure. Several epidemiological studies show positive associations between cadmium exposure and development of cancer [66-80] with little contradictory evidence [68, 81-84]. Exposure to cadmium led to increased risk for cancer of the breast [67, 73], of nose and nasal sinuses [76, 77], of the prostate [68, 77], of the lung [69, 78-80, 85], of the kidney [70-72, 74], and of the pancreas [75]. Cadmium exposure in humans occurs from several mediums that are both occupational and non-occupational but lead to carcinogenesis likely because of the long half-life of cadmium in the body.

HEAVY METALS AND EGFR SIGNALING

As a member of the ERBB family of receptor tyrosine kinases (RTKs), EGFR, or ERBB1, is often over-expressed in several types of human cancers and this over-expression is often associated with a poor prognosis [92-95]. Ligand binding to EGFR induces both homo-dimerization with EGFR or hetero-dimerization with ERBB2 (HER2), ERBB3 (HER3), or ERBB4 (HER4). Dimerization leads to

conformational changes allowing auto-phosphorylation of the cytoplasmic C-terminal segment of the receptor. After the intracellular domain is auto-phosphorylated, several adaptor proteins bind to the phospho-tyrosines leading to activation of several pathways including MAPK and PI3K-AKT pathways. ERBB receptor signaling can activate several transcriptions factors including c-fos, c-Jun, c-myc, STAT, and NF-kB [96]. These pathways regulate cell proliferation, migration, metastasis, evasion of apoptosis, and angiogenesis [97]. Phosphorylated EGFRs can bind proteins containing an SH2 domain or PTP domain. PTP domains are often located in phosphotyrosine phosphatases, which remove the phosphate from the EGFR to terminate EGFR signaling [98].

Arsenic

Arsenic exposure to cells can induce the phosphorylation of EGFR, which indicates the activation of EGFR [99-109]. Arsenic can also increase the phosphorylation levels of EGFR with concurrent ultraviolet (UV) exposure [110]. Further evidence of EGFR activation is that arsenic can induce phosphorylation of Shc and increase association of Shc with Grb [100, 106, 107], two well-known adapter proteins to EGFR. Many studies have shown increased phosphorylation levels of EGFR, but few studies have assessed EGFR kinase activity.

Arsenic exposure to epithelial cells induces proliferation that is mediated by EGFR [105, 108]. Several transcription factors and signaling pathways likely contribute to arsenic-induced proliferation *via* EGFR. Cyclin D1 is the final target of EGFR signaling induced by arsenic as cyclin D1 knockdown arrests arsenic-induced proliferation [111] and arsenic-induced cyclin D1 is blocked with EGFR inhibitors [99]. The transcription factors c-fos and c-jun, which promote proliferation, are increased with arsenic exposure in an EGFR-dependent fashion [100]. Arsenic also increased β-catenin, an oncogenic member of the Wnt signaling pathway, and this response was blunted with inhibitors to EGFR [105]. This response may be critical to arsenic-induced proliferation as a dominant negative to β-catenin decreased proliferation in cells exposed to arsenic [105]. EGFR-dependent arsenic induction of signaling pathways includes the ERK1/2 and PI3K pathways [99-103, 107-109, 112]. Other members of the MAPK signaling pathway activated by arsenic are Raf1, MEK1/2, JNK1/2, and p38 [107, 109, 113]. Activation of MAPK pathways by arsenic is likely mediated by EGFR-Ras signaling as arsenic activates Ras in an EGFR-dependent manner [105, 109] and arsenic-induced activation of MEK1/2 and ERK1/2 is prevented with interruption of Ras signaling [109]. Matrix metalloproteinases (MMPs) break down extracellular matrix and are critical for cancer cell invasion. Arsenic can induce MMP7/9 in an EGFR-dependent fashion [101, 105]. Clearly, arsenic induces many pathways throughout the cell leading to several changes in cell behavior resembling cancer cells.

The mechanism by which arsenic activates EGFR is not fully understood. EGF autocrine signaling was ruled out as monoclonal antibodies to EGF did not affect arsenic-induced EGFR activation [105, 106]. A more recent study observed increased production of heparin binding-EGF (HB-EGF) mRNA in response to arsenic exposure [99], but its role in EGFR activation by arsenic has not been studied. Perhaps the most likely candidate for mediating arsenic-induced EGFR activation is Src. A Src kinase inhibitor prevented EGFR activation upon arsenic exposure [106]. Furthermore, arsenic-induced activation of ERK1/2 was prevented with a Src inhibitor [101, 106] and arsenic can activate Src [112]. This would then raise the question of how arsenic activates Src. One report showed that arsenic bound to and altered the conformation of Src [114], but whether this alteration increases Src activity and mediates arsenic-induced EGFR activation has yet to be assessed. One final candidate for mediating EGFR activation by arsenic is reactive oxygen species (ROS) as N-acetyl cysteine (NAC) treatment prevented arsenic-induced phosphorylation of EGFR [106]. EGFR has previously been shown to be activated by ROS [115], but the source of ROS from arsenic exposure and the molecular mechanisms remain to be studied.

EGFR appears to be important for arsenic-induced cancer formation. Total EGFR expression is increased in cells chronically treated with arsenic [116], suggesting a possible amplification of the EGFR gene. This was also seen in animals showing an increase in EGFR mRNA levels with arsenic consumption [117], although another report did not detect a similar increase [118]. Increases in total EGFR expression were seen in newborn animals whose mothers were consuming arsenic while pregnant [119]. In humans, plasma samples from persons in Bangladesh with high concentrations of arsenic in local well water showed higher EGFR extracellular domain (EGFR-ECD), a marker of increased EGFR protein expression [120]. Furthermore, human lung tumors from persons with elevated toenail arsenic concentrations showed an increased total EGFR, although this was not significant [99]. However, this study did find a significant association between higher arsenic exposure and higher p-EGFR staining in lung tumor sections from humans [99] suggesting increased EGFR activation in tumors from persons with high arsenic exposure. More recently, liver cancer patients living in an endemic area of arsenic intoxication showed higher serum EGFR levels compared to patients living outside this endemic area [121], indicating EGFR may also be a biomarker for arsenic exposure. Together, these data suggest arsenic activates EGFR and this membrane receptor regulates many of the cell behaviors induced by arsenic.

Chromium

Chronic exposure (up to 38 passages) of an immortalized bronchial epithelial cell line to chromium induced malignant transformation of the cells that could grow tumors when injected into nude mice [122]. Subclonal cell lines from these chromium-mediated immortalized cells found a consistently higher expression of EGFR. Alternatively, one published report did not find increased EGFR expression after only 24hrs of chromium exposure [123]. This same study did find increased expression of EGFR family members ERBB2 and ERBB3 but whether that is an acute response to chromium exposure or a chronic adaptation has not been studied yet [123]. Despite the finding of increased EGFR expression in chromium-treated cells [122], there

have been no published reports showing chromium-induced EGFR phosphorylation or increased kinase activity. Chromium exposure has been seen to activate PI3K-AKT, MAPK [124], and STAT [125] signaling, but whether chromium utilizes EGFR to activate these pathways has not been studied. There are few studies published assessing the role of EGFR in chromium carcinogenesis but the evidence suggesting EGFR is amplified in chromium-induced tumors may warrant further investigation in the future.

Nickel

Nickel exposure to primary cells can induce cell immortalization leading to increased proliferative potential [126]. Cells immortalized by nickel exposure have an increased number of EGF receptors present on their cell membranes, suggesting EGFR may participate in nickel-induced immortalization [127]. Nickel-immortalized cells do not show increased phosphorylation of EGFR but they do show increased sensitivity to EGF compared to normal epithelial cells [127]. This may suggest EGFR signaling is enhanced rather than activated by nickel. A more recent study found that non-tumorigenic lung epithelial cells showed increased phosphorylation of EGFR with nickel exposure [128]. These results likely point out that the effect of nickel on EGFR is largely dependent on the cellular context.

A recent study may indicate nickel can alter EGFR location and function. Bronchial epithelial cells exposed to several different kinds of carcinogens from tobacco smoke and cells exposed to mixtures including nickel exhibited a shift of EGFR from the cell membrane to the nucleus [129]. This could be of great significance as EGFR has known capabilities of translocating to the nucleus and directly interacting with gene co-activators to regulate gene expression [130]. However, the significance of nuclear translocation of EGFR with nickel exposure is currently unknown. Nickel has also been shown to activate PI3K-AKT and MAPK signaling including ERK1/2 [131, 132], p38 [133, 134], and JNK1/2 [133, 135]. These are common pathways downstream of EGFR, but whether EGFR regulates nickel-induced signaling through MAPK pathways has not been published. Nickel is clearly a carcinogen and a few studies that have assessed the role of EGFR in nickel carcinogenesis provide interesting results. Nickel can induce several changes to EGFR and downstream signaling, but whether these changes contribute to nickel carcinogenesis and nickel-induced activation of growth and proliferative signaling requires further investigation.

Cadmium

The link between cadmium exposure and EGFR is circumstantial with the current literature. A recent study observed elevated EGFR expression levels, as well as several pro-inflammatory cytokines, with cadmium exposure [136]. Another report showed cadmium exposure leads to increases in Grb2, Shc, SOS, and Raf-1 [137], all of which are direct signaling components downstream of EGFR. Our lab has shown cadmium can activate AKT, ERK1/2, and HIF-1 signaling *via* reactive oxygen species [138], all of which can be upregulated by EGFR. Cadmium appears to affect the estrogen receptor, which may explain its link to breast cancer [139]. Chronic low-dose cadmium treatment on breast epithelial cells induces cell transformation characterized by tumor formation in nude mice [140]. This transformation also induced a loss of HER2 (ERBB2). One report showed cadmium-induced proliferation was blunted with pretreatment of inhibitors to EGFR [141]. However, further study of EGFR activation and its role in cadmium-induced cell signaling was not included in this study. Considering cadmium upregulates EGFR expression, upregulates EGFR adaptor proteins, and evidence suggesting EGFR may regulate cadmium-induced proliferation, further study of EGFR in cadmium carcinogenesis is warranted.

HEAVY METALS AND PI3K/AKT SIGNALING

Phosphitidylinositol 3-kinases (PI3Ks) in mammalian cells are composed of Classes I, II, and III. Class I PI3Ks have two subfamilies: class IA, which are activated by receptor tyrosine kinases (RTKs), and class IB, which are activated by G-protein-coupled receptors. Class IA PI3Ks are most understood to participate in regulating cell functions such as proliferation, growth and survival [142, 143]. These PI3Ks consist of heterodimers of a catalytic subunit, p110, and a regulatory subunit, p85. PI3K catalyzes the conversion of phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-trisphosphate (PIP3). This phosphate can be removed by phosphatase and tensin homolog deleted on chromosome 10 (PTEN) thereby negatively regulating PI3K signaling. PIP3 produced from PI3K activity recruits AKT, a serine/threonine kinase, and phosphoinositide-dependent kinase 1 (PDK1) to the plasma membrane by binding to their pleckstrin homology (PH) domains. Upon recruitment of PDK1 and AKT to the membrane, PDK1 phosphorylates AKT in its kinase domain (Thr308). Full activation of AKT comes with phosphorylation of its carboxy-terminal hydrophobic motif (Ser473) by PDK2 [144-146]. Following activation, AKT is released from the plasma membrane and moves to the cytoplasm and nucleus to phosphorylate many molecules that regulate many of the cell functions controlled by PI3K signaling. The main biological consequence of AKT activation related to cancer cells is survival, proliferation, and growth [147]. Activated RTKs, including EGFR, can interact with the p85 regulatory subunit to increase catalytic activity of the p110 subunit [148-150]. The p85 regulatory subunit can also bind to intracellular proteins including protein kinase C, SHP1, Rac, Rho, Ras, and Src to regulate PI3K activity [151]. AKT can activate mTORC1 indirectly by inhibiting TSC2, thereby allowing Rheb-GTP to activate mTORC1 signaling [152].

Arsenic

Arsenic exposure leads to activation of PI3K signaling. Specifically, arsenic has been shown to increase enzyme activity of PI3K [153-156]. Downstream, arsenic exposure also leads to increased phosphorylation of AKT [108, 111, 153-163] that is dependent on PI3K activity [111, 153-156, 159-162]. These studies strongly support that arsenic can activate PI3K signaling.

Arsenic alters cell behavior and many of these changes are regulated by the PI3K-AKT pathway. Our lab has shown

that chronic arsenic exposure can increase cell proliferation and anchorage-independent growth, and both of these can be reduced with interruption of the PI3K-AKT pathway [163]. Other labs have also shown that arsenic exposure increases cell proliferation [111, 155, 156, 158, 161], which can be dependent on PI3K signaling [111, 158]. Arsenic also increases the ability of cells to proliferate independent of attachment in a PI3K-dependent manner [116, 164]. Chronic exposure of cells to arsenic can lead to increased ability for migration and invasion, which can also be dependent on PI3K signaling [157]. Arsenic-induced proliferation has been shown to be dependent on cyclin D1 [111, 156]. In addition, arsenic increased cyclin D1 levels [155, 156, 165] *via* mechanisms dependent on PI3K-AKT signaling [111, 155, 156]. Several other signaling molecules may participate in arsenic-induced cell growth and proliferation. Arsenic exposure increases beta-catenin due to arsenic-induced phosphorylation of GSK-3β [163, 167], which is PI3K-dependent [108, 160, 161, 167]. Arsenic likely induces NF-κB signaling as IKKα and IKKβ are decreased with arsenic treatment [155]. Arsenic also increased levels of COX-2, an oncogenic enzyme [168] that is regulated by PI3K [116, 154]. Arsenic also increased phosphorylation of p70S6K1, which was PI3K-dependent [155, 169]. In addition to p70S6K1 signaling, arsenic activates the MAPK pathway JNK1/2 in a PI3K-dependent manner [165]. Another mechanism that may participate in arsenic-induced carcinogenesis is through epigenetic mechanisms. One report showed arsenic can induce phosphorylation of histone H3 that was dependent on PI3K and ERK signaling [170]. Furthermore, arsenic exposure can induce the phosphorylation of EZH2, [171], the catalytic subunit of polycomb-repressive complex 2 (PRC2) that alters methylation of histone H3 leading to wide changes in expression of tumor suppressors and oncogenes. Arsenic-induced phosphorylation of EZH2 required expression of STAT3, JNK, and AKT [171].

Arsenic also promotes tumor growth *via* promoting angiogenesis with low concentration exposure [172-175]. Our lab has shown that arsenic can induce angiogenesis *in vivo* [174]. We, and others, have shown arsenic can activate HIF-1 signaling [153, 164, 174] and upregulate VEGF expression [153, 173, 174] in a PI3K-dependent fashion [153, 174]. Furthermore, interruption of PI3K-AKT signaling reduced *in vivo* angiogenesis resulting from arsenic exposure [174]. Together these results suggest that arsenic has wide-ranging effects and PI3K-AKT is a crucial signaling pathway mediating many cellular changes with arsenic exposure.

The mechanism by which arsenic activates PI3K is not well understood. Arsenic-induced activation of PI3K-AKT signaling may be mediated by ROS as the ROS scavengers NAC and catalase prevented arsenic-induced AKT activation [153, 160, 174] although one report showed ROS inhibitors could not prevent arsenic-induced phosphorylation of AKT [162]. ROS-mediated activation of AKT appears to be important for arsenic-induced carcinogenesis as inhibition of ROS prevents arsenic-induced cell transformation [163] and *in vivo* angiogenesis [174]. Other factors may also play a role in PI3K activation such as MAPK signaling as a p38 inhibitor prevented arsenic-induced AKT phosphorylation [162] though this mechanism is not well understood. Inhibition of JNK or STAT3 also prevented arsenic-induced activation of AKT [176] likely indicating arsenic can activate AKT by multiple mechanisms. Considering the evidence suggesting arsenic activated EGFR, it is possible that arsenic-induced activation of PI3K-AKT signaling is mediated by EGFR, but this has yet to be studied. Lastly, inhibition of c-Met, which is the receptor for hepatocyte growth factor, also reduced arsenic-induced activation of AKT [159]. PI3K and AKT are clearly activated upon arsenic exposure and are critical for many of the carcinogenic effects. Future studies should seek to understand how arsenic activates PI3K-AKT and how this pathway regulates many of the cellular behaviors participating in carcinogenesis, including angiogenesis.

Chromium

There is little study of the effect of chromium on the PI3K-AKT pathway. There are a few studies that show chromium exposure leads to AKT phosphorylation [124, 177, 178]. However, there are no reports of the effects of chromium on PI3K activity. Furthermore, whether PI3K-AKT participates in any aspect of chromium carcinogenesis has not been published. Considering the lack of data, no conclusions may be accurately made regarding the role of the PI3K-AKT pathway in chromium carcinogenesis.

Nickel

Similar to arsenic, nickel can increase activity of PI3K [179] and induce phosphorylation of AKT [134, 179-181, 182] that is dependent on PI3K activity [179-181]. Despite clear evidence that nickel activates PI3K signaling, further study regarding the role of PI3K-AKT in nickel-induced carcinogenesis has been little. Nickel exposure does lead to increased COX-2 [181], increased IL-6 release [181], and activation of p70S6K1 [179] in a PI3K-dependent fashion. All three factors: COX-2 [168], IL-6 [183] and p70S6K1 can be oncogenic. In addition to this, nickel can induce the phosphorylation of GSK-3β, FOXO3A, and FOXO1A [180], all of which are AKT targets. GSK-3β phosphorylation releases the oncogenic β-catenin and FOXO3A phosphorylation decreases its pro-apoptotic activity. In addition, inhibition of AKT in nickel-transformed cells decreased expression of the anti-apoptotic proteins Bcl-2 and Bcl-xL [182]. This may suggest nickel carcinogenesis utilizes these mechanisms for cell proliferation and survival. However, further studies are required to understand the role of PI3K-AKT signaling in nickel carcinogenesis.

Cadmium

Our lab and others have shown that cadmium exposure leads to AKT phosphorylation [137-138, 184-191] that is dependent on PI3K activity [138, 191]. Cadmium can lead to phosphorylation of the p85 subunit thereby activating PI3K [137] further suggesting cadmium can activate the PI3K-AKT pathway. In addition, cadmium-transformed cells that display enhanced ability to grow in soft agar and migrate have these abilities significantly reduced with inhibition of AKT [192]. There is some evidence that cadmium-induced activation of AKT can be mediated by multiple molecules. One potential mediator between cadmium and activation of

PI3K and other signaling pathways is ROS. Our lab, and others, have shown cadmium can induce ROS generation [138, 185-186]. Inhibition of ROS reduces activation of AKT by cadmium [138, 185, 186]. Our lab has further shown that chronic cadmium exposure can lead to cell transformation characterized by primary tumor growth *in vivo* and tumor angiogenesis [138]. We also found cadmium can lead to increased HIF-1α and VEGF expression that is dependent on PI3K activity [138]. Other factors may also contribute as cadmium activation of AKT has been shown to be dependent on Src and EGFR kinase activity [191]. As mentioned above, cadmium appears to act on the estrogen receptor. Further evidence of this hypothesis comes from studies showing inhibition or knockdown of estrogen receptor prevents cadmium-induced AKT activation [141, 189]. There is a direct link of the regulatory p85 subunit of PI3K with the estrogen receptor [193] providing a likely mechanism for cadmium-induced PI3K-AKT activation *via* estrogen receptor. These data strongly suggest cadmium can induce cell transformation and tumor angiogenesis and that PI3K-AKT signaling may play a significant role in these processes. Further study is warranted to clarify if cadmium can induce cell migration, invasion, and proliferation, among other cancer cell characteristics and to what extent the PI3K-AKT signaling pathway is involved.

HEAVY METALS AND MTOR/P70S6K1 SIGNALING

Mammalian target of rapamycin (mTOR) has two complexes, mTORC1 and mTORC2, with distinct regulation and function in the cells. mTORC1 contains raptor and PRAS40 with raptor acting as a scaffold protein to link mTOR kinase to substrates. mTORC2 contains rictor, mSin1, and PRR5/protor with rictor and mSin1 promoting mTORC2 assembly and signaling. Both mTORC1 and mTORC2 contain mLST8/GβL and deptor with mLST8/GβL binding to the mTOR kinase domain in both complexes and deptor is an inhibitor subunit to both complexes. mTORC1 mediates protein synthesis, cell proliferation, growth, and metabolism in response to growth factors and changes in nutrient levels [194]. Low levels of growth factors and cell stress reduce mTORC1 action on biosynthesis. Raptor binds directly to mTOR motifs on downstream molecules such as p70S6K1 and 4EBP1 [195-197]. mTORC1 is sensitive to rapamycin whereas mTORC2 is not. TSC1/2 can regulate mTORC1 activity as TSC2 has a GTPase activating domain that converts Rheb to a GDP-bound state preventing its activation of mTORC1 signaling [194, 198]. Alternatively, TSC1/2 are activators of mTORC2 by a yet undefined mechanism. PI3K and MAPK signaling inhibits TSC1/2 allowing increased mTORC1 activity. AMP activated protein kinase (AMPK) is a master regulator of cellular energy metabolism. If AMPK is activated by a high AMP/ATP ratio, AMPK will phosphorylate TSC2 to decrease its inhibition of Rheb further depressing mTORC1 activity [199]. Amino acid insufficiency rapidly induces a decrease in mTORC1 activity but the mechanism is not well understood. There is a well understood feeback loop where mTOR1/p70S6K1 signaling leads to phosphorylation of insulin receptor substrate (IRS-1) uncoupling the insulin receptor from PI3K thereby reducing PI3K signaling [200]. Whereas AKT is an activator of mTORC1, AKT is a substrate for mTORC2 promoting cell proliferation, survival, and migration.

Arsenic

Accumulated evidence suggests arsenic activates mTOR/p70S6K1 signaling. Our lab and others have shown arsenic exposure leads to increased activation of p70S6K1 [123, 154, 155, 163, 164, 169, 201, 202] in an mTOR-dependent manner [155, 164, 169, 201, 202]. Arsenic can also increase the phosphorylation of 4E-BP1 in an mTOR-dependent manner [202] further suggesting arsenic is upregulating this pathway. Furthermore, arsenic increased the binding of eIF4E with 4E-BP1 [202, 203], which is commonly induced by mTOR activation. Another molecule that is regulated by mTOR appears to be HIF-1α as our lab has shown arsenic increases HIF-1 signaling in an mTOR-dependent manner [164].

Signaling through mTOR/p70S6K1 also appears crucial to arsenic-induced cell transformation. Our lab has recently shown that bronchial epithelial cells exposed to arsenic for 26 weeks leads to increased proliferation and anchorage-independent growth [163]. These cells can also form primary tumors *in vivo* (unpublished observations[1]). Both mTOR and p70S6K1 were constitutively activated in these cells and interruption of this pathway reduced both cell proliferation and anchorage-independent growth [163]. These results would suggest the mTOR/p70S6K1 pathway is not only activated with arsenic exposure, but also plays a critical role in arsenic-induced cell transformation.

Multiple mechanisms may mediate arsenic-induced mTOR/p70S6K1 signaling. Our lab has shown that p70S6K1 activation by chronic arsenic exposure is dependent on ROS as catalase could inactivate p70S6K1 under these conditions [163]. Arsenic-induced p70S6K1 activation is dependent on PI3K activity [155, 164, 169, 202, 204], consistent with previous results that AKT can activate mTOR-p70S6K1 signaling [205-209]. ROS may also play a role in arsenic-induced p70S6K1 activation as ROS scavenging prevents arsenic-induced p70S6K1 activation [169, 204]. Calcium chelators also prevented arsenic-induced p70S6K1 activation [169]. MAPK pathways may also participate as a p38 MAPK inhibitor partially inhibited arsenic-induced p70S6K1 activation [202]. Details regarding the mechanism of arsenic-induced activation of mTOR/p70S6K1 signaling are not clear, but these studies suggests upstream signaling pathways mediate mTOR/p70S6K1 activation with arsenic exposure. There is no evidence to this point that would suggest arsenic directly activates mTOR/p70S6K1 molecules.

Chromium

There are no published reports regarding chromium and mTOR/p70S6K1 signaling. Considering chromium has been shown to activate PI3K/AKT signaling [124, 177, 178] and AKT is a well-known activator of mTOR/p70S6K1 signaling [152, 205-209], it is unexpected to see no reports of chromium-induced mTOR activation yet.

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[1]Cells transformed in culture by arsenic exposure were observed to form tumors subcutaneously in nude mice.

Nickel

The only evidence nickel can activate mTOR/p70S6K1 signaling is nickel induction of p70S6K1 phosphorylation [179] but there is no published evidence that nickel activates mTOR or increases its kinase activity. Nickel-induced p70S6K1 activation was prevented with PI3K inhibitors [179], consistent with previous reports showing PI3K/AKT signaling can activate mTOR/p70S6K1 signaling [205-209]. Downstream of mTOR, rapamycin reduced nickel-induced VEGF induction and HIF-dependent transcriptional activity [210]. This is an interesting finding considering nickel can directly induce HIF-1 expression *via* inhibition of prolyl hydroxylases [211]. If mTOR signaling participates in nickel-induced hypoxia signaling, further investigation is needed to understand the role of mTOR in nickel carcinogenesis.

Cadmium

Published reports show that cadmium exposure can lead to phosphorylation of mTOR [186, 187, 212], p70S6K1 [137, 138, 186, 187, 212, 213], and 4E-BP1 [212], which is downstream of mTOR. Our lab has shown cadmium can induce cell transformation and these transformed cells can form primary tumors and induce tumor angiogenesis [138]. We also found cadmium increased expression levels of HIF-1α and VEGF, with HIF-1α expression dependent on p70S6K1 activity [138]. The mechanism by which cadmium activates mTOR and downstream signaling is not clear, but ROS and PI3K signaling likely contributes to that. We found cadmium could induce ROS generation and reduction of ROS levels with either catalase or diphenylene iodonium (DPI) prevented activation of p70S6K1 by cadmium [138]. Others have reported similar findings [186]. We also found cadmium-induced activation of p70S6K1 was dependent on PI3K activity [138], which was expected as PI3K-AKT signaling is known to activate mTOR signaling. Similar finding have been shown elsewhere with overexpressed PTEN, which prevents PI3K signaling, preventing cadmium-induced p70S6K1 activation [186]. These studies provide intriguing evidence for cadmium carcinogenesis considering mTOR and p70S6K1 can be strongly activated by cadmium. However, whether this pathway regulates any aspect of cadmium carcinogenesis is not yet known. Further investigation into the role of mTOR/p70S6K1 signaling in cadmium carcinogenesis is warranted.

CHEMOPREVENTION AND THERAPY

Naturally occurring compounds are often proposed for prevention of disease and some of these compounds may have application to prevention of heavy metal-induced cancers. Apigenin is a naturally occurring flavone that is consumed *via* fruits and vegetables. Our lab has shown apigenin can inhibit cell growth and tumor angiogenesis *via* inhibition of VEGF and HIF-1 [214-217]. We also showed apigenin inhibition of VEGF and HIF-1 was *via* inhibition of PI3K-AKT-p70S6K1 signaling [215]. In addition, apigenin has been shown to inhibit EGFR [218]. Resveratrol is a naturally occurring phytoalexin that exists in the skin of grapes, with lower levels in wine and grape juice, as well as other fruits and nuts. Our lab has shown resveratrol inhibits HIF-1 and VEGF expression in cancer cells [219]. Resveratrol has further been shown to inhibit EGFR-ERK1/2 and PI3K-AKT-mTOR signaling while enhancing anti-tumor effectiveness of the mTOR inhibitor rapamycin [220-222]. In addition, resveratrol can decrease cell proliferation and tumor angiogenesis while also increasing cell apoptosis [223, 224]. Several heavy metals can induce ROS and our lab has shown resveratrol can scavenge oxidative radicals and reduce cellular responses to ROS generation [225]. Curcumin is a naturally occurring polyphenol derived from the Indian spice turmeric. Curcumin has been shown to inhibit several signaling molecules including EGFR, AKT, cyclin D1, NF-κB, and AP-1 among others [226]. Curcumin also decreases cell proliferation, tumor growth, and tumor angiogenesis while increasing apoptosis [224]. Evidence indicates several heavy metals utilize signaling molecules that can be regulated by naturally occurring substances such as apigenin, resveratrol, and curcumin. While the main complication for these compounds is *in vivo* bioavailability, they may provide a viable preventative option to persons with a high risk of heavy metal exposure.

Treatment of cancers formed from exposure to heavy metals is an important area for future research efforts. As mentioned above, EGFR, PI3K, and mTOR signaling are often activated in cells exposed to heavy metals, making these pathways attractive targets for therapy of metal-induced cancer. Several different drugs that antagonize these pathways are currently undergoing clinical trials. Both gefitinib and erlotinib inhibit the EGFR tyrosine kinase domain with some success in clinical trials and may provide therapy to cancers with EGFR activating mutations [227]. Cetuximab is a monoclonal antibody directed against EGFR that may provide a therapeutic option to cancers with EGFR amplification, which has been seen in cells exposed to heavy metals [116, 122, 127]. Many inhibitors to the PI3K pathway have problems with toxicity as they target the ubiquitous α and β p110 isoforms. The drug often used in basic research to target PI3K is LY294002, however this compound has several issues to prevent *in vivo* efficacy. SF1126 is a prodrug of LY294002 designed to be more soluble, target tumor tissue, and have increased tolerability in patients [228]. SF1126 is currently undergoing a Phase I clinical trial to determine its safety and tolerability in patients. Temsirolimus, an ester of the mTOR inhibitor rapamycin, was seen to specifically decrease mTOR activity [229] and improve survival in patients with metastatic renal-cell carcinoma compared to interferon-α [230]. mTOR has negative feedback on AKT activation, thus inhibition of mTOR increases activity of AKT providing a possible mechanism for resistance to mTOR inhibition [231]. This led to development of compounds such as NVP-BEZ235, which is an mTOR-PI3K dual inhibitor [232]. NVP-BEZ235 has shown effectiveness with treating tumors in animals [233-236], and is currently in clinical trials for solid tumor types. Considering the propensity for some heavy metals to augment signaling through EGFR, PI3K, and mTOR, these compounds may be useful in patients with cancers from heavy metal exposure.

FUTURE PROSPECTUS

Literature clearly shows exposure to heavy metals alters flux through signaling pathways, but future research should

answer how this signaling relates to metal-induced cancer and how metals are activating these pathways. Metals could directly activate these pathways such as nickel replacing iron in prolyl hydroxylases leading to stabilization of HIF-1α protein [211]. However, instances of direct activation of signaling molecules are infrequent with different metals. Much effort in future research will likely focus on cellular changes that lead to increased signaling such as metal-induced epigenetic alterations and changes in microRNA expression. Several metals have shown to induce chromatin alterations, which could lead to overexpression and potential amplification of certain genes that are part of proliferative signaling pathways. While ongoing research on miRNA function and targets is plentiful, there are still considerable discoveries to be made about the contribution of miRNAs to normal cell function as well as its role in disease development such as cancer. Heavy metal exposure is likely to alter expression of certain miRNAs leading to changes in signaling causing cellular changes leading to a cancer cell phenotype. Another interesting set of molecules are free radicals and reactive oxygen and nitrogen species. Several heavy metals induce oxidative stress, but the precise role that plays in heavy metal-induced cancer and the mechanisms by which oxidative stress is increased are not well understood. To elucidate promising drug targets, molecular targets must be studied for their ability to control an *in vivo* tissue response such as tumor initiation, tumor growth, and tumor angiogenesis when exposed to heavy metals.

CONFLICT OF INTEREST

The authors confirm that there is no conflict of interest in this article.

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ABBREVIATIONS

DPI	=	Diphenylene Iodonium
EPA	=	Environmental Protection Agency
IARC	=	International Agency for Research on Cancer
MCL	=	Maximum Contaminant Level
NAC	=	N-Acetyl Cysteine
ROS	=	Reactive Oxygen Species
RTK	=	Receptor Tyrosine Kinase
UV	=	Ultraviolet

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