TOXICOLOGICAL HIGHLIGHT

Potential Clinical Significance of EGFR-Mediated Signaling following Inorganic Arsenic Exposure in Human Lung

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In the late 1960s, arsenic-rich drinking water was found to induce carcinogenesis, and arsenic contamination became an international environmental health issue. The pleiotropic nature of arsenic-induced toxicity has become evident due to several epidemiological studies which have demonstrated and defined exposure levels relevant to human health. Evidence from these epidemiological studies suggests that arsenic simultaneously causes the promotion and progression of several diseases, including lung cancer (National Research Council, 1999). In addition to the ability of arsenic alone to induce carcinogenesis, there is also compelling evidence that arsenic acts synergistically with other carcinogens, such as tobacco or polycyclic aromatic hydrocarbons (IARC, 2004; Rosman, 2003). The complexity of the interaction of arsenic with other carcinogens, coupled with the evidence supporting multiple modes of actions, makes the mechanisms surrounding arsenic-induced carcinogenesis in the human lung unclear. Arsenic has been shown to be a potent activator of diverse signal transduction pathways even at subcytotoxic exposures. Until recently, few models of low-level arsenical-induced cancer have been established in animals, so in vitro methods have been widely used to study subcytotoxic arsenic exposures.

In this issue of Toxicological Sciences, Andrews et al. identifies the involvement in both Beas-2B cells and human lung tumors of the epidermal growth factor receptor (EGFR) pathway following exposure to arsenic. This comparison between in vitro methods and human lung tissue makes this study a valuable contribution to the investigation into the mode of action behind arsenical-induced lung cancer. The highlighted article by Andrews et al. reports the activation of the EGFR (EGFR/ErbB1/HER1) pathway by arsenic at contamination levels which are pertinent to the U.S. population. In this study, arsenic exposure (0.01–10μM sodium arsenite) was shown to increase levels of EGFR phosphorylation, increase the levels of the pro-ligand heparin-binding EGF (HB-EGF), and also increase the protein levels of downstream cyclin D1, and extracellular signal-regulated kinase (p-ERK) in human bronchial epithelial cells (Beas-2B cells).

EGFR belongs to the ErbB/HER-family of tyrosine kinase receptors, a class of transmembrane proteins which are activated after binding with peptide growth factors of the EGFR family of proteins. Several peptide growth factors show binding affinity to EGFR: EGF, transforming growth factor alpha, amphiregulin, betacellulin, HB-EGF, epiregulin, and heregulins. Following ligand binding, a conformational change is induced and EGFR homo- or heterodimerizes with other members of the HER-family of proteins. Through these different interactions, the EGFR family of proteins maintains a diverse network of signal transduction pathways in combination with other transmembrane receptors (Bianco et al., 2007).

The EGFR pathway has been identified to be important in both the regulation of normal cell processes such as growth as well as the induction of human cancers. During formation of a malignancy, EGFR plays a pivotal role in increased cell proliferation, dysregulation of apoptotic cell death, angiogenesis, and metastatic spread due to the numerous signal transduction pathways which are activated following EGFR phosphorylation (Bianco et al., 2007). One of the most widely studied downstream pathways to EGFR is that of Ras/Erk signaling cascade. Several studies have identified the activation of this pathway following iAs exposure at levels relevant to those seen in drinking water (< 10μM) in multiple tissue types, including lung, bladder, and prostate (Barchowsky et al., 1999; Drohna et al., 2002; Simeonova et al., 2002). Persistent EGFR activation, such as that which occurs following chronic, low-level arsenical exposure, can lead to aberrant signaling and constitutively increased EGFR protein levels. High levels of EGFR protein have been identified in several solid human tumors, including those of the lung. Several studies have established that EGFR expression is prevalent in non–small cell lung carcinoma, the tissue type studied by Andrews et al. (Graus-Porta et al., 1997).

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Andrews et al. (2009) utilized several well designed studies to identify the interaction between As(III) exposure at relevant levels (0.01–10 μM, or 0.75–75 μg/l), EGFR activation and downstream mitogen-activated protein kinase (MAPK) signal transduction. By utilizing both the Beas-2B cell line, a well established human lung epithelial cell line, as well as tumor tissue from histologically confirmed non–small cell lung cancer (NSCLC), a link between low-level arsenic exposures and cancer induction in humans has been identified. By using subcytotoxic concentrations of As(III), not cytotoxic concentrations, the authors provided data which has significant bearing on the mechanism of arsenic carcinogenesis in the lung.

Andrews et al. (2009) showed that EGFR degraded following EGF but not As(III) treatment, and that As(III) increased the amount of phosphorylated EGFR at 1, 5, and 10 μM As(III). In addition, the Beas-2B cells exposed to 5 μM As(III) and the EGFR-tyrosine kinase inhibitor (TKI), Tarceva, no longer showed increased phosphorylation at Tyr 1173. By utilizing the EGFR TKI, the authors further validated the importance of EGFR activation following arsenic exposure, as this is a currently marketed reversible TKI which is approved for the treatment of patients with NSCLC (Bianco et al., 2007). By using a currently marketed drug and studying its interactions with arsenic-induced signal transduction, this study provides a beginning for the investigation into clinical utility of this treatment for As(III) associated lung tumors.

Following the identification of increased EGFR phosphorylation, the authors begin investigating downstream pathways that are being activated in Beas-2B cells by arsenical exposure. Levels of cyclin D1, which can be regulated by the EGFR pathway, were elevated after 3 h exposure to As(III). Again, addition of Tarceva blocked the effects of As(III) exposure by inhibiting the TK activity of EGFR and blocked the increased cyclin D1. Defects in cyclin D1 expression by As(III) are important to investigate as dysregulation of cyclin D1 and cell cycle control may allow some cells to overcome cell cycle checkpoints and may contribute to carcinogenesis by continual cell cycle progression.

In order to further determine the specific pathway activation, and how arsenic is triggering the EGFR protein, the highlighted article describes a study to identify the ligand responsible for the activation and the downstream pathways that are activated following As(III) exposure. As(III) induced the expression of the EGFR pro-ligand HB-EGF and triggered increased phosphorylation of Erk. Erk phosphorylation following arsenical-induced EGFR signaling is not limited to lung, as other studies have identified this pathway and its importance in bladder cells as well (Eblin et al., 2007; Simeonova et al., 2002). Since arsenical-induced MAPK signaling through the EGFR receptor has been reported following long-term exposure to As or its metabolites in multiple tissues, this pathway appears to be a common mechanism of action and also provides clinical implications for treatment options.

The final portion of the study by Andrews et al. (2009) was to determine if there was a correlation between arsenic concentration in human toenails and the amount of EGFR protein present in the NSCLC tumor samples. Immunohistochemistry identified a significantly higher percent of cells which stained positive for EGFR protein in the specimens from individuals with elevated arsenic identified in the toenail clippings. By using logistic regression, the authors demonstrated an association between arsenic exposure with the increased EGFR protein staining.

The results from the study by Andrews et al. (2009) have several implications as EGFR can activate many diverse signal transduction pathways. They show both the relationship between EGFR activation and As(III) exposure in human tumor samples, as well as in vitro studies which show that the Food and Drug Administration–approved Tarceva inhibits the activation of EGFR by As(III) exposure. By both identifying the relationship between As(III) exposure and EGFR phosphorylation in cell culture and human lung tumor specimens, the authors provide the basis for several additional studies. Further identification of the key players in the arsenic-induced EGFR signaling need to be identified, as well as how As(III) exposure triggers the increase in HB-EGF. The role of cyclin D1 in the arsenic associated cancer should also be identified. Further immunohistochemistry of tumor samples should be able to identify if there is an association between cyclin D1 expression and arsenical exposure. Another important finding of this study is the lack of degradation of EGFR following As(III) activation. This is in contrast to the rapid degradation that occurs following EGF exposure, suggesting that As(III) is also inhibiting some step in the degradation pathway, another area of future study. This highlighted study provides the basis to begin investigating the clinical utility of using EGFR-TKIs to treat arsenic associated cancers of the lung or possibly other tissues.

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REFERENCES


