Building an Adverse Outcome Pathway Network for Arsenic-Induced Bladder Cancer (Poster 2)

Ingrid L. Druwe1, J. Allen Davis2, Lyle Burgeno3, Jeff Gift1, Ila Cote1, Janice S. Lee1

1 EPA, Office or Research and Development, National Center for Environmental Assessment – Research Triangle Park. 2 EPA, Office or Research and Development, National Center for Environmental Assessment – Cincinnati. 3 United States Army Engineer Research and Development Center, 3001 Halls Ferry Road, Vicksburg, MS 39180

Purpose and Scope

➢ 2015 Inorganic Arsenic (iAs) Assessment Development Plan laid out plans to:

  - Develop network analyses for endpoints considered to be causally or likely causally associated with specific adverse outcomes. Based on National Research Council (NRC) recommendations, extensive Mode of Action (MOA) analysis were also conducted for bladder cancer to better understand human variability and the possible use of mechanistic data to inform low dose extrapolation.

  - The utility of these analyses were evaluated in the context of EPA’s 2005 Cancer Guidelines recommendations on use of MOA framework to address:

    - Human relevance of animal tumor responses: MOA analyses are usually applied for chemicals with insufficient human data. iAs is a chemical with a large amount of epidemiological evidence. Hence, MOA is not needed for establishing human relevance.

    - Differences in anticipated response among humans: extensive information of risk modifiers in humans are available in the epidemiologic database. Hence, a MOA analysis to address potential differences in response across human populations was not considered essential.

  - Decisions about the anticipated shape of the dose-response relationship: Given the availability of low dose epidemiological studies, mechanistic data (which is largely based on animal and in vitro studies) is not considered critical for low dose extrapolation. However, as recommended by NRC, EPA inventoried mechanistic evidence (Protocol, Appendix A) and conducted a case study MOA analysis for idiopathic bladder cancer to assess its utility for reducing uncertainties in dose-response analysis. Bladder cancer was selected due to its extensive evidence base as compared to other priority iAs health outcomes.

Adverse Outcome Pathway Network (AOPn) Development

In order to develop network analyses we decided to use the Adverse Outcome Pathway (AOP) framework. AOPs are chemically agnostic representations that identify the sequence of biochemical events required to produce an adverse effect or outcome. AOPs begin with a molecular initiating event (MIE) and link to a series of key events (KE) that traverse biological complexity starting at the molecular level, through cellular, organ, and organismal events and culminate in an adverse outcome (AO).

Step 2: Identifying Arsenic-specific Modification in the Bladder Cancer Network

➢ After establishing a general disease-based network for bladder cancer; information on arsenic-specific alterations in the pathway was integrated from published literature on arsenic-induced bladder cancer, principally derived from epidemiological, in vivo, and in vitro studies that analyzed effects of iAs or its metabolites (e.g., monomethylarsonic acid (MMAIII) and dimethylarsinous acid (DMAIII)) in vitro when the test system is known not to have metabolizing capability) at concentrations ≤ 100 µM. The postulated bladder cancer AOPn (Figure 3) indicates activation of the FGFR and HRAS oncogenes, as well as activation of the ErbB2 receptor as molecular initiating events (MIE) in the progression of bladder carcinoma. Activation of Ras was identified as a key event (KE). Activation of Ras triggers a number of molecular events such as stimulation of the MAPK, VEGF, PI3K-AKT, and JAK/STAT pathways which culminate in cell proliferation, angiogenesis, cell survival, and ultimately bladder tumor formation.

➢ Evaluating the arsenic-specific evidence in relation to the disease-based bladder cancer AOPn, we identified several KE in iAs-induced bladder carcinoma. Specifically, iAs may activate Ras signaling through production of reactive oxygen species (ROS), imbalance of oxidative signaling, or through activation of the ErbB2 receptor and lead to cell proliferation, angiogenesis and metastasis. Ras activation was also identified as a KE in the progression of idiopathic bladder carcinoma.

➢ Additionally, iAs-produced ROS can damage DNA and lead to p53 dysregulation, stimulation of matrix metalloproteinases (MMPs), and ultimately angiogenesis and metastasis (Figure 3, Table 1).

Step 1: Establishing the Disease-Based Biological Pathway for Bladder Cancer Development in Humans

➢ To delineate a postulated mode of action for arsenic-induced bladder cancer, the molecular basis for bladder tumor development, irrespective of a specific chemical insult, was first established.

➢ The information for building this AOPn was principally derived from current literature reviews.

➢ Several key events were identified in the progression of bladder cancer; including activation of the Ras-MAPK, PI3K and JAK-STAT pathways. Activation of these pathways was associated with genetic alterations in the HRAS and FGFR oncogenes that induced constitutive activation of these genes. For example, Ras was identified as a key event (KE) in the progression of bladder carcinoma (Figure 1).

➢ The AOPn was compared to the KEGG (Kyoto Encyclopedia of Genes and Genomes) database for bladder carcinoma in humans to ensure concordance (see Figure 2).

Conclusions

➢ The bladder cancer-based AOPn framework to support the iAs MOA was created using literature reviews of bladder cancer idiopathic disease as a starting point.

➢ Information from published literature on arsenic induced bladder cancer was integrated into the bladder cancer AOPn and nodes in the network that arsenic acted upon were identified. In this way, we created a bladder cancer-based AOP analysis of iAs MOA (Figure 3; Table 1).

➢ While the MOA evaluation identified arsenic-specific mechanisms and risk modifiers likely to increase risk of human bladder cancer, the impact and utility of mechanistic information on dose-response analyses was minimal.

➢ Much of the primary MOA evidence is based on in vitro studies which raises concerns about their applicability to informing low-dose effects.

➢ Ample epidemiological data is available for dose-response, and many studies included observations down to US background exposure levels.

➢ Conducting a similar analysis for other prioritized outcomes is hindered by the lack of a complete MOA for any health outcome and the likelihood that most, if not all, health outcomes associated with arsenic exposure involve multiple interactive MOAs.

References can be found in HERO (https://heroe.epa.gov/heroe/index.cfm/project/page/project_id/22313).

Figure 1: AOPns for idiopathic bladder cancer in humans.

Figure 2: KEGG pathway for human bladder cancer induction.

Table 1. Representative evidence and references where iAs has been shown to affect the AOPn in bladder cancer.

<table>
<thead>
<tr>
<th>AOPn Event</th>
<th>Evidence</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53 Mutation</td>
<td>Increased protein expression of p53 in vitro</td>
<td>El-Shaikh et al. 2006; El-Shaikh and Running 2007</td>
</tr>
<tr>
<td>Cell proliferation, cell survival, angiogenesis</td>
<td>Increased gene expression related to angiogenesis and cell survival</td>
<td>Cheng et al. 2011; Cote et al. 2012</td>
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Figure 3: Postulated AOPn for iAs-induced bladder cancer in humans.

The majority of the evidence comes from research groups that examined immunohistochemical and/or metabolism studies of arsenic species tested in these biological systems were varied but predominantly include skin, lung, and bladder (to which arsenic exposure is particularly sensitive).