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

Ingrid L. Druwe[1], J. Allen Davis[2], Lyle Burgoon[3], Jeff Gift[1], Ila Cote[1], Janice S. Lee[1]

[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, 3909 Halls Ferry Road, Vicksburg, MS 39180

Ingrid L. Druwe | email druwe.ingrid@epa.gov | 919-541-2452

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 frameworks 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 organism effects and culminate in an adverse outcome (AO).

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 **(see Figure 1).**
- Inactivation of key tumor suppressor genes, p53 and Rb1, were identified as key events (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).**

Adverse Outcome Pathway Network (AOPn) Development

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., monomethylarsonous acid (MMAIII) and dimethylarsonous 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).**

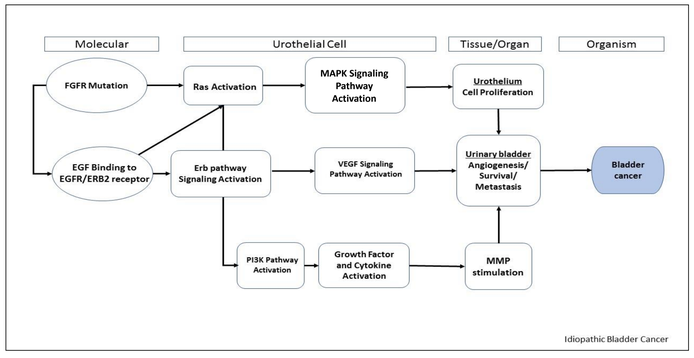

Figure 1: AOPn for idiopathic bladder cancer in humans.

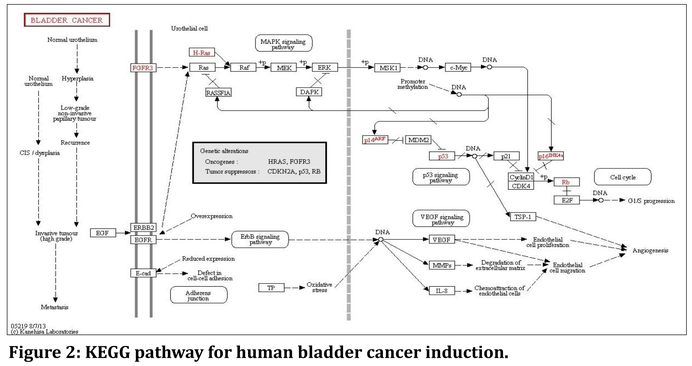

Figure 2: KEGG pathway for human bladder cancer induction.

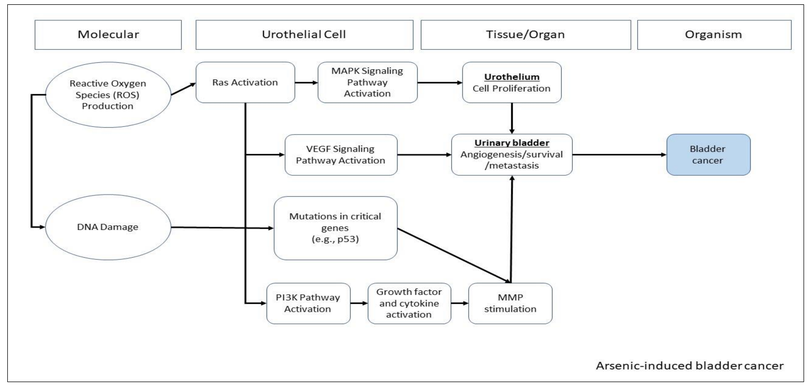

Figure 3: Postulated AOPn for iAs-induced bladder cancer in humans.

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

Key Event	Evidence	References
EGFR, ErbB2 activation	• Upregulation of EGFR in human urothelial cell line (UROtsa) following chronic exposure to iAs metabolite MMA(III) (50nM)	• Eblin et al., 2007
	• Upregulation of EGFR in vivo	• Simeonova et al 2002 • Simeonova and Luster 2002
Oxidative stress, ROS generation; imbalance of oxidative signaling	• Activation of AP-1, NFkB in vitro	• Felix et al., 2005 • Barchowsky et al., 1996 • Kaltreider et al., 1999 • Wijeweera et al., 2001
	• ROS generation from iAs & its metabolites (eg., DMA) lead to oxidative stress in vivo	• Yamanaka et al 1990, • Yamanaka et al 1989
	• ROS generation from iAs & its metabolites (eg., DMA) lead to oxidative stress in vitro	• Liu et al., 2001, • Hei et al 1998, • Wang et al 2001
Ras signaling; MAPK activation	• Activation of MAPK signaling in human urothelial cell lines	• Bailey et al., 2012 • Wang et al., 2013 • Eblin et al., 2007
	• Increased expression of MAPK proteins in mouse bladder at 0.01% arsenite (in vivo)	• Luster and Simeonova, 2004
p53 Mutation	• Increased protein expression of p53 in vitro	• Naranmandura et al., 2011 • Huang et al., 2004 • Flora et al., 2011
Metallotheoionein activation	• Increased metallotheoionein transcriptional expression in human urothelial cell lines	• Eblin et al., 2006, • Eblin et al., 2008, • Wnek et al., 2011 • Clewell et al., 2011
Cell proliferation, cell survival, angiogenesis	• Increased gene expression related to epithelial-to-mesenchymal transition, inflammation, DNA damage, apoptosis/survival and proliferation in vitro and in vivo	• Yager et al 2013 • Clewell et al., 2011 • Flora et al., 2011 • Gentry et al., 2010 • Clewell et al., 2014 • Vizcaya-Ruiz et al., 2009

The majority of the evidence comes from research groups that examined immortalized human urothelial cell lines and human bladder cancer cell lines (UROtsa, EJ-1), although evidence for gene expression changes in rodent bladder are also available. Disruption of the pathway and signaling has been demonstrated at the level of transcriptional expression as well as protein expression. The arsenic species tested in these biological systems were varied but predominantly include iAsIII and MMAIII (to which UROtsa cell lines are particularly sensitive).

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://hero.epa.gov/hero/index.cfm/project/page/project_id/2211)