TOXICOLOGICAL HIGHLIGHT

Backing into Cancer: Effects of Arsenic on Cell Differentiation

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The article highlighted in this issue is “Sodium Arsenite Inhibits and Reverses Expression of Adipogenic and Fat Cell-Specific Genes during in Vitro Adipogenesis” by E. M. Wauson, A. S. Langan, and R. L. Vorce (pp. 211–219).

Although the carcinogenicity of arsenic in humans has been unequivocally demonstrated (IARC, 1980) and limited evidence of animal carcinogenicity is available (Kitchin, 2001), an understanding of the mechanism(s) responsible for these effects remain elusive. Among the numerous studies performed to gain a better understanding of mechanisms of arsenic carcinogenicity, a few have involved analyses of potential contributions from intracellular metabolism (including reduction and methylation of arsenic), induction of oxidative stress, depletion of glutathione, and the generation of reactive oxygen species. In contrast, many more studies have focused primarily on genetic mechanisms. While arsenic fails to induce mutations in bacteria or Chinese hamster cells (Rossman et al., 1980), it does cause gene amplification (Lee et al., 1988) and sister chromatid exchange (Lerda, 1994). It has been suggested that these clastogenic and gene expression dysregulating effects might be due to modulation of normal DNA methylation patterns (Mass and Wang, 1997; Zhao et al., 1997; Zhong and Mass, 2001) arising, in part, from altered repair of methylated bases (Lee-Chen et al., 1993) or increased methylation of cytosine (Yamanaka et al., 1997). Though not a direct mutagen, arsenic is also known to act as a comutagen with select agents (i.e., UV radiation, X-rays, alkylating agents) (Jha et al., 1992). This comutagenic effect is apparently mediated by inhibition of DNA repair (Abernathy et al., 1999) arising from effects of arsenic on DNA ligases II and I (Li and Rosman, 1989).

Clearly, damage to DNA and/or effects upon RNA transcription/translation present an acceptable grouping of mechanisms that explain, in part, how environmental carcinogens act to bring about their ultimate pathologies. However, for an atypical carcinogen like arsenic that is neither a classic initiator nor a promoter, many investigators have concluded that alternative approaches to study cell transformation and eventually cancer needed to be postulated. One alternative viewpoint that evolved was that in order to explain the carcinogenicity of arsenic, a basic examination of macrocellular processes that might be perturbed by exposure, including regulation of cell proliferation or responsiveness to exogenous signals, needed to be undertaken in appropriate in vitro systems or using cells from arsenic-exposed hosts.

One tenet that became a basis for studies in support of these macrocellular pathways was that cancer could be viewed as an imbalance that arises in the cellular homeostatic control over two antagonistic processes—proliferation and differentiation. The relationship between these processes is complex. For example, any cell that is differentially competent will normally remain undifferentiated in the presence of proliferative stimuli; however, these same cells will undergo differentiation and arrest of proliferation in the presence/absence of appropriate biologic signals. Since proliferation is halted during cell differentiation, it is reasonable to conclude that exiting from the cell cycle is absolutely essential; in fact, for cells to undergo differentiation, cells must exit the cycle at Go/G1 and enter into mitogenically quiescent states (Scott et al., 1982; Wier and Scott, 1986). Thus, as noted in the highlighted article and in previous publications (Trouba et al., 2000a,b), if an agent is able to uncouple these antagonistic processes, block differentiation outright, and/or inhibit differentiation by preventing arrest in the required stage of the normal cell cycle, these cells could then be maintained in mitogenically responsive states. Under this scenario, the cells would display increased proliferative activities, a characteristic evident in the earliest stages of most cancers.

It is therefore not surprising that the effect of arsenic on both processes has recently been receiving considerable attention. Normally, inhibition of proliferation involves inhibition (by transcriptional targets of p53, including Gadd45 and p21) of Cdc2, a cyclin-dependent kinase whose activation is required for a cell to enter mitosis. Under acute exposure scenarios, significant induction of growth inhibitory protein p53 (and to a lesser extent p21) was noted in different cell types following arsenite treatment (Salazar et al., 1997; Vogt and Rossmann,
As both p53 and p21 have short half-lives and are degraded within proteosomes, and the inhibitor MG132 was found to induce both p53 and p21 in these same cells, it was hypothesized that acute exposure to arsenic may interfere with the cell cycle and proliferation, through blockage of normal proteosomal function(s). An earlier series of studies showing that arsenite inhibits two steps in the ubiquitin-dependent proteolytic pathway (Klemperer and Pickart, 1989) provides suggestive evidence for that particular mechanism. This effect on p53 and p21 could help to explain the increasing utility of arsenic compounds as anticancer agents in clinical trials. For example, treatment of newly diagnosed and relapsed patients with acute promyelocytic leukemia with arsenic trioxide has been found to result in remission rates of 85–93% (Wang, 2001). The ability of an acute exposure to arsenic to block proliferation, and in effect, shift the balance toward differentiation, is borne out in studies that have shown that treatment with arsenic at low doses could trigger differentiation of blood cells (Calleja and Warrell, 2000; Zhang et al., 2001).

Interestingly and in contrast, as a result of long-term arsenic exposure (more akin to what would occur under real-world exposure scenarios), p21 levels did not increase in cells in response to radiation; moreover, arsenite was found to suppress radiation-induced p21 induction, but did not affect induction of p53 (Vogt and Rossman, 2001). Wauson et al. (2002) found that arsenic blocks the normal regulation of p21 during a process in which it is normally upregulated, adipogenesis. As yet, it is not clear whether similar mechanisms are involved in arsenic-induced p21 suppression in response to radiation or during cellular differentiation.

In addition to suppression of p21 induction during adipocyte differentiation, it was demonstrated that long-term arsenic treatment resulted in inhibited expression of important transcription factors, including C/EBPα (CCAAT-enhancer binding protein-α), which is part of a positive feedback loop in the regulation of the expression of a “master switch” in adipogenesis, nuclear receptor PPARγ (peroxisome proliferative activated receptor-γ), and the adipocyte differentiation marker fatty acid binding protein aP2 (Wauson et al., 2002). Although the study clearly showed that arsenic exposure led to reductions in expression of PPARγ, whether the observed effects on adipogenesis might be related to an induction of cellular kinases, specifically mitogen-activated protein (MAP) kinases, which can lead to phosphorylation (and hence, inactivation) of this receptor (Hu et al., 1996) also were analyzed. Using the MEK-1 and -2 specific inhibitor U0126 in conjunction with one of several known mitogenic stimuli, they found that the presence of the inhibitor did not block the ability of arsenic to inhibit adipogenesis. Based upon these results, it was suggested that arsenic-induced increases in cell proliferation could be the result of a shift away from normal differentiation, and that the latter is more likely due to direct mechanisms (i.e., effects on molecular events) involved in differentiation processes rather than the result of an induced ability to maintain cells in a mitogenically competent state.

Overall, this highlighted work clearly provides evidence supporting the proposition that inhibition of differentiation could be one mechanism by which arsenic might cause increased cell proliferation and thus promote carcinogenesis. Interestingly, however, some of the results also provide data in support of another potential mechanism where arsenic may exert its carcinogenicity by altering normal cell receptor biology. For example, in a finding critical for determining how skin cancer may be induced by exposure, arsenic was shown to cause decreased β2-adrenergic receptor expression (without affecting affinity) in keratinocytes (Chang et al., 1998). A β2-adrenergic-adenylate cyclase-cAMP system in epidermal tissue is thought to play a crucial role in proliferative and differentiative homeostasis; therefore, reduced β2-adrenergic receptor expression might result in increased skin cell proliferation. This would be in keeping with other proliferation-based skin diseases (i.e., psoriasis) wherein β2-adrenergic receptor hypersensitivity is evident. In addition, in studies to discern underlying reasons why MAP kinases were routinely activated in arsenic-exposed cells (Wu et al., 1999; Trouba et al., 2000), Chen et al. (1998) showed that arsenic caused an increase in the phosphorylation of epidermal growth factor (EGF) receptors. Though the study did not directly examine EGF receptor expression or binding activity, the results clearly indicated that the initial phosphorylation event was essential for subsequent activation of the MAP signal cascades. Lastly, a recent study by Chen et al. (2001) has provided suggestive evidence that altered receptor biology/expression might be a factor in arsenic carcinogenicity. Using microarrays, it was demonstrated that genes for receptors for follicle-stimulating and growth hormones were downregulated, while that for estrogen was upregulated, in arsenic-transformed cells. The latter finding was not unexpected as earlier studies indicated that arsenic induced uterine hyperplasia and increased estrogen receptor expression in situ (Waalkes et al., 2000).

Though not pertaining to cancer in terms of the process of cell transformation per se, but of import to the disease process in situ, the cited microarray data also provided evidence of differential effects of arsenic on several genes for cytokine receptors. Cell receptors for interleukin-2, -10, and -15, and to a much lesser extent, tumor necrosis factor-α were shown to be downregulated; conversely, those for IL-1, -6, and -7 receptors were all increased. These alterations in cytokine receptor expression are in agreement with the results of studies of immune system cells from individuals with arsenic-induced Bowen’s disease. In these patients the ability of the affected host to launch immune responses against the transformed cells is altered by effects upon the inducible expression of mononuclear cell interleukin-2 receptors (IL-2R, α and β subunits). This results in a state wherein the immune cells of these patients are hypersensitive to IL-2 and less able to undergo immunoproliferation and differentiation (Yu et al., 1998).
Even with the important information provided by this highlighted study, it is clear that future studies are still needed to address the effects of arsenic on cell differentiation and to advance our understanding of the cellular and molecular effects that are at the root of the carcinogenicity of arsenic.

REFERENCES


