DNA Damage Response Dysregulation in Keratinocytes Chronically Treated with Low Dose Arsenic

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Abstract

Background: Chronic arsenic exposure results in multi-organ cancers, skin being the major target. Arsenic is a clastogen and exposure to arsenic leads to double strand break (DSB) accumulation, an important step in carcinogenesis[1,2]. The Ataxia-telangiectasia mutated (ATM) protein is an apical kinase that is required for high-fidelity DSB DNA damage response recruitment. Following successful DSB repair, phosphatases (PP2A and PP5) dephosphorylate ATM.

Hypothesis: Arsenic exposure suppresses DSB repair response leading to accumulation of double-strand DNA breaks. To examine this hypothesis, the DSB repair response was analyzed in immortalized human keratinocytes (HaCaT and Ker-CT cells) chronically and acutely exposed to sodium arsenite (iAs^{3+}).

Materials and Methods: HaCaT and Ker-CT cells were chronically exposed to iAs^{3+} (0.1 μM; 7 and 8 weeks respectively). HaCaT cells were also acutely exposed to increasing doses of iAs^{3+} (0-5 μM; 24 h). Cell lysates were collected and subjected to immunoblot analysis for ATM (activated and total), its downstream protein targets (CHK2, TP53, CDKN1A; activated and total) as well as phosphatases (PP2ACA, PPP2R2C, PP5). Densitometric data was analyzed employing unpaired student's two-tailed t-test with a Welch's correction (chronic exposure) or One-way ANOVA followed by Tukey's multiple comparisons post-hoc test (acute exposure).

Results: ATM phosphorylation was significantly reduced upon chronic iAs^{3+} exposure in both cell lines. Such exposure also led to induction of PPP2R2C and PP5C (mRNA and protein) in HaCaT cells but suppression of PPP2R2C and PP5C (protein) in Ker-CT cells. Acute iAs^{3+} exposure in HaCaT cells induced PPP2R2C protein expression.

Conclusions: Chronic iAs^{3+} exposure suppressed ATM activation irrespective of the cell line tested. Suppressed ATM activation may lead to an impaired DSB repair response that acts as a harbinger for iAs^{3+}-induced skin carcinogenesis. However, the phosphatase expression profile is opposite in the two cell lines, suggesting that ATM activation might be modulated by upstream regulators rather than phosphatases exclusively.

Background

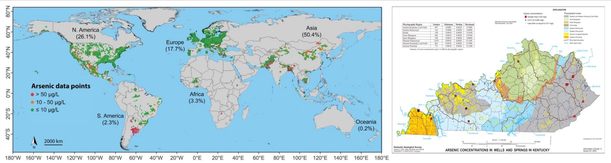


Figure 1. Arsenic contamination in drinking water worldwide and Kentucky[3,4].

- Arsenic exposure occurs primarily through contaminated drinking water that exceeds the World Health Organization (WHO) and Environmental Protection Agency (EPA) maximum contaminant level concentration (10 ug/L) (Figure 1)
- Chronic exposure to low doses of arsenic induces cardiovascular and pulmonary disease, diabetes, and multi-organ cancers, including skin cancer (Figure 2)
- Arsenic is a clastogen – leads to accumulation of chromosomal aberrations

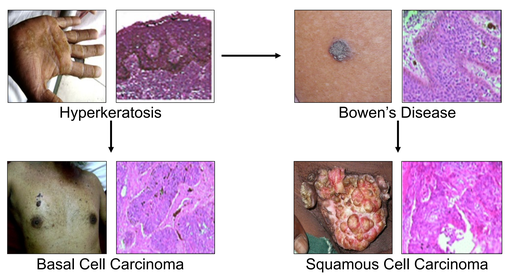


Figure 2. Chronic arsenic exposure can lead to the development of skin cancer[5].

Background

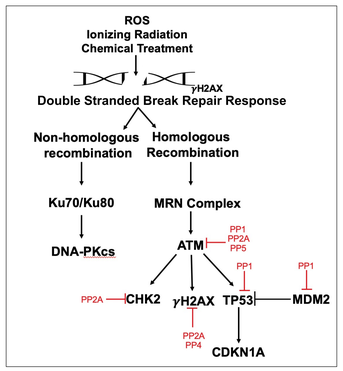


Figure 3A. Two pathways involved in the DSB repair response

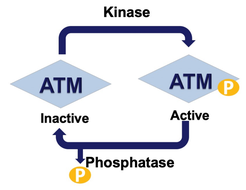


Figure 3B. Catalytic regulation of ATM activation

Hypothesis and Aims

Hypothesis: **Arsenic exposure suppresses the DSB repair response leading to accumulation of double-strand DNA breaks.**

Aims:

- Determine whether the double-strand DNA repair response is perturbed in cells chronically or acutely treated with toxicologically relevant concentrations of iAs^{3+}.
- Assess whether commonalities exist between double-strand DNA repair responses across keratinocyte cell lines as a result of chronic iAs^{3+} exposure.

Materials & Methods

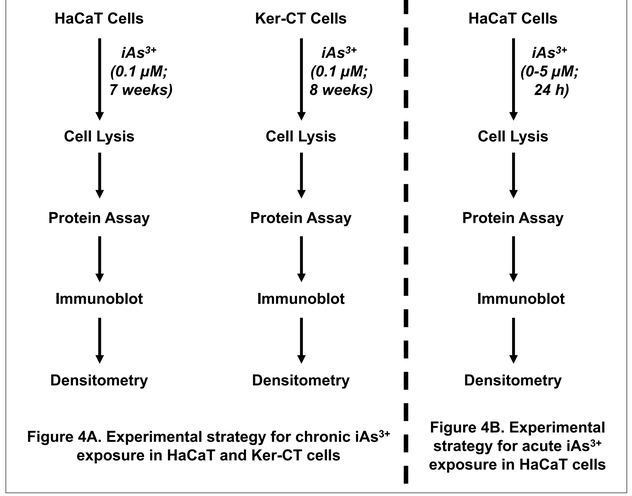


Figure 4A. Experimental strategy for chronic iAs^{3+} exposure in HaCaT and Ker-CT cells

Figure 4B. Experimental strategy for acute iAs^{3+} exposure in HaCaT cells

Results

ATM pathway activation is suppressed by chronic iAs^{3+} exposure in HaCaT cells

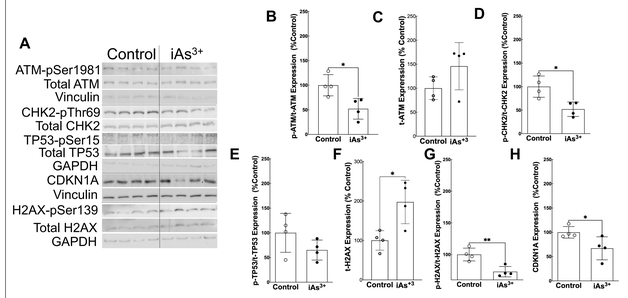


Figure 6 A. Immunoblots for ATM pathway molecules in chronically iAs^{3+} exposed HaCaT cells. B-H. Densitometric analysis for activated ATM (B), total ATM (C), activated CHK2 (D), activated TP53 (E), total H2AX (F), activated H2AX (G) and CDKN1A (H). *p<0.05, **p<0.01 by unpaired, two-tailed t-test with Welch's correction.

Chronic iAs^{3+} exposure induces expression of phosphatases in HaCaT cells

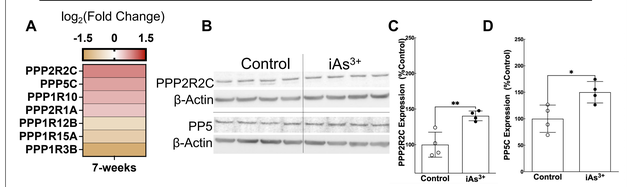


Figure 7A. Modulation of mRNA expression of phosphatases by chronic iAs^{3+} exposure. B. Immunoblots for selected phosphatases C-D. Densitometric analysis for PPP2R2C (C) and PP5 (D). *p<0.05, **p<0.01 by unpaired, two-tailed t-test with Welch's correction.

Chronic iAs^{3+} exposure suppressed ATM activation but induces phosphatase expression in Ker-CT cells

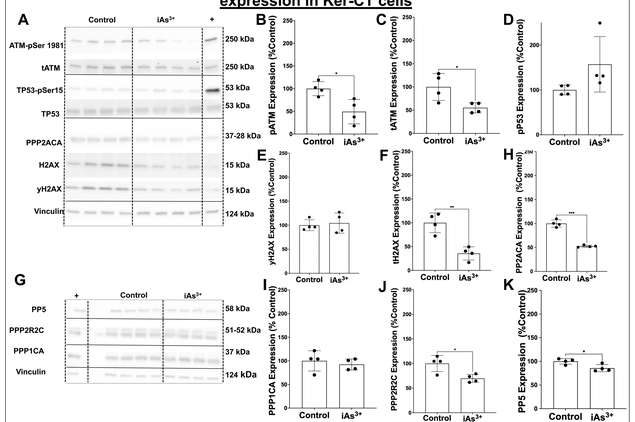


Figure 8 A. Immunoblots for ATM pathway molecules in chronically iAs^{3+} exposed Ker-CT cells. B-F. Densitometric analysis for activated ATM (B), and total ATM (C), activated TP53 (D), activated H2AX (E) and total H2AX (F). G. Immunoblots for selected phosphatases. H-K. Densitometric analysis for PP2ACA (H), PPP1CA (I), PPP2R2C (J) and PP5 (K). *p<0.05, **p<0.01, ***p<0.001 by unpaired, two-tailed t-test with Welch's correction (Panels B-F and H-K).

Results

Acute iAs^{3+} exposure does not alter ATM activation or phosphatase expression in HaCaT cells

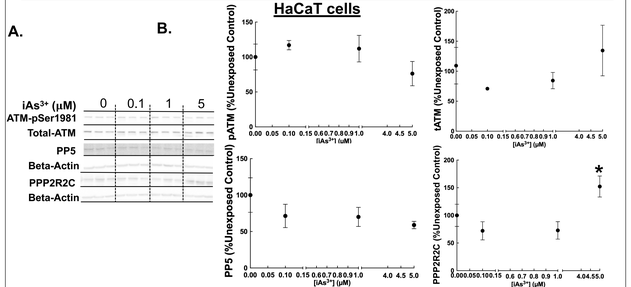


Figure 9A. Immunoblots for ATM (phosphorylated and total) and selected phosphatases in HaCaT cells exposed acutely to increasing doses of iAs^{3+}. B-E. Densitometric analysis for activated ATM (B), total ATM (C), PP5 (D) and PPP2R2C (E). *p<0.05 for One-way ANOVA with Tukey's multiple comparisons post-hoc test.

Conclusions

Reduced ATM activation due to chronic iAs^{3+} exposure is a common mechanism across two keratinocyte models

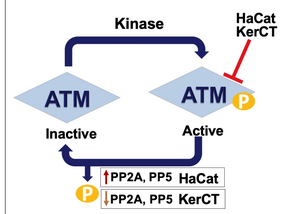
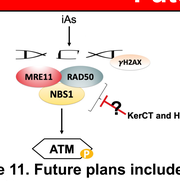


Figure 10. Schematic representation of reduced ATM activation in HaCaT and Ker-CT cell lines by iAs^{3+} exposure

- **Suppression of ATM activation is a common mechanism across two different keratinocyte cell lines**
- **Lack of correlation with phosphatase expression suggest upstream mechanism**
- **Suppression of ATM activation likely leads to impaired DNA repair response and accumulation of DSB, explaining the clastogenic effect of chronic iAs^{3+} exposure**

Future Directions



- RAD50: binds DNA following DSB
- NBS1: activates ATM
- MRE11: binds RAD50 and NBS1

Figure 11. Future plans include investigating how chronic iAs exposure affects the MRN complex, a key player required for ATM activation.

References

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Acknowledgements

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