Arsenic and hsa-miR-186 Overexpression Impair the DNA Damage Response Pathway in Human Keratinocytes

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Abstract

Background: Chronic arsenic exposure in drinking water is a global public health problem affecting >200 million people. Arsenic exposure induces cardiovascular disease and cancer. Skin is a major target organ for arsenic and skin cancers are common in people chronically exposed to arsenic. Several mechanisms of arsenic-induced carcinogenesis have been proposed, including inhibition of DNA repair, miRNA dysregulation, oxidative stress and aneuploidy. Our data indicate hsa-miR-186 is overexpressed in As-induced squamous cell carcinoma (SCC) relative to As-induced hyperkeratosis (HK). We investigated whether elevated hsa-miR-186 could affect any of these proposed mechanisms. **Hypothesis:** Chronic As exposure induces hsa-miRNA-186 dysregulation that targets the DNA damage response pathway components which could lead to carcinogenesis. **Methods:** Potential targets of hsa-miR-186 were investigated using DIANA-miRPath. HaCaT transfected with pEP-hsa-miR-186 expression vector or empty vector were maintained with puromycin in the media to select stable clones. Three hsa-miR-186 overexpressing clones and three empty vector clones with low hsa-miR-186 expression were maintained with 0 or 100 nM $NaAsO_2$ for 2 months. Total RNA was purified and hsa-miR-186 expression levels assessed by RT-qPCR. Cell lysates were collected and western blot assay performed to evaluate hsa-miR-186 target proteins levels. Data were analyzed by two way ANOVA; significant difference at $p<0.05$. **Results:** DIANA-miRPath analysis suggested that ATM and CDKN1A, which are DNA damage response pathway components, are targeted by hsa-miR-186. ATM levels were decreased in both hsa-miR-186 overexpressing clones and arsenic-exposed empty vector clones, but levels were mixed in arsenic-exposed hsa-miR-186 overexpressing clones. Arsenic exposure induced phosphorylation of TP53 on serine 15 in both empty vector transfected clones and hsa-miR-186 overexpressing clones. CHK1 and CDKN1A levels remained constant under all conditions. **Conclusions:** The results suggest that both arsenic and hsa-miR-186 overexpression interfere with components of the DNA damage response pathway. This dysregulation can negatively impact DNA repair capability and cell cycle control. These effects could explain apparent inhibition of DNA repair by arsenic exposure.

Introduction

- Arsenic contamination in groundwater is a global public health issue (Fig. 1A)
- > 200 million people in over 70 countries are exposed
- Chronic exposure to arsenic leads to skin tumors
- hsa-miR-186 is overexpressed in As-induced Squamous Cell Carcinoma relative to pre-malignant As-induced Hyperkeratosis (Fig.1B)

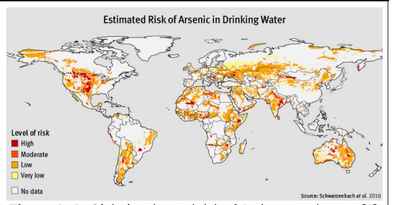

Figure 1. A. Global estimated risk of As in ground water [1].

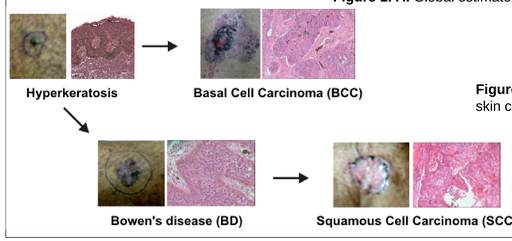

Figure 1.B. Arsenic-induced skin cancer progression [2].

DNA Damage Response Pathway

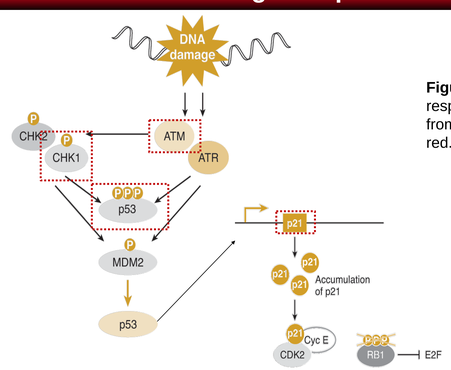

Figure 2. DNA damage response pathway [3]. Targets from investigation are boxed in red.

Hypothesis

Chronic As exposure induces hsa-miR-186 dysregulation that targets the DNA damage response pathway components which could lead to carcinogenesis

Methods: Overview

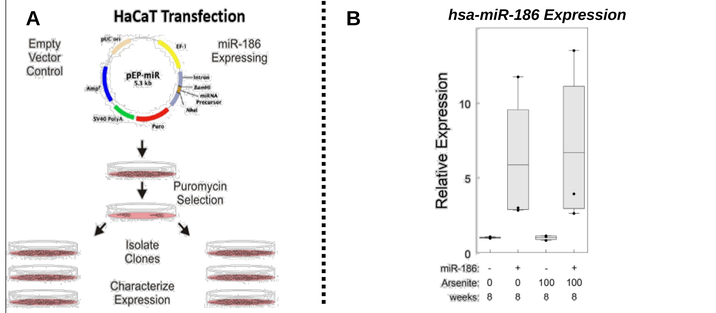

Figure 3. Methods overview. **A.** Plasmid construction and cell transfection; clones were exposed to 0 or 100 nM of As^{3+} for 8 weeks **B.** hsa-miR-186 expression and clone characterization

Results: hsa-miR-186 Targets

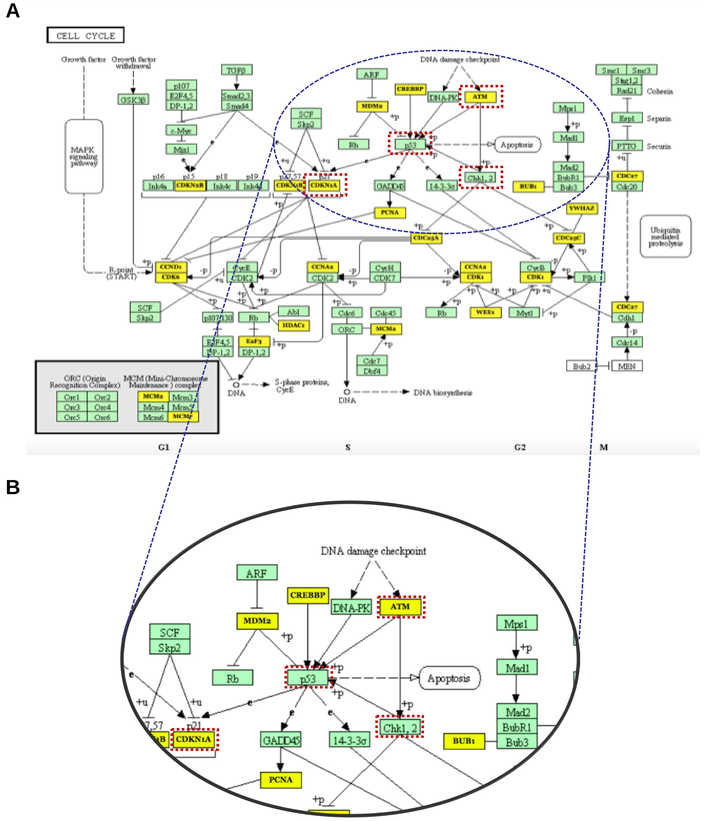

Figure 4. A. Targets of hsa-miR-186-5p in the cell cycle pathway identified using the DIANA miRpath3 results **B.** Magnification of the DNA damage response pathway components targeted by hsa-miR-186 [5].

Results

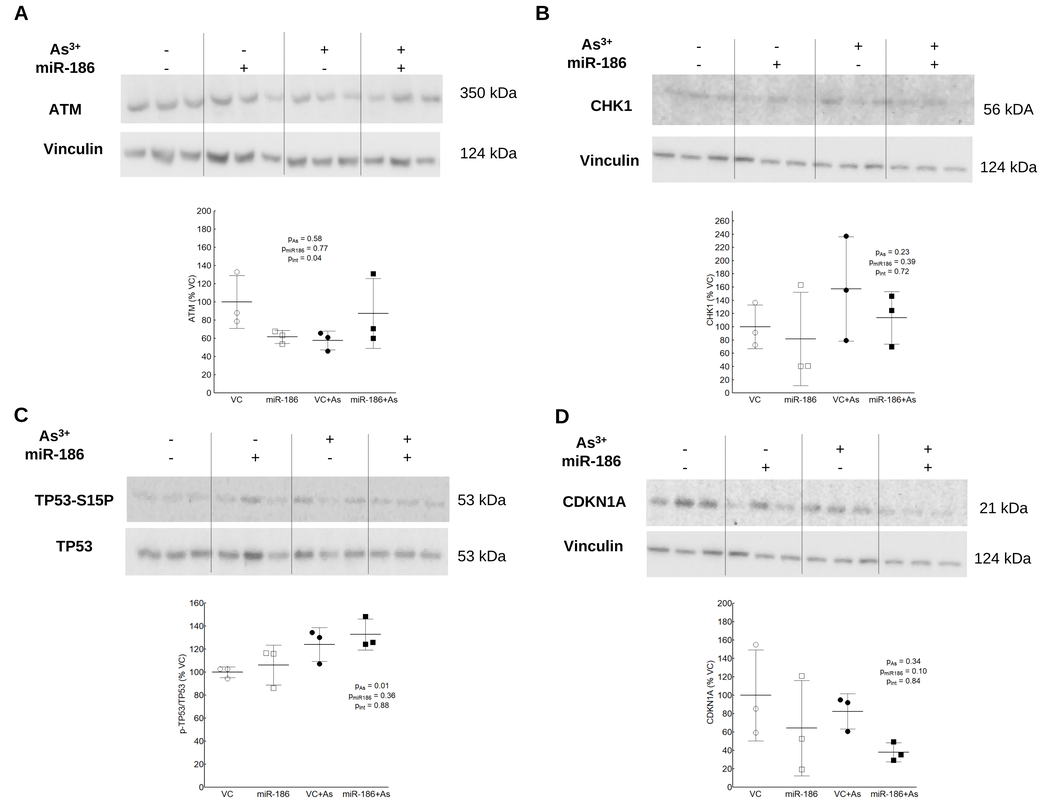

Figure 5. Investigation of the DNA damage response pathway components by Western Blot **A.** ATM **B.** CHK1 **C.** TP53-S15P, TP53, **D.** CDKN1A. hsa-miR-186 and As^3 both reduce ATM. As^{3+} induces TP53 phosphorylation in vector controls and in hsa-miR-186 overexpressing clones. CDKN1A is not increased in response to phosphorylation of TP53. Two way ANOVA followed by Bonferroni post-hoc test.

Discussion and Future Endeavors

- ATM is an apical kinase in the DNA Damage Response Pathway
- ATM phosphorylates targets such as TP53 which then can induce CDKN1A expression
- ATM levels are decreased in hsa-miR-186 overexpressing cells supporting predicted targeting of ATM by hsa-miR-186
- Arsenic induces phosphorylation of TP53 but CDKN1A response is mitigated by hsa-miR-186 overexpression supporting predicted targeting of CDKN1A by hsa-miR-186
- Arsenic and hsa-miR-186 overexpression interfere with some components of the DNA Damage Response Pathway
- To further investigate the effects of arsenic exposure and hsa-miR-186 overexpression, future endeavors include investigating other predicted hsa-miR-186 targets in the DNA damage response pathway such as DNA Pol/beta and MDM2, and exploring alternate mechanisms of TP53 and CDKN1A regulation

References

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