UV and Arsenic Exhibit Differential Modulation of MAPK Pathways in HaCaT and Ker-CT Cell Lines

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Abstract

Background: Skin cancer is very common across the US and world. UV exposure via sunlight and arsenic exposure via drinking water are the two most common skin carcinogens. However, differences in tumor development suggest differences in mechanisms of carcinogenesis. Previous studies and preliminary data have also shown that UV and arsenic may differently modulate activation of ERK, a mitogen-activated protein kinase (MAPK) that is often dysregulated in carcinogenesis. ERK, as well as p38, are involved in phosphorylating transcription factors like ELK-1, which helps regulate growth processes in keratinocytes. Our goal was to understand better the effects of acute UV and arsenic exposure by performing a comparison of modulations in ERK and p38 MAPK signaling across two keratinocyte models, HaCaT and Ker-CT.

Hypothesis: Sunlight and arsenite induce carcinogenesis via differential modulation of keratinocyte model signaling pathways.

Methods: HaCaT and Ker-CT cells were grown in culture for these experiments. For UV, cells were exposed to UVC from 0 to 20 J/m^2 and lysed after 6 h. For arsenic, cells were exposed to sodium arsenite from 0 to 100 μM for 2 h before lysis. Lysates were examined via immunoblotting for phosphorylated and total ERK, ELK-1, p38, GAPDH, and vinculin expression. Ratios of phosphorylated to total proteins were normalized to controls. Statistical analysis of treatments was performed via ANOVA followed by Tukey multiple comparison posttest. Differences were significant at p<0.05.

Results: In all experimental conditions, ERK and ELK-1 phosphorylation levels opposed each other. In response to each skin carcinogen, ERK and ELK-1 phosphorylation responses were not constant between cell lines. p38 phosphorylation was not induced in UV-treated HaCaT and KerCT cells, nor As^{3+}-treated Ker-CT cells, but a pattern of p38 phosphorylation mimicking ELK-1 phosphorylation was seen in As^{3+}-treated HaCaT cells.

Conclusions: Opposing phosphorylation of ERK and ELK-1 with either skin carcinogen suggests modulation of ELK-1 by modulated signaling components independent of ERK. Modulated p38 may enhance ELK-1 signaling in As^{3+}-treated HaCaT cells, but other known ELK-1 modulators need to be explored. Different responses to both UV and arsenic between cell lines suggests differences in molecular pathways of HaCaT and Ker-CT models and necessitates a comparative study against primary human keratinocytes.

Background

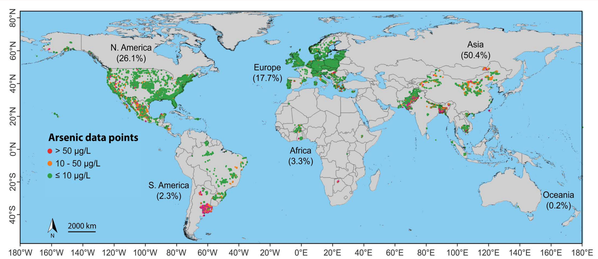


Fig 1A. Recorded concentrations of arsenic in groundwater across the world [1]

	Arsenic	Sunlight
Where on the body?	Palms of hands, soles of feet	Sun-exposed
Actinic keratosis	No	Yes
Hyperkeratosis	Yes	No
Basal cell carcinoma	Yes	Yes
Bowen's lesions (SCC in situ)	Yes	Rare
Squamous cell carcinoma	Yes	Yes
Malignant melanoma	No	Yes

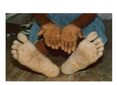


Fig 1B. Differences between UV- and arsenic-driven skin carcinogenesis

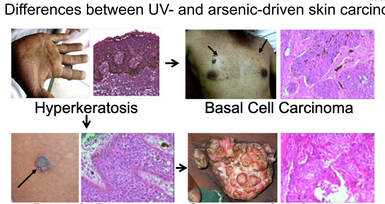


Fig 1C. Carcinogenic progression of arsenic-induced skin lesions [2]

Hypothesis

Sunlight and arsenite induce carcinogenesis via differential modulation of keratinocyte model signaling pathways

Objectives

- Determine if there are differences between the patterns of ERK1/2, ELK-1, and p38 phosphorylation induced by acute UVC and arsenite exposure.
- Investigate whether the responses to UVC and arsenite are constant between HaCaT and Ker-CT cell lines.

Materials & Methods

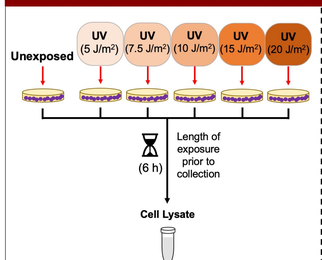


Fig 3A. Exposure of HaCaT and Ker-CT cells in duplicates to increasing doses of UVC (UV), followed by 6 h incubation (37 ˚C) prior to lysis

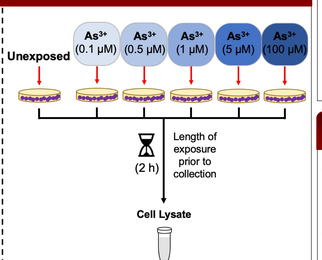


Fig 3B. Exposure of HaCaT and Ker-CT cells in triplicates to increasing doses of sodium arsenite (As^{3+}) for 2 h prior to lysis

Antibodies Used (1:1000 dilutions):

- Phosphorylated-ERK (**p-ERK**; CST #9101)
- Total-ERK (**t-ERK**; CST #9102)
- Phosphorylated and total-ELK-1 (**p-ELK-1** and **t-ELK-1**; CST #9182)
- **GAPDH** (CST #5174)
- Phosphorylated-p38 (**p-p38**; CST #9201)
- Total-p38 (**p38**; CST #9202)
- **Vinculin** (CST #13901)
- **Anti-Rabbit HRP** (1:2000; CST #7074)
- **Anti-Mouse HRP** (1:2000; CST #7076)

Background (cont.)

	Published Data	Preliminary Data
Skin Carcinogen	UV	As^{3+}
Exposure	Acute	Chronic
Cell Line	NTERT	HaCaT
Effect on ERK signaling	Decreased	Increased (at 19 weeks)

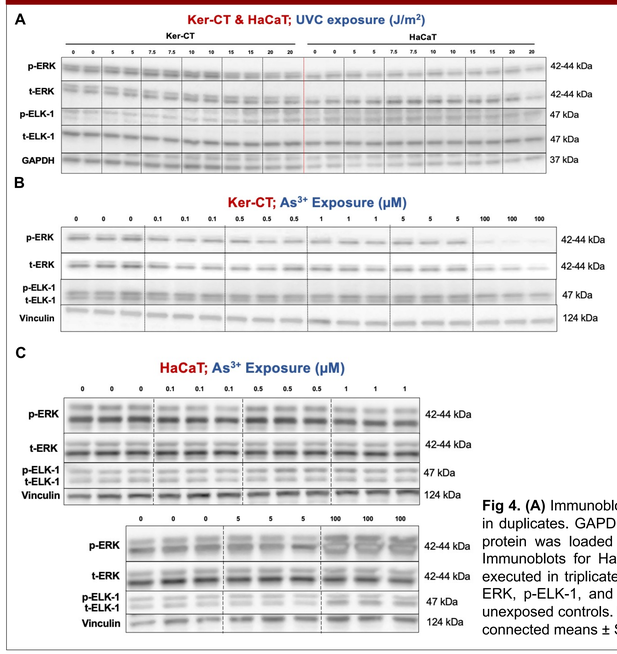
Fig 2A. Comparison of ERK phosphorylation with UV or As^{3+} exposure

Fig 2B. Overview of ERK1/2 MAPK cascade and ELK-1 activation

Immortalized Skin Cell Models		
	HaCaT	**Ker-CT**
In-vitro Model	Well-established	Recently developed
Origin	From margin of adult male melanoma	Neonatal foreskin
Immortalized?	Yes	Yes
Chromosomal Alterations	Pseudo-tetraploid	Near diploid

Fig 2C. Comparison of utilized skin cell models, HaCaT and Ker-CT

Results: UV- and As^{3+}-induced modulation of ERK and ELK-1 phosphorylation is not constant between cell lines



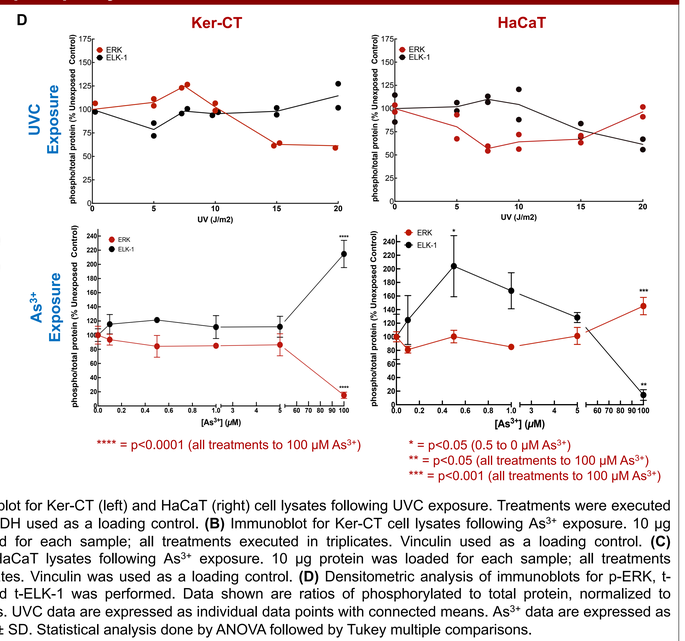


Fig 4. (A) Immunoblot for Ker-CT (left) and HaCaT (right) cell lysates following UVC exposure. Treatments were executed in duplicates. GAPDH used as a loading control. **(B)** Immunoblot for Ker-CT cell lysates following As^{3+} exposure. 10 μg protein was loaded for each sample; all treatments executed in triplicates. Vinculin used as a loading control. **(C)** Immunoblots for HaCaT lysates following As^{3+} exposure. 10 μg protein was loaded for each sample; all treatments executed in triplicates. Vinculin was used as a loading control. **(D)** Densitometric analysis of immunoblots for p-ERK, t-ERK, p-ELK-1, and t-ELK-1 was performed. Data shown are ratios of phosphorylated to total protein, normalized to unexposed controls. UVC data are expressed as individual data points with connected means. As^{3+} data are expressed as connected means ± SD. Statistical analysis done by ANOVA followed by Tukey multiple comparisons.

Results: As^{3+}-treated HaCaT cells express similar modulation of p38 and ELK-1 phosphorylation

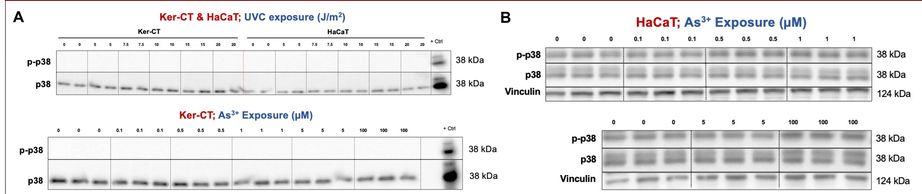
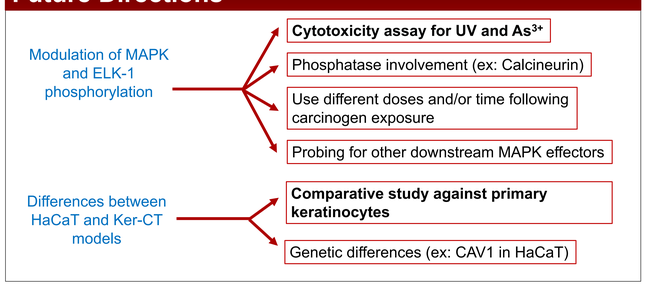


Fig 5. (A) Immunoblots for Ker-CT and HaCaT cell lysates following UVC exposure (top) and Ker-CT cell lysates following As^{3+} exposure (bottom). Cells treated with 50 mJ/cm^2 UVC followed by 30 min recovery served as positive control for p-p38 signal. **(B)** Immunoblots for HaCaT cell lysates following As^{3+} exposure. 10 μg protein loaded for each sample; all conditions in triplicates. Vinculin used as a loading control. **(C)** Densitometric analysis of As^{3+}-HaCaT blots for p-p38 and p38 was performed. Data shown are ratios of phosphorylated to total protein, normalized to unexposed controls. ELK-1 data shown are from blots in Fig 4C. Data are expressed as connected means ± SD. Statistical analysis done by ANOVA followed by Tukey multiple comparisons.

Conclusions

- ELK-1 is modulated by signaling components independent of ERK that are also modulated by UV and arsenic.
- In arsenic-treated HaCaT cells, enhanced ELK-1 signaling may be in part modulated by p38 activity, but this does not account for the patterns of ELK-1 signaling seen with the other experiments.
- Different signaling responses to UV and arsenic between HaCaT and Ker-CT models necessitates the need for a comparative study against MAPK responses in primary human keratinocytes.

Future Directions



References

1. Podgorski J, Berg M. Global threat of arsenic in groundwater. *SCIENCE*. 2020;368(6493):845-+. doi:10.1126/science.aba1510
2. Al-Eryani L, *et al.* miRNA expression profiles of premalignant and malignant arsenic-induced skin lesions. PLoS One. 2018; 13(8):e0202579.

Acknowledgements

This research was supported in part by the National Institute of Health grants R01ES027778, P30ES030283 and R25CA134283.