Zinc Mitigates Arsenic-Induced Dysregulation of ZRANB2 Splice Function

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Abstract

Background: Chronic arsenic exposure in drinking water is a global issue that affects over 225 million people. Arsenic is a Group I carcinogen and directly linked to both basal and squamous cell carcinomas. There are multiple hypotheses around the mechanism of arsenic-induced carcinogenesis, one of which being dysregulation of alternative splicing. An apical protein involved in alternative slice site regulation is ZRANB2. Previously, we showed that arsenic could displace the zinc in the zinc fingers of ZRANB2. This displacement led to upregulated ZRANB2 protein levels and disrupted ZRANB2 splice function in HaCaT cells. Additional data from that project showed that zinc could displace arsenic and restore ZRANB2 folding in a cell-free environment. Taken together, these data suggest an equilibrium between zinc and arsenic for the binding of the two zinc finger motifs of ZRANB2.

Hypothesis: Zinc supplementation can shift the equilibrium between zinc and arsenic in favor of zinc binding to the zinc fingers of ZRANB2 in HaCaT cells, restoring its structure and function.

Methods: HaCaT cells were supplemented with 1mM zinc acetate one hour prior to acute exposure with 100 nM sodium arsenite (iAs). Cell lysates were obtained six hours after arsenic exposure. ZRANB2 and TRA2ß protein levels were evaluated by immunoblotting. RNA isolation and Reverse Transcription PCR using primers for TRA2ß transcript was used to evaluate ZRANB2 splice function. PCR products were eluted from the gel and cloned in a TA vector. Clones were screened using PCR and the inserts sequenced. Immunoblotting and RT-PCR data were analyzed via two-way analysis of variance (ANOVA) followed by Tukey's post hoc test. p-values less than 0.05 were considered significant.

Results: Immunoblotting experiments showed that zinc supplementation significantly reduced the arsenic-induced expression of ZRANB2. Zinc supplementation restored the β3:β1 TRA2β isoform ratio, which was lowered after iAs exposure. Sequencing results from the cloning experiment confirmed β1 and β3 transcripts of TRA2β.

Conclusions: Zinc supplementation prevents arsenic-induced dysregulation of ZRANB2 expression and splice function.

Background: I

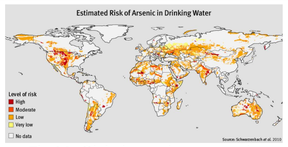


Fig 1A. Global arsenic exposure[1]

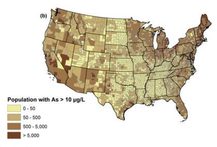


Fig 1B. US population exposed to arsenic[2]

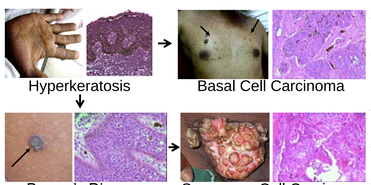


Fig 1C. Arsenic-induced skin cancer progression[3]

- Arsenic is a Group I human carcinogen
- >225 million people in ~70 countries are exposed; 2.9 million in USA
- Variety of associated diseases and malignancies
- Second leading cause of skin cancer: First is sunlight
- Etiology: Not dependent on sunlight exposure

Background: II

- ZRANB2 is an apical splice site regulator with two C4 zinc finger motifs
- iAs displaces zinc from ZRANB2 zinc finger motifs, inducing ZRANB2 expression and suppressing its splice function
- Zinc can displace iAs bound to ZRANB2 zinc finger motifs, suggesting an equilibrium[4]

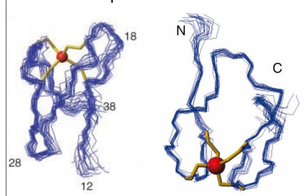


Fig 2A. ZRANB2 Zinc Finger Motifs[5,6]

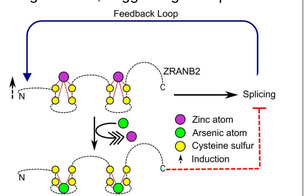


Fig 2B. Effect of As^{3+} on ZRANB2 Expression and Splice Function[4]

Hypothesis & Objectives

Zinc supplementation can shift the equilibrium between zinc and arsenic in favor of zinc binding to the zinc fingers of ZRANB2 in HaCaT cells, restoring its structure and function.

- Does zinc supplementation prevent the arsenic-induced expression of ZRANB2?
- Does zinc supplementation prevent the arsenic-induced decrease in the ratio of β3 to β1 isoforms of TRA2β?

Methods: Experimental Design

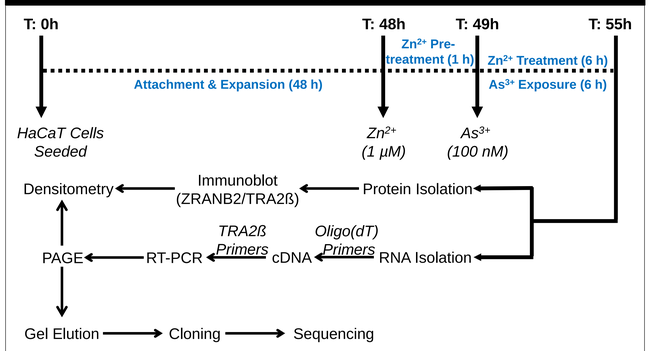


Fig 3. Experimental design to determine the effect of zinc supplementation on iAs-induced dysregulation of ZRANB2 expression and splice function

Results: Zn^{2+} Reverses As^{3+} Mediated ZRANB2 Induction

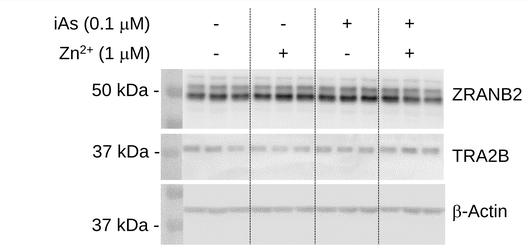


Fig 4A. Immunoblot Analysis of ZRANB2 and TRA2B

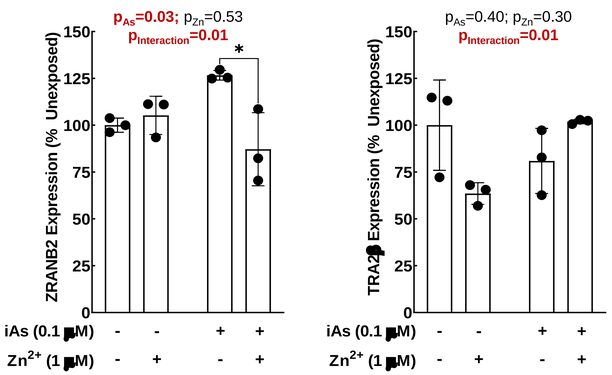


Fig 4B. Densitometric Analysis of ZRANB2 and TRA2ß expression (*p<0.05 by Tukey's post-hoc test following two-way ANOVA)

Results: Zn^{2+} Restores ZRANB2 Splice Function

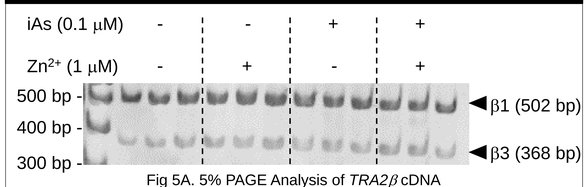


Fig 5A. 5% PAGE Analysis of *TRA2β* cDNA

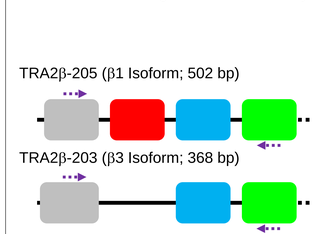


Fig 5C. PCR primer location[4]

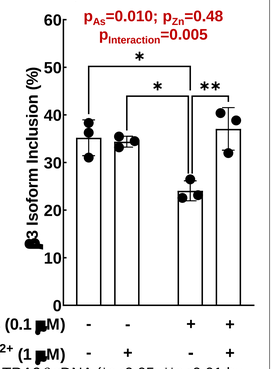
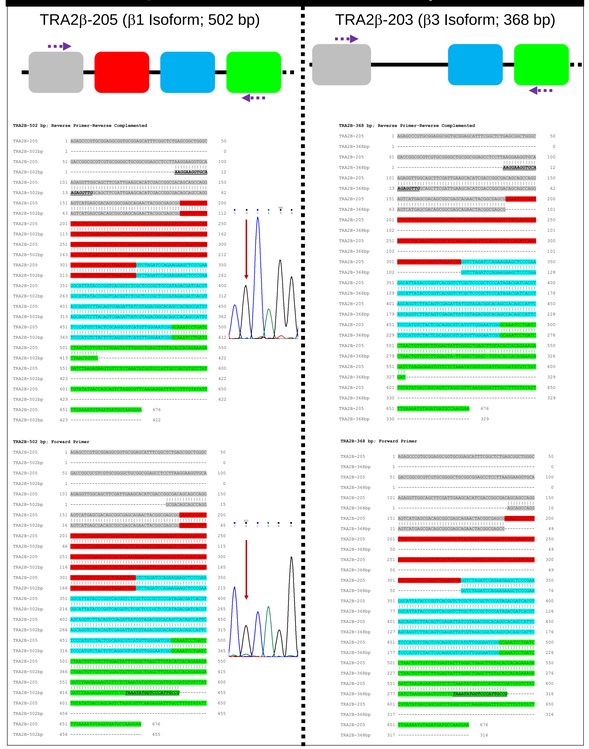


Fig 5B. Densitometric Analysis of *TRA2β* cDNA (*p<0.05; **p<0.01 by Tukey's post-hoc test following two-way ANOVA)

Results: Sequence Validation of TRA2β Isoforms



Conclusions

- Zinc supplementation prevents arsenic-induced dysregulation of ZRANB2 expression and splice function
- Zinc supplementation could be a mitigation strategy for exposed population

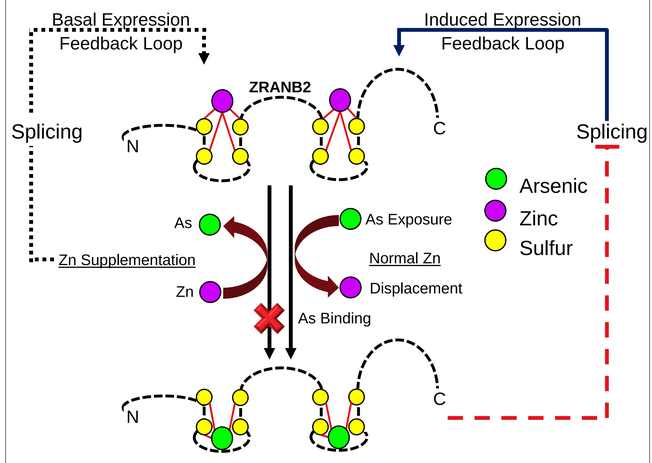


Fig 7. Zinc Supplementation Prevents iAs-Induced Dysregulation of ZRANB2 Expression and Splice Function

Future Directions

- Quantification of iAs and zinc bound to ZRANB2 in vitro with and without Zn supplementation employing immunoprecipitation and ICP-MS
- Examine whether iAs exposure disrupts global splicing profile of ZRANB2 and if Zn supplementation can restore it to the basal level
- Repeat in a different cell line: Ker-CT

References

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Acknowledgements

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