CHANGES IN GENE EXPRESSION PROFILE OF HUMAN HEPATOCELLULAR CARCINOMA CELL LINE (HepG2) INDUCED BY BORIC ACID AT HALF MAXIMAL INHIBITORY CONCENTRATION (IC_{50})

Ayşegül Tombuloğlu[1], Hülya Çöpoğlu[2], Tülin Güray[2], Yeşim Aydın Son[1]
[1]Middle East Technical University, Graduate School of Informatics, Health Informatics Department
[2]Middle East Technical University, Biological Sciences Department

MIDDLE EAST TECHNICAL UNIVERSITY
INFORMATICS INSTITUTE
BIOINFORMATICS PROGRAM

ABSTRACT

Boron is an essential micronutrient for many biological processes in plants and animals. In humans, adverse health outcomes are reported for both boron deprivation and excessive boron intake. Humans might get exposed to high doses of boron by consuming boron containing products or by working at boron enriched workplaces. In this study, the aim is to find out about the cytotoxicity mechanisms of boric acid, the most abundant form of boron containing compounds in human blood, on human hepatocellular carcinoma (HepG2) cell line. XTT cell proliferation assay was carried out to assess the effect of boric acid treatment at varying concentrations on the viability of HepG2 cells and to determine the half maximal inhibitory concentration (IC_{50}) of boric acid. Comet assay and microarray experiments were performed at IC_{50} concentration to examine boric acid induced DNA damage and changes in gene expression profile. Boric acid inhibited cell growth in a dose dependent manner with an IC_{50} value of 24 mM. Microarray analysis revealed a total of 785 gene as differentially regulated in response to boric acid treatment. Analyses of differentially expressed transcripts for enriched gene ontologies and pathways using Database for Annotation, Visualization and Integrated Discovery (DAVID) Functional Annotation Tool suggest cell cycle arrest at mitotic phase and controlled cell death as potential mechanisms of inhibited cell growth. Our overall findings suggest, at IC_{50} concentration boric acid might be inhibiting DNA replication and possibly interfering with lipid and amino acid metabolism.

METHODS

Cell Culture

XTT Cell Proliferation Assay
- 0-40 mM Boric acid
- 24 hours incubation
- Absorbance at 415 nm

Alkaline Comet Assay
- 24 mM Boric acid
- 24 hours incubation
- Untreated complete medium: negative control
- Hydrogen peroxide (40 µM) : positive control
- Tail moment

RNA Isolation and cDNA Preparation

Microarray Experiments
- 24 mM Boric acid
- 24 hours incubation
- 6 Affymetrix Human Gene 1.0 S.T arrays
- 3 hybridizations : BA treatment
- 3 hybridizations: Untreated control

Quality Control of Microarray Data

Microarray Data Preprocessing
- Limma package[1]
- RMA summarization
- Only «main» probeset category is kept.

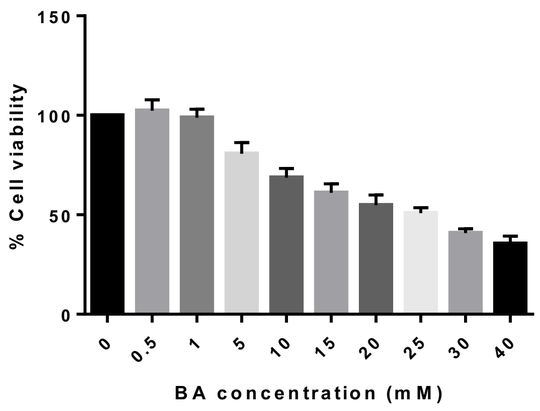
Identification of Differentially Expressed Genes
- e-bayes moderated t-test
- Limma package[1]
- BH adjusted p<0.05
- FC>2

Pathway Enrichment Analysis
- DAVID Online Bioinformatics Platform[2] -KEGG Pathways
- Upregulated and downregulated genes as separate lists
- BH adjusted p<0.05

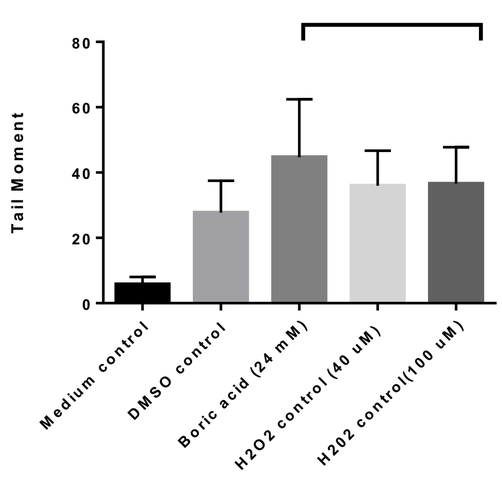
GO Enrichment Analysis
- DAVID Online Bioinformatics Platform[2] –GO BP, MF, CC
- Upregulated and downregulated genes as separate lists
- BH adjusted p<0.05

RESULTS

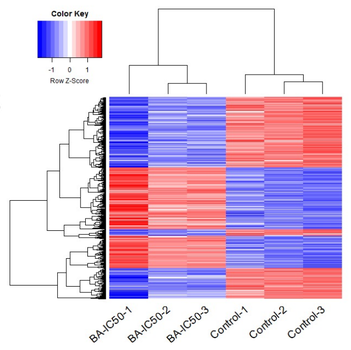
Boric acid Cytotoxicity
- Boric acid inhibits HepG2 Cell Growth in a dose dependent manner.
- IC_{50} is determined as 24 mM.



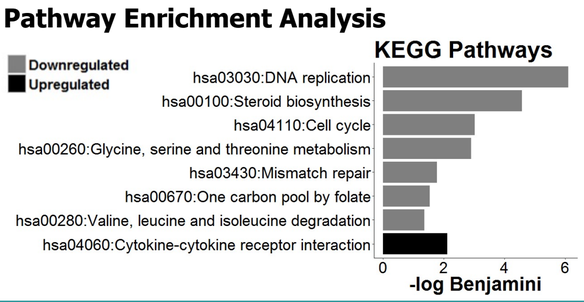
Boric acid Genotoxicity at IC50 concentration
- Boric acid induces significant DNA damage at 24 mM.



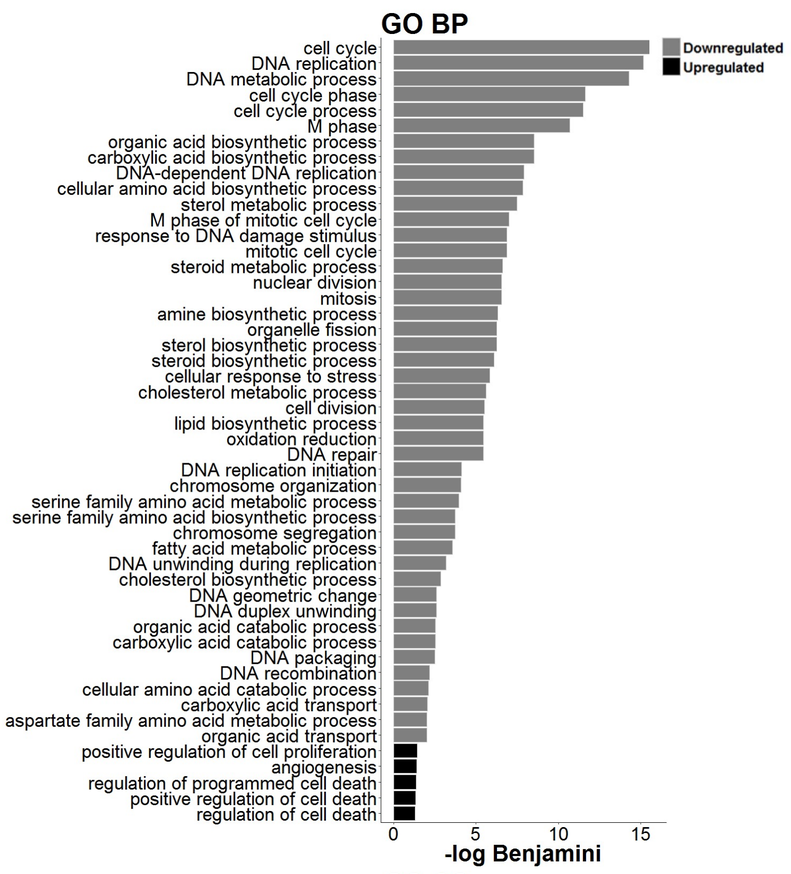
Microarray analysis revealed 785 differentially expressed genes.
- BH adjusted p<0.05
- FC>2
- 365 upregulated
- 420 downregulated



Pathway Enrichment Analysis



Gene Ontology Enrichment Analysis
- Downregulated genes indicate cell cycle arrest and metabolic changes. Cell cycle arrest is the most significant category and is a possible primary mechanism for cell growth inhibition.
- Binding ATP, Nucleotide and DNA are among molecular functions of downregulated genes.
- Downregulated genes are mostly localized to chromosome.
- Controlled cell death is enriched among upregulated genes.



CONCLUSION / FUTURE STUDIES

- Few studies aim to understand interactions and roles of boric acid at the molecular level in human cells[3,6].
- Molecular mechanisms of the predominant chemical form of boron at half maximal inhibitory concentration are being investigated in this study.
- Boric acid displayed dose dependent cytotoxicity and reached half maximal inhibitory concentration (IC_{50}) at 24 mM.
- According to the analyses boric acid influences metabolic pathways especially lipid metabolism and amino acid metabolism.
- Hypothetical mechanisms of growth inhibition at IC_{50} is cell cycle arrest and programmed cell death.
- The next step is to reconstruct and analyze boron-induced gene networks which will provide a detailed and holistic map of crucial pathways and genes.
- Network analysis might provide further insight about underlying regulatory mechanisms.
- Analysis of a reconstructed network might also be useful for revealing any possible link between metabolic machinery and cell cycle regulation.
- After *in silico* analyses, genes which are most likely to be central in boron induced cytotoxic responses will be validated through qRT-PCR experiments.
- Other than understanding molecular cytotoxic mechanisms, gene profile revealed in large scale experiments and analyses might be useful for discovering the potential of boric acid as a candidate drug for various cancer types or metabolic diseases.

1. Smyth, G. K. (2005). limma: Linear Models for Microarray Data. In Bioinformatics and Computational Biology Solutions Using R and Bioconductor (pp. 397–420). New York: Springer-Verlag.
2. Huang, D. W., Sherman, B. T., Tan, Q., Kir, J., Liu, D., Bryant, D., … Lempicki, R. A. (2007). DAVID Bioinformatics Resources: Expanded annotation database and novel algorithms to better extract biology from large gene lists. Nucleic Acids Research, 35(SUPPL.2).
3. Wei, Y., Yuan, F., Zhou, W., Wu, L., Chen, L., Wang, J., & Zhang, Y. (2016). Borax-induced apoptosis in HepG2 cells involves p53, Bcl-2, and Bax. Mol. Res, 15(2).
4. Barranco, W. T., & Eckhert, C. D. (2006). Cellular changes in boric acid-treated DU-145 prostate cancer cells. British Journal of Cancer, 94(6), 884–890.
5. El-Dakdoky, M. H., & Abd El-Wahab, H. M. F. (2013). Impact of boric acid exposure at different concentrations on testicular DNA and male rats fertility. Toxicology Mechanisms and Methods, 23(5), 360–367.
6. Jin, H., Xu, Z., Li, D., & Huang, J. (2014). Antiproliferative activity and therapeutic implications of potassium tris(4-methyl-1-pyrazolyl) borohydride in hepatocellular carcinoma. Chemico-Biological Interactions, 213, 69–76.

INFORMATICS INSTITUTE