

Research article

Transcriptional profiling of livers from different strain of mice treated with Asparaginase

Rana Jaber Tarish

Department of Physiology, College of Veterinary Medicine, University of Al-Qadisiyah, Iraq
Corresponding Author Email: Rana.tarish@qu.edu.iq

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Abstract

Asparaginase (ASNase) is widely used to treat acute lymphoblastic leukemia (ALL) in children but it causes metabolic complications related to liver toxicity. ASNase results in synthesis of some factors such as ATF4. The eIF2-ATF4 pathway is essential for cell survival during amino acid starvation conditions. Activation of the AAR in liver requires the eIF2 kinase called general control nonderepressible 2 kinase (GCN2). To what extent activation of the GCN2-eIF2-AAR is mediated by ATF4 is unknown. Our objective and hypothesis are addressed in our aim to describe the liver response to ASNase in mice deleted for Atf4. RNA sequencing alongside complementary biochemical approaches were performed in the livers of mice treated with eight daily injections of ASNase or saline excipient. Differences in gene expression were evaluated. We also explored the relationship between the different treatment groups and strains. This research provides insight into the importance of genetic background of patients in choosing ASNase as a treatment.

Keywords: Asparaginase, ASNase, Mice, Liver.

Introduction

Asparaginase (ASNase) is a drug that is used to treat Acute lymphoblastic leukemia (ALL) (1), and it is widely recommended as it improves remission induction rate (2). Nevertheless, ASNase has many deleterious side effects such as liver failure (3). Our laboratory was the first to demonstrate that ASNase reduces liver protein synthesis by increasing phosphorylation of eukaryotic initiation translation factor 2 (eIF2) (4) via the general control nonderepressible 2 kinase (GCN2) (5). Phosphorylation of eIF2 by GCN2 dampens global protein synthesis rates while simultaneously promoting gene-specific translation of protein factors. ATF4 is described in many research articles as a master regulator of metabolism in response to many cellular stressors. It was found to play critical role in amino acid deficiency as a member of the GCN2-eIF2-ATF4 AAR pathway (6). The goal of this research is to

explore the transcriptional profiling after injecting mice with phosphate buffered saline (PBS) or ASNase. Assessing ATF4 deletion mRNA profile will determine whether the role of GCN2 is fully mediated by ATF4 or shared with other factors. This will help to better identify the causes of drug toxicity and perhaps reveal new treatment and prevention approaches.

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 442

Animals from Jackson Laboratories, Bar Harbor, ME were used in these experiments. All mice were individually housed in clear plastic cages with corncob bedding. Mice were freely provided commercial diet.

Protocols of animal use were according to Rutgers (IACUC). Animals were bred and maintained at the Rutgers Bartlett animal care facility.

Design of the study.

Mice were administered once-daily intraperitoneally injected with ASNase (Deerfield, Illinois) after the start of the light cycle as previously detailed (Wilson et al., 2015). The doses are based on our previous work as described in (4) and enzyme activity was determined prior to injection by the Nesslerization technique by detecting the level of ammonia as was described (4, 5).

Sample Collection.

Mice from all treatment groups were euthanized by decapitation ~8 h after the

eighth daily injection. Trunk blood collected to obtain serum. Frozen samples were stored at -80°C until analysis.

RNA

Frozen liver samples were processed to obtain high quality RNA for RNA-Sequencing (RNA-Seq). Total RNA was extracted from frozen livers using NucleoSpin® RNA Kit (Macherey-Nagel, Newmann-Neander, Germany) followed by DNase treatment. The A260/280 and 260/230 absorbance ratios were identified using NanoDrop1000 (Thermo Fisher Scientific, Wilmington, DE).

Statistics

R using cummeRbund was employed to do statistics for this study with FDR<0.1.

Results

The results of the current study showed that there are big differences between all the treated groups regarding the profiling of the genes of the livers that were analyzed using RNA seq. Figures (1-A), (1-B), and (1-C) showed the absence of *Gcn2* and *Atf4* genes in the *Gcn2* mutants and *Atf4* mutants

respectively confirming our study design. In addition, the results showed the samples were well suited this study since there was no considerable variances between the samples of the same group as in Figures (2-A) and (2-B).

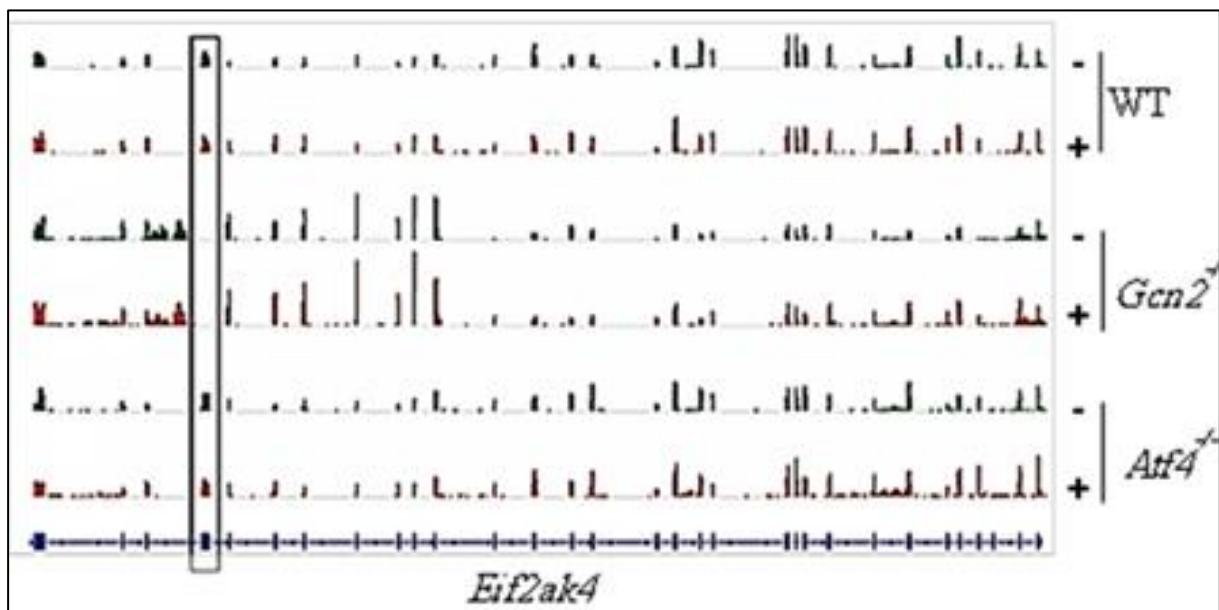


Figure (1-A): *Eif2ak4* (*Gcn2*) gene expression in WT, *Gcn2*^{-/-} and *Atf4*^{-/-} mice treated with PBS or asparaginase

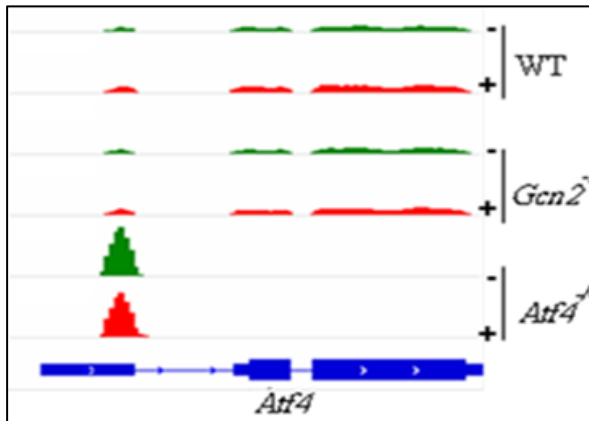


Figure (1-B): *Atf4* gene expression in WT, *Gcn2*^{-/-} and *Atf4*^{-/-} mice treated with PBS or asparaginase

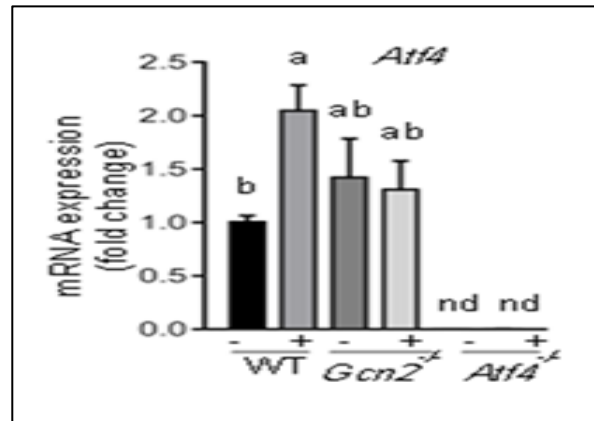


Figure (1-C): *Atf4* mRNA expression in WT, *Gcn2*^{-/-} and *Atf4*^{-/-} mice treated with PBS or asparaginase

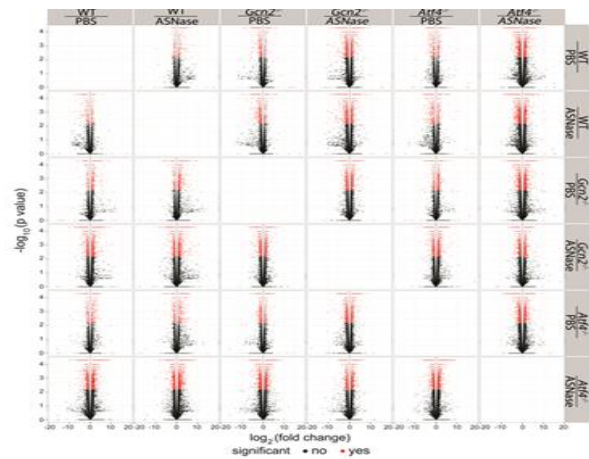


Figure (2-A): Volcano plot in WT, *Gcn2*^{-/-} and *Atf4*^{-/-} mice treated with PBS or asparaginase

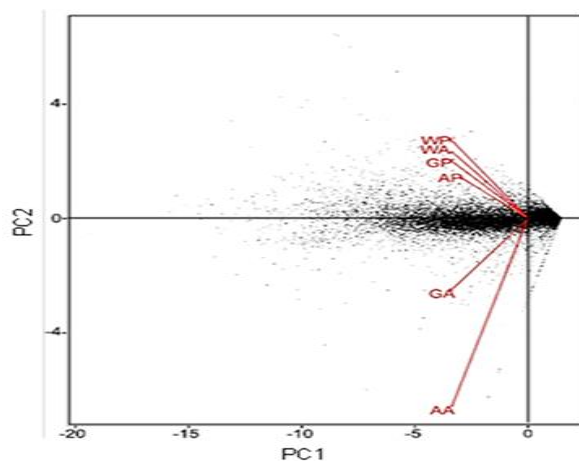


Figure (2-B): Principle Component Analysis (PCA) plot in WT, *Gcn2*^{-/-} and *Atf4*^{-/-} mice treated with PBS or ASNase. WP: Wildtype treated with PBS, WA: Wildtype treated with ASNase, GP: *Gcn2*^{-/-} treated with PBS, GA: *Gcn2*^{-/-} treated with ASNase, AP: *Atf4*^{-/-} treated with PBS, AA: *Atf4*^{-/-} treated with ASNase

Discussion

We assessed here the transcriptional profiling of livers that were dissected from WT, *Gcn2* mutants and *Atf4* mutant mice treated with PBS or ASNase. First, we confirmed that our protocol of deleting the *Gcn2* and *Atf4* genes was successfully accomplished. We showed this confirmation of *Gcn2* and *Atf4* deletion genes in mice by using the Integrity Genome Viewer (IGV) software. The IGV plots Figures (1-A) and (1-B) show the absence of *Gcn2* and *Atf4* exons in *Gcn2*^{-/-} and *Atf4*^{-/-} mice respectively after being aligned to the mouse genome mm10. We also confirmed that *Atf4*^{-/-} livers were not expressing any considerable amount

of the gene by conducting RT-qPCR as shown in Figure 1C. In the same time, the other genetic strains (WT and *Gcn2*^{-/-} mice) expressed significant amount of the *Atf4* gene comparing to the *Atf4*^{-/-} mice ($P < 0.05$). This indicates that this gene was intact in those strains, and that losing of *Gcn2* gene caused high level of *Atf4* gene expression. This might be due to compensatory mechanism those mice exerted to manage the loss of *Gcn2* in their bodies. Additionally, the Volcano plot Figure (2-A) shows the genes that differ significantly (highlighted in red) among PBS and ASNase treatment conditions. From this, we conclude that

Gcn2^{-/-} and *Atf4*^{-/-} mice exert different genomic profiles compared to the WT. In addition, the PCA plot Figure (2-B) with X-axis represents PC1. Y-axis represents PC2, the second component points in the direction of highest variance. This plot indeed shows that gene expression profiles are different among the three strains. It also shows that the PBS treated groups tend to locate closer to each other along with the WT group treated with ASNase that surprisingly locate in close position. On the other hand, the plot also reveals that the *Gcn2* null and *Atf4* null mice

treated with ASNase have different gene sets compared to the WT. Moreover, the *Atf4*^{-/-} gene sets were positioned in the furthest spot comparing to all other groups. This was the first study to be done to compare these different strains genetically using the RNA-Seq technique. Furthermore, this was the first study to reveal that *Gcn2*^{-/-} and *Atf4*^{-/-} mice have different transcriptional profiling when treated with ASNase. This study is worth to follow and considered in choosing ASNase to treat patients with different genetic backgrounds.

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