Targeting Branched-Chain Amino Acid Metabolism for the Treatment of Chronic Myeloid Leukemia

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Abstract

Chronic myeloid leukemia (CML) is caused by a chimeric fusion of human chromosomes 9 and 22. This produces a hybrid BCR-ABL tyrosine kinase that drives abnormal myeloid cell proliferation. BCR-ABL positive CML is treated with tyrosine kinase inhibitors (TKIs). Mutations in the BCR-ABL gene chimera reduce the efficacy of TKIs. Our study aimed to target genes influencing CML metabolism as a new approach to overcome CML resistance to TKIs. We focused on the branched-chain amino acid (BCAA) metabolism since it stimulates CML growth. By using the eukaryotic promoter database (EPD) we found that the transcription factor JUN-B has putative binding sites in the promoter regions of 4 genes in BCAA metabolism, *BCAT1, BCAT2, BCKDHA* and *DBT*. JUN-B induces apoptosis, but it is silenced in CML. CML specimens, collected before (baseline, n=133) or after treatment with 400mg (n=30) and 800mg (n=33) Imatinib (TKI), were obtained from the R2-genomic analysis platform. T-test analysis tested the difference between the gene expression of JUN-B and the BCAA metabolic genes. In baseline specimens, there was a significant negative correlation between the expression of JUN-B and BCAT1/BCAT2 suggesting that JUN-B is their negative regulator in CML. Treatment with Imatinib reduced the expression of *BCAT1* but not that of *BCAT2, BCKDHA or DBT*. Results suggest that *BCAT1* and *BCAT2* are downstream targets of JUN-B and Imatinib may reduce the burden of CML by downregulating the expression of *BCAT1*. These findings provided the background to investigate the mechanism of targeting BCAA metabolism in CML in TKI resistant patients.

Objective

Use bioinformatic data to compare the expression of Jun-B with that of genes related to the BCAA metabolic pathway before and after a treatment with imatinib in patients with CML.

Background

The **BCAA metabolic pathway** is responsible for the degradation of branched chain amino acids (leucine, isoleucine, and valine) into their branched chain keto acids (BCKAs) by the branched chain aminotransferase (BCATc and BCATm) enzymes, which are encoded by *BCAT1* and *2* genes. The BCKAs are used as a source of TCA cycle intermediates after they are irreversibly decarboxylated to branched-chain acyl-CoAs by the branched chain ketoacid dehydrogenase complex encoded by the *BCKDHA* and *DBT* genes.[2] Upregulation of this pathway has been shown in several cancers, including CML, as it is a major source of energy for cancer cells.

Imatinib and other TKI's are considered the baseline treatment for **CML**.[4] The interaction between these drugs and the BCR-ABL tyrosine kinase is well studied. *However little research has been done on the effect of treatment on gene expression in the BCAA metabolic pathway.*

JUN-B is a transcription factor associated with cellular proliferation. When JUN-B is expressed, it activates cell cycle inhibitors, such as p21 and INK4 which prevents cellular proliferation. In some cancer subtypes, however, JUN-B expression is inhibited, to aid in malignant proliferation of cancer cells.[3]

Methods

Initial screening for transcription factors effecting the BCAA metabolic pathway was conducted on the Eukaryotic Promoter Database[1]. The human promoter of *BCAT1, BCAT2, BCKDHA,* and *DBT* was screened for transcription factor binding sites relevant to CML.The screened promoter area was between -2000 and +100 bp relative to the transcription start site [TSS] for each gene of interest.

A human dataset originating from the Wolf[6] study on CML was taken from the R2 genomics database[5]. The dataset was divided into three groups, pre-treatment (n=133), 400 mg Imatinib treatment (n=31), and 800 mg Imatinib treatment (n=33).

The treatment groups were compared to the pre-treatment group by two tailed T-tests. A p-value less than 0.05 indicated a significant difference in gene expression before and after treatment. The "correlate 2 genes" function in R2 was then used to show the relationship between JUNB as well as BCR and ABL (genes involved in CML chimera) and the BCAA metabolic genes.

Results

Gene Expression (Imatinib treatment vs. Control)

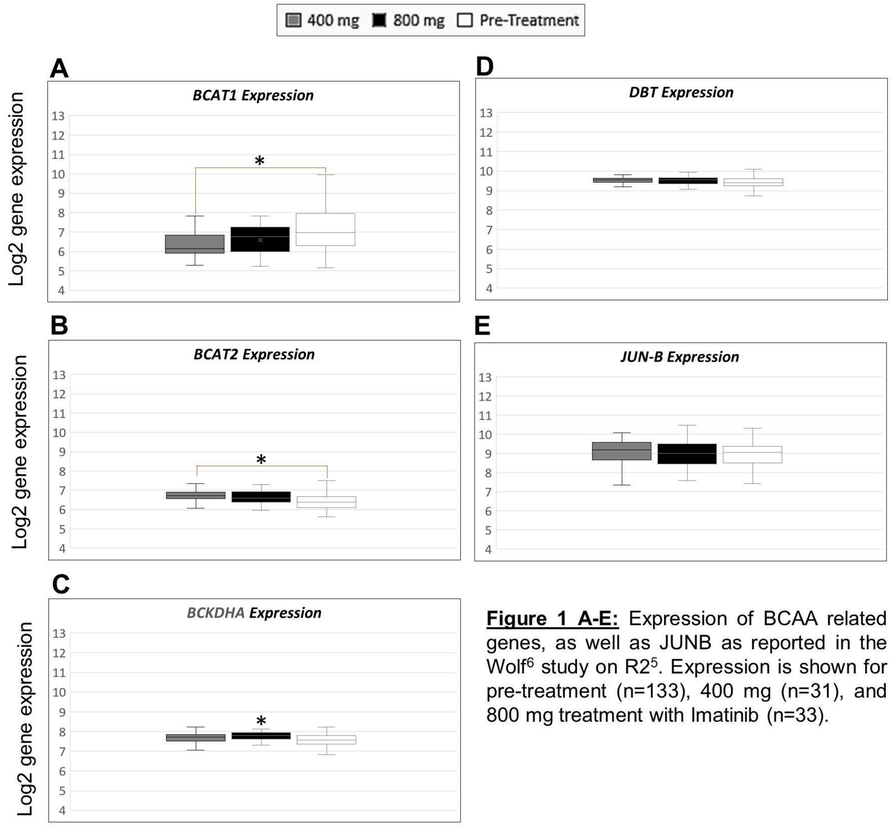

Figure 1 A-E: Expression of BCAA related genes, as well as JUNB as reported in the Wolf[6] study on R2[5]. Expression is shown for pre-treatment (n=133), 400 mg (n=31), and 800 mg treatment with Imatinib (n=33).

Correlation of gene expression between treatment and pre-treatment groups (P- values)					
Treatment	*BCAT1*	*BCAT2*	*BCKDHA*	*DBT*	*JUN-B*
400 mg	0.00018*	0.00043*	0.02707*	0.39543	0.25137
800 mg	0.01291*	0.00345*	0.00075*	0.53832	0.87741

Table 1: Results of t-tests conducted between the control, and two imatinib treatment groups, for the expression data shown in figure 1. * displays the data where there is a statistically significant change in expression between the pre-treatment and treatment groups.

BCAA Metabolic Genes Correlation Analysis

JUN-B Correlation Analysis				
	Pre-Treatment		Treatment	
Gene:	**R-value:**	**P-value:**	**R-value:**	**P-value:**
BCAT1	**-0.297**	**0.0004***	0.021	0.164
BCAT2	**-0.285**	**0.0007***	-0.193	0.129
BCKDHA	0.074	0.3950	-0.039	0.763
DBT	0.013	0.878	-0.118	0.357

BCR Correlation Analysis				
	Pre-Treatment		Treatment	
Gene:	**R-value:**	**P-value:**	**R-value:**	**P-value:**
BCAT1	-0.111	0.200	-0.148	0.246
BCAT2	**0.236**	**0.005***	**0.501**	**0.00002***
BCKDHA	**0.385**	**0.000004***	**0.292**	**0.020***
DBT	0.164	0.057	0.068	0.598

ABL Correlation Analysis				
	Pre-Treatment		Treatment	
Gene:	**R-value:**	**P-value:**	**R-value:**	**P-value:**
BCAT1	0.116	0.181	-0.058	0.651
BCAT2	0.073	0.400	**0.382**	**0.002***
BCKDHA	**0.174**	**0.0440***	**0.279**	**0.027***
DBT	**0.241**	**0.004***	0.122	0.340

Table 2 Data was collected from the R2[5] "Correlate 2 genes" function. Table 2 shows the BCAA metabolic genes and their correlation with JUN-B or the BCR-ABL chimera. * indicates values with statistical significance (P≤ 0.05). R-value shows the direction, and magnitude of the correlation between the two genes tested.

JUN-B Promoter Binding Sites

JUN-B Putative Binding Sites		
Gene:	**Location (p-value=0.001)**	**Location (p-value=0.0001)**
BCAT1	-195, -559, -1217, -1286, -1621	-559
BCAT2	-196	N/A
BCKDHA	-1695	N/A
DBT	675, 615, 613, 284, -1290, -1419, -1461, -1744	-1461

Table 3: Eukaryotic Promoter Database[1] was used to find putative binding sites (with different specificity) of JUN-B located at the promoter regions of the selected genes in the BCAA metabolic pathway (screened region was between -2000 and +100 bp relative to TSS). JUN-B is shown to bind with high specificity to both *BCAT1* and *DBT*, and with a lesser specificity to all 4 genes involved.

Conclusions and Limitations

Conclusions:

- Treatment of CML cells with Imatinib leads to: 1) a decrease of expression of *BCAT1*, 2) an increase of expression of *BCAT2* and *BCKDHA*.
- In pre-treated CML cells, there is a negative correlation between: 1) the expression of *BCAT1* and *JUN-B*, 2) the expression of *BCAT2* and *JUN-B*.
- There was a significant positive correlation between: 1) the expression of *BCAT2* and BCR-ABL chimera, 2) the expression of *BCKDHA* and BCR-ABL chimera regardless of the applied treatment.
- **Overall, the main findings suggest that *the BCAA metabolic* are downstream targets of JUN-B and Imatinib may reduce the burden of CML by downregulating the expression of *BCAT1.***

Limitations:

- Physical experimentation is required to further explore the interaction between the genes identified in this study of genomic database information.
- This analysis was based on a single human dataset.

1 – Eukaryotic Promoter Database (https://epd.expasy.org/epd)

2 – Jung MK, Okekunle AP, Lee JE, Sung MK, Lim YJ. Role of Branched-chain Amino Acid Metabolism in Tumor Development and Progression. J Cancer Prev. 2021 Dec 30;26(4):237-243. doi: 10.15430/JCP.2021.26.4.237. PMID: 35047449; PMCID: PMC8749315.

3 –Ren FJ, Cai XY, Yao Y, Fang GY. JunB: a paradigm for Jun family in immune response and cancer. Front Cell Infect Microbiol. 2023 Sep 4;13:1222265. doi: 10.3389/fcimb.2023.1222265. PMID: 37731821; PMCID: PMC10507257.

4 – Sacha T. Imatinib in chronic myeloid leukemia: an overview. Mediterr J Hematol Infect Dis. 2014 Jan 2;6(1):e2014007. doi: 10.4084/MJHID.2014.007. PMID: 24455116; PMCID: PMC3894842.

5 – 'R2: Genomics Analysis and Visualization Platform (http://r2.amc.nl)'.

6 – Wolf, D. (2021, December 31). *Gene expression-based imatinib response prediction in chronic phase CML*. National Center for Biotechnology Information. https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=gse44589