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OVER EXPRESSION OF THE FATTY ACID SYNTHASE IS A STRONG PREDICTOR OF POOR PROGNOSIS AND CONTRIBUTES TO GLUCOCORTICOID RESISTANCE IN B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Abstract – *Background:* *Glucocorticoid response, as one of the most reliable prognostic factors, plays a pivotal role in predicting clinical outcome in the acute lymphoblastic leukemia (ALL). Hence, identifying potential biomarkers of glucocorticoid resistance can help to decide on the best treatment for ALL. On the other hand, the relationship between increased fatty acid synthase (FASN) expression and poor clinical prognosis has been reported in various kinds of cancer. So here, we aimed to evaluate the prognostic value of FASN in ALL.*

Materials and Methods: *In this study, we investigated FASN expression level in glucocorticoid-resistance and sensitive leukemia cell lines, REH and NALM-6, before and after glucocorticoid (prednisolone and dexamethasone) treatment using quantitative RT-PCR. Then, we evaluated the prognostic and the diagnostic value of FASN in bone marrow samples of children with acute lymphoblastic leukemia.*

Results: *Our results indicated there was no significant difference in expression level of FASN in REH resistance cells after glucocorticoids treatment, while decreased FASN expression was observed in NALM-6 sensitive cells. Additionally, we reported FASN overexpression in ALL samples in comparison with non-cancerous controls, particularly in high-risk samples ($p < 0.002$).*

Conclusions: *This study suggests that increased expression of FASN may play a pivotal role in the development of ALL. Also, FASN could be considered as a potential marker of the poor prognosis in pediatric ALL.*

KEYWORDS: *ALL, FASN, Prognosis, Diagnosis.*

INTRODUCTION

Evolution of multiagent chemotherapy and independent risk factors have improved survival rates approximately 80% in children with acute lymphoblastic leukemia. Although, 20-30% of patients will be relapsed within the treatment process[1].

Outcome assessment based on risk factors could remarkably help to prevent relapse at this malignancy[2]. Hence, ALL patients based on risk factors such as age, white blood cell count, cytogenetic abnormalities, response to glucocorticoids and PCR-based and flow cytometric MRD (minimal residual disease) are divided into high-risk and low-risk groups[3]. Response to glucocorticoids is considered as a strongest independent factor in predicting ALL patient outcome[4]. Therefore, identification of glucocorticoid resistance markers is the beneficial tools for improvement of prognostic and diagnostic strategies in this malignancy.

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Human fatty acid synthase has been found to act as an oncogene and is a key multi-functional enzyme in the lipogenesis pathway[5]. It has been demonstrated that lipogenesis process contributes in signal transduction of malignant cells[6]. In addition, high expression levels of FASN correlate with tumor progression, metastasis, chemoresistance, and survival rate in numerous malignancies[7]. Furthermore, inhibition of FASN expression dramatically promotes apoptosis and suppresses cell growth and metastasis[8].

In the present study, we evaluated FASN expression in glucocorticoid-resistance and sensitive ALL cell lines before and after glucocorticoids treatment. Afterwards, we indicated the expression of FASN in ALL samples and determined its prognostic significance in childhood ALL. To our knowledge, this is the first time that clinically prognostic role of FASN in childhood ALL is reported.

MATERIALS AND METHODS

Cell culture and drug treatment

Precursor B-ALL cell lines, namely REH and NALM-6, was acquired from the Pasteur Institute of Iran. Cells were grown in RPMI-1640 culture medium supplemented with 10% FBS, 1% streptomycin, 1% penicillin and were maintained at 37°C in a humidified atmosphere containing 5% CO_2. Cell concentration of 30,000 cells/ml were treated with 1 µM prednisolone and 200 nM dexamethasone for 48h. In our previous work, the cytotoxic effects of two concentrations of prednisolone and dexamethasone were assayed using MTT assay, and data are available upon request.

Patient selection

We selected bone marrow samples at diagnosis from 17 children with newly diagnosed B-lineage ALL (10 standard risk and 7 high risk) and 7 non-cancerous bone marrow samples which were admitted to Mofid Hospitals (Tehran, Iran). Our study was approved by the Clinical Research Ethics Committees of the Mofid Hospitals, and all samples were collected with informed consent. The patients were divided into two groups contain high-risk and standard-risk based on age, cytogenetic abnormalities and flow cytometry-based detection of MRD on day 33. Clinical characteristics of these 17 ALL patients are summarized in Table 1.

TABLE 1. Distribution of childhood ALL patients, 10 standard risk patients and 7 high risk patients were involved in this study.

	Standard risk n=10	*High risk n=7*
Gender		
Male	7	5
Female	3	2
Age at diagnosis		
<1 year	0	0
1–9 years	10	5
≥10 years	0	2
Chromosome translocation		
Non-detected	8	5
ETV6-RUNX1	1	0
BCR-ABL	0	0
Down syndrome	0	1
Hypodiploidy	0	1
Hyperdiploidy	1	0
Flow-MRD at day 33		
<0.1	10	0
≥0.1	0	7
Status		
Complete remission	10	4
Relapsed	0	1
Induction failure	0	1
Died	0	1

RNA extraction and cDNA synthesis

Briefly, mononuclear cells were separated and harvested from bone marrow aspirates using Ficoll-Paque density gradient centrifugation. Total RNA was extracted from the cells using the total RNA isolation solution (RiboExTM; GeneAll, Seoul, Korea) according to the manufacturer's guidelines. The cDNA was synthesized using a RevertAid H Minus First Strand cDNA synthesis kit (Fermentas, USA), The cDNA synthesis reaction was incubated at 25 °C for 10 min, 45 °C for 60 min followed by enzyme inactivation at 75 °C for 5 min and stored at -20 °C until used.

FASN mRNA expression analysis

The quantitative real-time PCR was performed with the SYBR Green PCR master mix 2x (Takara, Japan) using a Rotor-gene 6000 instrument (Corbett, Germany). Each reaction was performed in duplicate. The ABL was used as an internal control to normalize differences in the amount of total RNA in each sample. In this study, for high specificity and high efficiency, each primer pair was designed to span exon junctions, thus avoiding the amplification of genomic DNA. Also, product verification was investigated

TABLE 2. Sequences of primers for real-time PCR.

Primer	*Sequence*
ABL-F	CTTCTTGGTGCGTGAGAGTGAG
ABL-R	GACGTAGAGCTTGCCATCAGAAG
FASN-F	TGGTAGTGAGTGGGAAGGTGTAC
FASN-R	CAGACGCAGCTCCTTGTAAACTT

by analyzing the melting curve of PCR samples. Since, we had primers with different efficiency, we used Pfaffl method for relative quantification[9]. The amplicon sizes of FASN and ABL were 134 and 115 bp respectively. Sequences of the primers listed in Table 2.

Statistical Analysis

All relevant statistical analyses were computed by the software of SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± SD. $p < 0.05$ was considered statistically significant.

RESULTS

Expression of FASN in REH cells

Expression of the FASN gene was examined in glucocorticoid resistance REH cells. Results showed that FASN expression level in prednisolone-treated cells had no significantly different than untreated cells. Additionally, FASN expression level in dexamethasone treated cells had slightly different than untreated cells (Fig. 1).

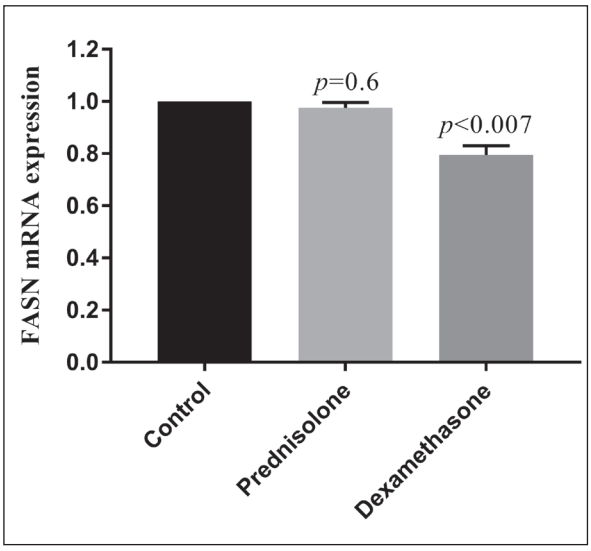

Fig. 1. Relative mRNA expression levels of FASN in REH resistance leukemia cells before and after glucocorticoid (prednisolone and dexamethasone) treatment.

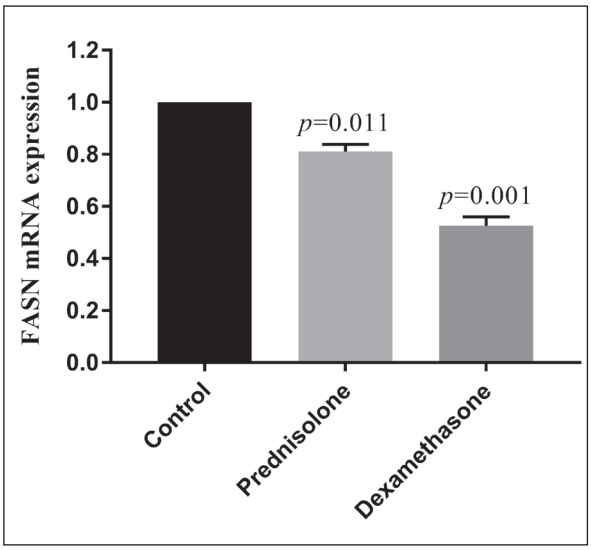

Fig. 2. Relative mRNA expression levels of FASN in NALM-6 sensitive leukemia cells before and after glucocorticoid (prednisolone and dexamethasone) treatment.

Expression of FASN in NALM-6 cells

Q-PCR results in NALM-6 cells indicated decreased expression of FASN after glucocorticoids treatment, particularly after dexamethasone treatment (p =0.001, Fig. 2).

Expression of FASN in ALL patient samples and non-cancerous controls

Expression of the FASN gene was examined in leukemia cells from 17 patients with B-ALL (10 standard risk and 7 high risk) and 7 non-cancerous samples as controls. Quantification of mRNA expression after ABL normalization revealed that the median levels of FASN expression were remarkably higher in childhood ALL patients than those in the control group ($p < 0.05$, Fig. 3).

The high-FASN expression is related to poor prognosis in ALL patients

Based on FASN expression analysis, we found that cancer patients in the high-risk group indicated significantly higher FASN expression in comparison with the low-risk group (p = 0.008, Fig. 3).

DISCUSSION

Response to glucocorticoids has been recognized as one of the strongest predictors of outcome assessment in childhood with acute lymphoblas-

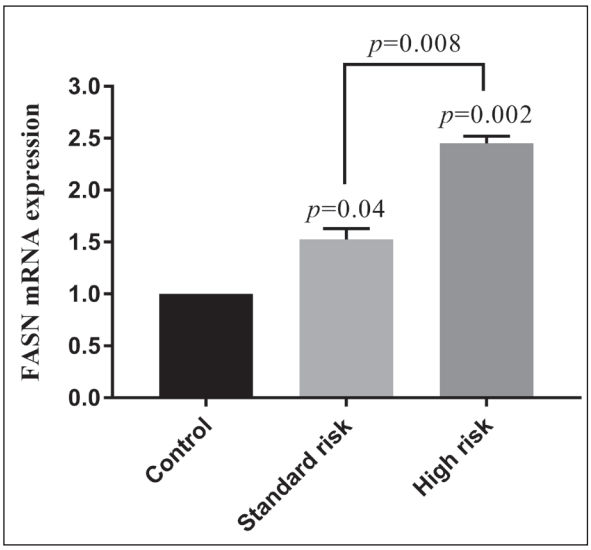

Fig. 3. Relative mRNA expression levels of FASN in bone marrow samples of children with acute lymphoblastic leukemia (10 standard risk and 7 high risk) and non-cancerous samples as control.

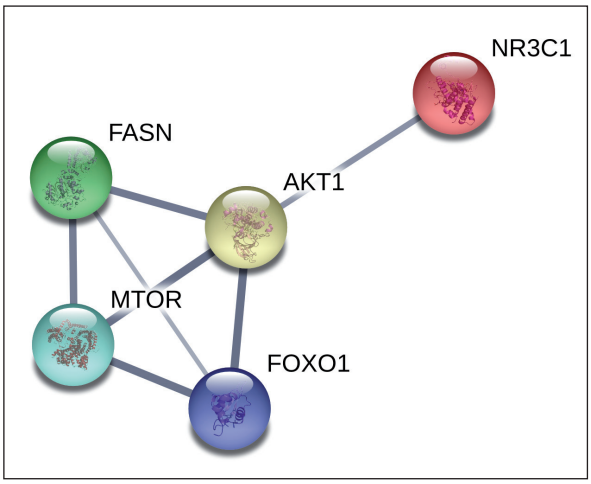

Fig. 4. In silico analysis of FASN and NR3C1 interactions. The protein-protein interactions of FASN were searched in the STRING database (http://string-db.org/).

tic leukemia[4]. Consequently, the identification of markers associated with glucocorticoid resistance could develop improved prediction of medical outcome in ALL patients.

Over expression FASN as a prognostic factor has been reported in some cancers such as colorectal, prostate, breast, gastric, and bladder carcinomas[10-16]. These studies have indicated the pivotal function of FASN in tumor progression. However, results concerning the role of the FASN in ALL remain unknown. Increased lipogenesis in cancer cells is associated with the increased FASN expression as a key enzyme in this pathway[17]. In addition, dysregulation fatty acid synthesis has been observed in some hematologic malignancies[18-20].

In the present study, our results showed no significant alterations in FASN expression after glucocorticoids treatment in resistant cells. However, decreased expression of FASN was observed after glucocorticoid treatment in sensitive leukemia cells. These data indicated that FASN gene is targeted by the glucocorticoids. It has been reported that glucocorticoids regulate FASN expression in many tissues, including lung, adipose, and liver and also glucocorticoid treatment reduces lipogenesis[21]. There is limited evidence to support the association between abnormal lipid metabolism and leukemogenesis. Here, we indicated glucocorticoids could have effects on lipogenesis of leukemic cells. We also suggested a crosstalk between glucocorticoid resistance and lipogenesis based on alterations in FASN gene regulation.

Additionally, our results indicated overexpression FASN in ALL patient samples, particularly in high-risk cases. Therefore, these data demonstrate that the FASN expression is significantly associated with poor prognosis in pediatric ALL, suggesting that high expression of FASN contribute to ALL development.

Finally, we investigated the protein-protein interaction of FASN and NR3C1 glucocorticoid receptor using the STRING database. Our results indicated the role of the FASN/AKT1 / NR3C1 pathway in this study. It has been reported that AKT1 is a major negative regulator of the NR3C1 in ALL[22]. Additionally, it has been indicated downregulation of FASN suppress cell migration by targeting AKT[16]. According to these results, we hypothesized that FASN inhibition suppresses AKT activation and consequently balance NR3C1 expression. Together, this protein interaction analysis indicated the role of FASN and AKT1 in developing ALL resistance.

CONCLUSIONS

These findings suggest that FASN inhibition may be a target for treatment of the glucocorticoid-resistant subset of ALL and generate better treatment efficacy. To further explore the diagnostic and the prognostic value of FASN in B-ALL, further studies in large sample size will be required to demonstrate this hypothesis.

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Author Disclosure Statement

The authors declare no competing financial interest.

Authors' Contribution:
All authors contributed to the conception and design of the work, conducting the study, analysis and interpretation of data, drafting and revising the draft, approval of the final version of the manuscript, and agreed to all aspects of the works.

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