Review Article:

A systematic review of proteomic biomarkers associated with risk stratification in pediatric acute lymphoblastic leukemia

Nasrin Dehghan-Nayeri[1], Peyman Eshghi[1], Kourosh Goudarzi Pour[1], Ahmad Gharehbaghian[1,2]*

[1]Pediatric Congenital Hematologic Disorders Research Center, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Department of Hematology and Blood Bank, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

* Corresponding author:email address: gharehbaghian@sbmu.ac.ir (A. Gharehbaghian)

ABSTRACT

Risk-based therapy protocols have dramatically improved survival rates in more than 80% of childhood acute lymphoblastic leukemia (chALL). Prognostic biomarkers could be valuable for predicting the relapsed ALL patients and may therefore contribute to improving ALL outcome. Presently, there are little data on the role of prognostic biomarkers in the risk stratification of ALL. The aim of the present systematic review is to survey the identified prognostic biomarkers of chALL. In this study, protein-protein interaction of identified biomarkers was evaluated to reveal the biological pathways related to high risk chALL. To pursue this goal, firstly all relevant studies were collected through the PubMed and Google Scholar databases with no restrictions. Then, the biomarkers of high risk patients were recorded and finally protein-protein interaction of biomarkers was analyzed through using the STRING database. After screening 82 abstracts, three studies were included with 36 high risk and 33 low risk B-ALL participants. Totally, 142 biomarkers were investigated in this study. Protein interaction network analysis of biomarkers revealed two main pathways, namely ribosome and spliceosome. Dysregulation of two key pathways, ribosome and spliceosome can be associated with the high risk phenotype of childhood acute lymphoblastic leukemia.

Keywords: Acute lymphoblastic leukemia; Prognostic biomarker; Ribosome pathway; Spliceosome pathway

INTRODUCTION

Despite improvements in risk-based therapy regimens, nearly 20-30% of the children with ALL suffer from a relapse [1]. Inappropriate classification of risk groups leads to poor outcome in patients. Indeed, the main reason for the complexity of precise risk stratification in chALL is due widely to its clinical heterogeneity [2]. Currently, significant risk stratification factors in chALL comprise age, white cell count, genetic abnormalities, prednisolone response, and determination MRD (minimal residual disease) by flow cytometry or PCR [3]. In addition to these risk factors, predictive markers can provide clinically important prognostic information for optimal utilization of chemotherapy strategies [4]. Additionally, risk-stratification biomarkers as a complementary approach can help avoid inessential intensive chemotherapy in low-risk patients and selection of optimal chemotherapy regimens in high risk individuals [5]. To the best of our knowledge, there has been no reported systematic evaluation of the prognostic biomarkers in chALL. Accordingly, a systematic review of available manuscripts was performed to investigate the

prognostic biomarkers and also pathways involved in these proteins.

MATERIAL AND METHOD

Studies eligible for review

PubMed and Google Scholar databases were searched using the terms 'acute lymphoblastic leukemia', 'proteomic', as well as one of the following title/abstract phrases 'childhood' or 'pediatric', 'proteomics', 'biomarker', 'prognosis', and 'risk factor' with no language restriction. Animal studies, adult studies, those which applied proteomics to different ALL groups (e.g. with T-ALL, AML, …) or which presented their results as peaks and not as named proteins were excluded. Studies were deemed eligible if a) patient population was B-ALL subset of chALL; b) patient samples (BM or PB) were collected at diagnosis; c) patients divided into two groups of high and low risk; d) the studies were investigated through using proteomic methods.

Data Abstraction

Titles and abstracts of collected articles were screened and the full text of selected literatures was evaluated. Additionally, the handsearching of references from all the researches was planned to identify any other potentially relevant studies. The search ended in August 2016. The search findings were independently assessed by 2 of the authors (ND and AG). This process has been summarized in Figure 1.

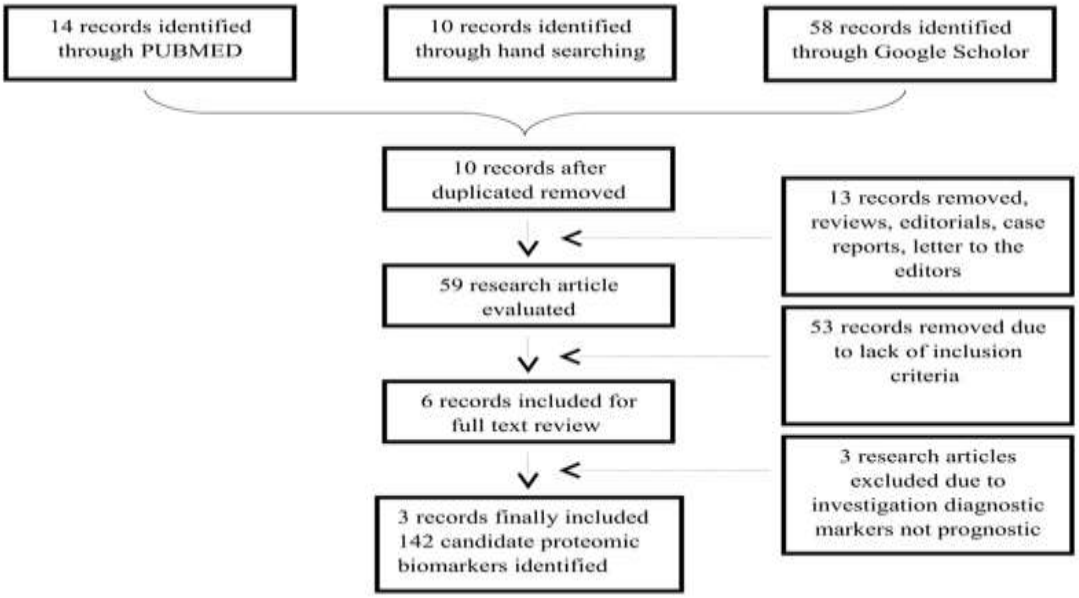

Figure 1. A flow chart of summarizing the selection process of the literatures

The main characteristics of the articles

The selected articles were screened and specific characteristics of the studies were recorded. These characteristics include: type of sample collected at diagnosis (*e.g.* bone marrow (BM) or peripheral blood (PB)), number of participants in high risk or low risk groups, the age range of patients and type of proteomic technique. Finally, a list of differentially expressed proteins in high risk patients versus low risk cases was created (Table 1).

To minimize selection bias, screening of the studies was independently performed by 2 of the authors (KG and PE).

Construction of the protein-protein interaction network

Protein-protein interaction (PPI) network was constructed by STRING v10 database (http://string-db.org) with highest confidence (0.900).

Table 1. Three publications containing potentially prognostic chALL biomarker data that were analyzed.BM, bone marrow; PB, peripheral blood; HR, high risk; LR, low risk; 2DE, two-dimensional gel electrophoresis; LC-ESI-MS/MS, liquid chromatography-electrospray ionization-tandem mass spectrometry; MALDI-TOF MS, matrix-assisted Laser Desorption/Ionization Mass Spectrometry; SELDI-TOF MS, Surface-enhanced laser desorption ionization time-of-flight mass spectrometry; WB, western blot.

Study	Site of sample	Sample size	Age (years)	Proteomic technique	Proteomic biomarkers discovered in HR groups	Validation technique/ biomarkers
Xu 2016	BM	6HR 6LR	0.5-11	LC-ESI MS/MS	THRAP3 HIST1H4G FUBP1 HMHA1 XRCC5 COPA DCXR CD74 RANBP2 HNRNPL ETFA Q59GX9 H1FX C1QBP XRCC6 HNRNPM HADH RPS14 Q59F66 RPS18 SRSF7 SUN2 H2AFY PRPF8 B2RDY9 SPTBN1 GANAB SMC3 SF3B2 EVL NDUFS3 ARHGEF1 NUMA1 variant protein SMARCD2 KTN1 BANF1 HLA-DQA1 RPL27A TCL1A hnrnpc ARPC3 EPRS HNRNPA3 B4DR52 YBX1 NACA SMARCA4 f10 HLA-DR B RPS2 SRP19 HIST1H1D EIF4A3 AASDHPPT EEF1A1 RPL11 RPL11 PTMA HIST1H3A MDH2 HNRPK APEX1 VIM HIST1H1B RPLP0 HMGB1 EEF2 HNRNPH1 HIST1H4L HSPD1 TUBB NPM1 HNRNPA1 HIST2H3PS2 HSP90AB1 SERBP1 HNRNPA2B1 STMN1 PKM HIST1H2BJ HIST1H2BB PA2G4 STMN2 HSP90AA1 RAB21 PHB2 ENO1 HIST1H2BO	Immunoblotting (Hsp90β Hsp90α YBX1 DDX48 THRP3)
Braoudaki 2013	PB BM	26HR 19LR	0.4-7	2DE/MALDI-TOF MS	A1AT A2MG ACTB ACTB ACTC1 ACTG1 AFAM AFM AMBP AMBP ANGT ANT3 APOA4 APOA4 APOC2 APOE BICR1 CALL5 CATA CERU CFAB CH60 CLUS ENOA ENOB FCN3 FHR1 FIBA FIBB FIBG G3P GELS HEMO HPT IGHG1 IGHG2 KNG1 KPYM PLMN PRDX1 PSME1 S10A9 SAA THRB TRFE UBQL1 UBQL1 VTDB VTNC ZA2G	WB (CERU CLUS)
Lauten 2006	PB BM	4HR 8LR	1-9	2DE/SELDI-TOF MS	CAT TRIM3 VCP GPRC5D	WB (VCP)

RESULT

Literature search

Initial database searches provided 82 eligible articles within the literature for inclusion. After removing 10 duplicates, 72 exclusive abstracts were screened among which, 66 were excluded, mostly due to a lack of inclusion criteria, and finally the full text of 6 articles were evaluated. Three articles were excluded after reviewing the full text, essentially due to the investigation of ALL diagnostic biomarkers and not prognostic. Totally, three articles including 36 high risk and 33 low risk B-ALL participants provided the suitability criteria and were included in this study.

Protein-protein interaction network

Investigation of three studies provided a total of 142 biomarkers in ALL high risk patients. Analyzing protein-protein interaction of 142 biomarkers using STRING database revealed two main pathways, namely ribosome and spliceosome pathways (Figure 2).

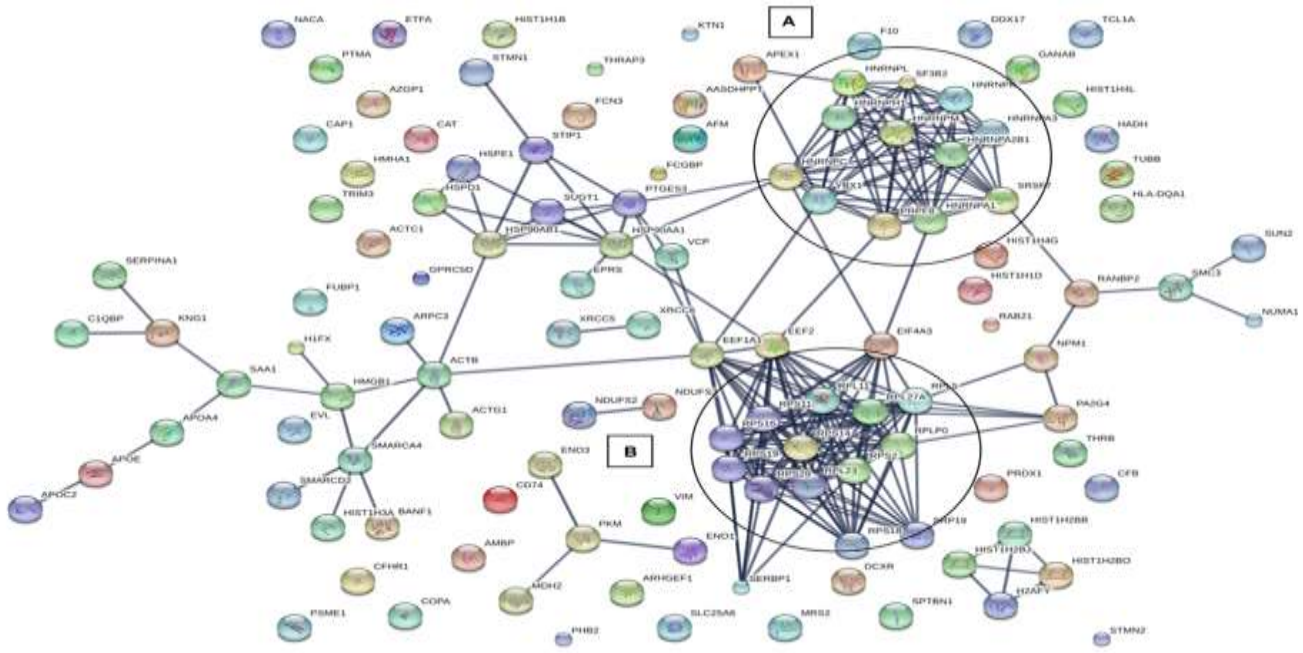

Figure 2. Protein-protein interaction network of biomarkers in high risk ALL patients using the STRING database (A) Proteins involved in spliceosome pathway (B) Proteins involved in ribosome pathway

DISCUSSION

The assessment of minimal residual disease (MRD) in acute lymphoblastic leukemia (ALL) is presently considered as a potentially valuable tool to evaluate the relapse rate of therapy [6]. Currently, multi-parameter flow cytometry and polymerase chain reaction are useful methods for identifying the MRD in ALL patients [7]. Prognostic markers of relapsed ALL are the new approach to the detection of MRD in high risk patients. The proteomic analysis can be used as one of the best tools to detect specific biomarkers in order to discriminate high risk and low risk patients with various degrees of remission [8]. Hence, in this systematic review, the identified biomarkers of high risk ALL patients were assessed through using the proteomic techniques. To better understand the signaling pathways involved in high risk ALL, protein-protein interaction of identified biomarkers were evaluated using the STRING database. STRING data revealed that a large number of proteins were localized in two pathways, namely ribosome and spliceosome.

Ribosome pathway plays a pivotal role in forming the most of cellular proteins and is essential for cell growth [9]. The mutation in ribosomal genes was recognized in the genome of hematologic malignancies, including T-cell acute lymphoblastic leukemia and chronic lymphocytic leukemia [10,11]. Additionally, ribosomal proteins as the poor prognostic factors are detected in various kinds of cancers such as colorectal, glioblastoma, pancreatic, ovarian and gastric cancers [12-16].

The other recognized pathway in this study was spliceosome pathway. Dysfunction of the splicing machinery is closely related to various human cancers, including acute myeloid leukemia (AML) [17]. Evaluating the expression level of the alternative splicing isoforms in tumors can distinguish tumor cells from the normal ones; moreover, it may predict patient survival [18]. Additionally, it has been demonstrated that splicing factors contribute to resistance of cancer cells to chemotherapy plus the fact that splicing factor inhibitors may be considered as target therapy in various cancers such as chronic lymphocytic leukemia (CLL) [19,20]. On the other hand, the mutation in splicing factors has been reported in T-cell acute lymphoblastic leukemia [21].

All in all, in this study, the potential prognostic role of ribosomal proteins and splicing factors in high risk group of B-ALL patients was indicated. Investigating the expression level of

these proteins in the clinical setting is needed to confirm this hypothesis.

CONCLUSION

To sum up, results of the present study revealed that the dysregulation of two key pathways, ribosome and spliceosome can be associated with the high risk phenotype of childhood acute lymphoblastic leukemia. These findings suggest that mutations in the ribosome and spliceosome pathways may lead to modifying the genetic program of the cancer cells to maintain survival. Investigation of proteins involved in these two pathways could provide prognostic, diagnostic and therapeutic tools for the monitoring and treatment of patients with ALL.

ACKNOWLEDGMENT

The current study is part of the Ph.D. thesis at Shahid Beheshti University of Medical Sciences. *"The authors declare no conflict of interest"*

REFERENCES

1. Kaspers GJ, Pieters R, Klumper E, de Waal FC, Veerman AJ. The treatment of recurrence in children with acute lymphatic leukemia. Current results and various developments. Tijdschr Kindergeneeskd. 1993: 6: 1-7

2. Dobson S, Waanders E, Grandal I, Gan O, McLeod J, Minden M, et al. Evolving heterogeneity in acute lymphoblastic leukemia. Exp. Hematol. 2014; 42: S31.

3. Pui CH, Carroll WL, Meshinchi S, Arceci RJ. Biology, risk stratification, and therapy of pediatric acute leukemias: an update. J Clin Oncol. 2011; 29: 551–565.

4. Geng H, Brennan S, Milne TA, Chen WY, Li Y, Hurtz C, et al. Integrative epigenomic analysis identifies biomarkers and therapeutic targets in adult B-acute lymphoblastic leukemia. Cancer Discov. 2012; 2: 1004–1023.

5. Felip E, Martinez P. Can sensitivity to cytotoxic chemotherapy be predicted by biomarkers? Annals of oncology. 2012; 23: 189-92.

6. Campana D. Minimal residual disease in acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program. 2010; 2010: 7-12.

7. Neale GAM, Coustan-Smith E, Stow P, Pan Q, Chen X. Pui CH, Campana D. Comparative analysis of flow cytometry and polymerase chain reaction for the detection of minimal residual disease in childhood acute lymphoblastic leukemia. Leukemia. 2004; 18: 934–938

8. López V, Elena W, Duojiao C, William C, Madero L, Wang X. Proteomics-based discovery of biomarkers for paediatric acute lymphoblastic leukaemia: challenges and opportunities. J Cell Mol Med. 2014; 18: 1239–1246.

9. Huang CJ, Yang SH, Lee CL, Cheng YC, Tai SY, Chien CC. Ribosomal proteinS27-like incolorectal cancer: a candidate for predicting prognoses. PLoS One. 2013; 24: e67043.

10. Goudarzi KM, Lindström MS. Role of ribosomal protein mutations in tumor development (Review). Int J Oncol. 2016; 48: 1313-24.

11. De Keersmaecker K, Atak ZK, Li N, Vicente C, Patchett S, Girardi T, et al. Exome sequencing identifies mutation in CNOT3 and ribosomal genes RPL5 and RPL10 in T-cell acute lymphoblastic leukemia. Nat Genet. 2013; 45: 186-90.

12. Chen J, Wei Y, Feng Q, Ren L, He G, Chang W, et al. Ribosomal protein S15A promotes malignant transformation and predicts poor outcome in colorectal cancer through misregulation of p53 signaling pathway. Int J Oncol. 2016; 48: 1628-38.

13. Yong WH, Shabihkhani M, Telesca D, Yang S, Tso JL, Menjivar JC, et al. Ribosomal Proteins RPS11 and RPS20, Two Stress-Response Markers of Glioblastoma Stem Cells, Are Novel Predictors of Poor Prognosis in Glioblastoma Patients. PLoS One. 2015; 27: e0141334.

14. Yan TT, Fu XL, Li J, Bian YN, Liu DJ, Hua R, et al. Downregulation of RPL15 may predict poor survival and associate with tumor progression in pancreatic ductal adenocarcinoma. Oncotarget. 2015; 10: 37028-42.

15. Tsofack SP, Meunier L, Sanchez L, Madore J, Provencher D, Mes-Masson AM, Lebel M. Low expression of the X-linked ribosomal protein S4 in human serous epithelial ovarian cancer is associated with a poor prognosis. BMC Cancer. 2013; 22: 303.

16. Yoshida S, Matsumoto K, Arao T, Taniguchi H, Goto I, Hanafusa T, Nishio K, Yamada Y. Gene amplification of ribosomal protein S6 kinase-1 and -2 in gastric cancer. Anticancer Res. 2013; 33: 469-75.
17. Adamia S, Haibe-Kains B, Pilarski PM, Bar-Natan M, Pevzner S, Avet-Loiseau H, et al. A genome-wide aberrant RNA splicing in patients with acute myeloid leukemia identifies novel potential disease markers and therapeutic targets. Clin Cancer Res. 2014; 1: 1135-45.
18. Hou HA, Liu CY, Kuo YY, Chou WC, Tsai CH, Lin CC, et al. Splicing factor mutations predict poor prognosis in patients with de novo acute myeloid leukemia. Oncotarget. 2016; 23: 9084-101.
19. Kashyap MK, Kumar D, Villa R, La Clair JJ, Benner C, Sasik R, et al. Targeting the spliceosome in chronic lymphocytic leukemia with the macrolides FD-895 and pladienolide-B. Haematologica. 2015;100: 945-54.
20. Larrayoz M, Blakemore SJ, Dobson RC, Blunt MD, Rose-Zerilli MJ, Walewska R, et al. The SF3B1 inhibitor spliceostatin A (SSA) elicitsapoptosis in chronic lymphocytic leukaemia cells through downregulation of Mcl-1. Leukemia. 2016; 30: 351-60.
21. Lo TC, Barnhill LM, Kim Y, Nakae EA, Yu AL, Diccianni MB. Inactivation of SHIP1 in T-cell acute lymphoblastic leukemia due to mutation and extensive alternative splicing. Leuk Res. 2009; 33: 1562-6.