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2DGE and DIGE based proteomic study of malignant B-cells in B-cell acute lymphoblastic leukemia

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ABSTRACT

With an objective of defining potential diagnostic and/or therapeutic markers and the mechanism of cellular transformation, we present a comparative proteomic study of B-lymphocytes from B-cell acute lymphoblastic leukemia (B-ALL) patients and normal controls, using two-dimensional gel electrophoresis and MALDI ToF/ToF tandem mass spectrometry. Our study led to the identification of 79 differentially regulated proteins in the malignant cells including proteins participating in proteostasis, cytoskeletal organization, redox homeostasis, and signal transduction pathways relevant to leukemogenesis. Principal component analysis displayed immunophenotype-/genotype-dependent variations in the malignant cell proteome. Our study adds new insights to the leukemogenic B-cell biology and prognostic stratifications.

Significance

Our work presents a comparative proteomic study of a homogeneous population of CD19⁺ B-lymphoblasts from 30 B-ALL patients and 13 normal controls that led to the identification of 79 differentially regulated proteins. Our 2DGE profiles of the B-lymphocytes together with the mass spectrometric protein identification data, deposited in the PRIDE database, will enrich the normal and leukemic Human B-lymphocyte proteome dataset available till date. Gene ontology (GO) based functional annotation revealed significant de-regulation of proteins participating in proteostasis, cytoskeletal organization, cellular energy and redox homeostasis, signal transduction, RNA splicing, and regulation of transcription, in the malignant B-cells. Multivariate statistics and principal component analysis (PCA) revealed immunophenotype-/genotype-dependent variations in the malignant B-cell proteome. Candidate proteins that exhibit opposite trend of de-regulation in myeloid and lymphoid leukemia hint towards prospective biomarkers. Our study adds new insights to the leukemogenic B-cell biology and

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prognostic stratifications. Overall, we believe our data will advance the field of B-cell proteomics.

1. Introduction

B-cell acute lymphoblastic leukemia (B-ALL) is the most common cancer in children, characterized by an accumulation of B-cell blasts that fail to differentiate. Immunophenotyping results establish that B-lineage leukemias dominate amongst lymphoid leukemias in both children (where >80% ALLs are B-ALLs) and adults (>75%). Gene fusions like TEL-AML1, BCR-ABL, E2A-PBX1 and MLL rearrangements, created by chromosomal translocations are involved in the genesis of B-ALL. In human B-ALLs, BCR-ABL and MLL-involving translocations presumably originate in early progenitors (HSC or lymphoid-myeloid progenitor), while TEL-AML1- and E2A-PBX1-harbouring leukemic cells seem to represent more differentiated, lymphoid-committed precursors [1]. Although identification of such genetic abnormalities has led to an improved understanding of B-ALL pathophysiology, the mechanisms responsible for B-cell developmental arrest remain unknown. The primary oncogenic events seem to require secondary cooperative changes to generate a fully transformed cell. Contemporary therapies cure more than 80% of children with B-ALL but, some patients fall in high risk or poor prognosis classes requiring intensive treatment, others exhibit drug resistance and many patients develop serious acute and late complications owing to drug toxicity. Furthermore, the survival rate for adults with B-ALL remains below 40% despite the use of transplantation, and treatment outcome is poor among patients who relapse on current front-line ALL regimens [2]. Current research focuses on the identification of new molecular targets to improve outcome for B-ALL patients with a dismal prognosis [3].

Proteomic studies for the identification of proteins aberrantly expressed in malignant B-cells can potentially be used to develop new diagnostic, prognostic or therapeutic targets. Transcriptome data on genome-wide expression does not necessarily translate to protein expression patterns, which warrants direct characterization of the malignant cell proteome. Proteomic studies of B-cell malignancies have made significant progress, but further studies are needed to increase the coverage of B-cell malignant proteome and to unveil new insights in B-cell biology [4]. Two-dimensional gel electrophoresis (2DGE) and, difference in-gel electrophoresis (DIGE) using fluorescent cyanide (Cy) dyes offer in essence a 2-step approach to separating complex protein mixtures in accurate quantitative details and identify differences between normal and aberrant cells.

The proteome is strongly influenced by cell differentiation. A simple comparison of mononuclear cells (MNCs) from different patients may result in the identification of proteins whose apparent change in expression actually reflects a difference in the cellular composition of the specimens. Polypeptide differences between abnormally proliferating cells could be lineage related or could reflect, in part, the selective activation of genes that are not expressed in normal cells. To prevent such a complication and eliminate population-shift effects, we compared the proteome profiles of B-lymphoblasts separated on the basis of their surface expression of CD19 and CD10. Comparison of such background-matched fractions should eliminate pseudopositive data resulting from different proportions of leukemic blasts or from differences in cell lineage to which the leukemic blasts are committed. Our choice of CD antigens was based on the information that early precursor-B cells express CD10 and CD19, and as the precursor-B stage ends, CD10 is no longer expressed [5].

With a 2DGE followed by MALDI ToF/ToF tandem mass spectrometry approach, our work presents a comparative proteomic study of a homogeneous population of $CD19^+$ cells from 27 B-ALL patients and 10 normal controls that led to the identification of 79 differentially regulated proteins. Gene ontology (GO) based functional annotation revealed significant de-regulation of proteins participating in proteostasis, cytoskeletal organization, cellular energy and redox homeostasis, signal transduction, RNA splicing, and regulation of transcription, in the malignant cells. Multivariate statistics and principal component analysis (PCA) revealed genetic anomaly dependent variations in the malignant cell proteome. We have discussed how the interactome of the observed de-regulations could propose research avenues for addressing the altered B-cell biology. Furthermore, candidate proteins that exhibit opposite trend of de-regulation in myeloid and lymphoid leukemia might not only point out prospective biomarkers but also enhance our knowledge on the regulation of differentiation of HSC into myeloid or lymphoid lineages.

2. Materials and methods

2.1. *B-ALL patient samples and enrichment of $CD19^+$ cells*

Bone marrow and peripheral blood samples were obtained from B-ALL patients with their informed written consents (guardians in case of minors), following the guidelines of the Institutional Ethical Committee of Vivekananda Institute of Medical Sciences, Ramakrishna Mission Seva Pratishthan and Institutional Animal & Bioethics committee of Saha Institute of Nuclear Physics. The research was conducted according to the principles expressed in the Declaration of Helsinki. Clinical characteristics of individual patient samples are provided in Supplementary Material 1. All patient samples were first analysed for their immunophenotypes and only samples with more than 60% of $CD19^+/CD10^+$ B-cells were selected for the study. The rationale for this selection criterion was to ascertain high sample homogeneity in terms of their immunophenotype, far-off from the normal range of <15% B-cells in healthy individuals. 4–10 ml of peripheral blood samples and 1–3 ml of bone marrow specimens from B-ALL patients were used for isolation of $CD19^+$ cells. Leukemic blasts were isolated by density gradient centrifugation using Histopaque®-1077 (Sigma, St. Louis, MO, USA) following the manufacturer's protocol; the $CD19^+$ cells enriched by immunomagnetic separation (Miltenyi Biotec, Germany) and were >95% pure as determined by flow cytometry employing anti-CD19-FITC antibody. For immunomagnetic separation, cells were washed with PBS and re-suspended in minimal volume of ice cold PBS, pH-7.2, 0.5% BSA, 2 mM EDTA. Next, cells were