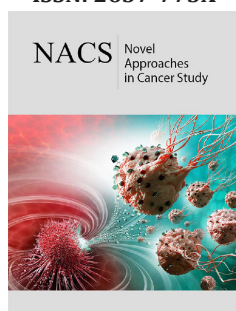# Proteomics Exploration of Chronic Lymphocytic Leukemia

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Abstract

Chronic Lymphocytic Leukemia (CLL) is an adult heme malignancy characterized by the presence of mature-appearing CD5+ B cells in the blood, bone marrow, and secondary lymphoid organs. CLL is heterogeneous in its progression and clinical outcomes. Factors that contribute to the heterogeneity include the immunoglobulin heavy chain (IGHV) status and chromosomal aberrations. CLL's heterogeneous behavior has made it difficult to understand its biology and find a cure. Systems biology approaches are commonly used to establish biomarkers for cancer. Because proteins are targets of FDA approved therapies, proteomics approaches have been used to decipher the c mplex behavior of CLL pathogenesis and identify actionable targets. In this minireview, we highlight factors that contribute to CLL, new insights discovered from proteomics studies, and future directions.

Introduction

Chronic Lymphocytic Leukemia (CLL) is an adult hematological malignancy characterized by the presence of mature-appearing CD5+ B cells in the blood, bone marrow, and secondary lymphoid organs [1]. In the United States, there will be an estimate of 20,720 new cases and 3,930 deaths according to the American Cancer Society statistics. Symptoms include swollen lymph nodes, frequent infections, and fatigue which negatively impacts the quality of life of people affected [1]. CLL is heterogeneous in its progression and clinical outcomes. Factors that contribute to the heterogeneity include the immunoglobulin heavy chain (IGHV) status and chromosomal aberrations [2,3]. The IGHV mutation status, stratified as > or < 2% of cell with the mutation[4], can be used to define two subtypes of CLL: Unmutated(U-CLL) and Mutated CLL(M-CLL) with 40% and 60% of patients respectively. U-CLL is characterized by the presence of CLL cells that have less than two percent of their IGHV mutated, whereas M-CLL cells have more than two percent mutated [4]. U-CLL is the more aggressive phenotype [2] having increased responsiveness to antigens that bind the B cell receptor (BCR) versus M-CLL cells [5]. M-CLL is the more indolent phenotype. Increased BCR signaling results in increased cell survival and proliferation [5].

Mutations that influence CLL's progression include the 17p deletion(p53), trisomy 12(NOTCH1), 13q deletion(mir15), deletion 11q(ATM), and SF3B1[6]. These mutations result in the loss of p53, increased NOTCH1 expression, increased BCL-2 expression, and loss of ATM. NOTCH1 is mutated in 10-15% of patients diagnosed with CLL. NOTCH1 has a role in the development of different types of blood cells. NOTCH1 amplification results in faster progression, a less favorable outcome, and have increased risk of Richter's transformation, the process of CLL cells transforming into diffuse large B cell lymphoma [7]. SF3B1 is a splicing factor subunit and has a role in alternative mRNA splicing and formation of certain proteins in CLL and other hematological malignancies [8]. The SF3B1 mutation is commonly found in patients with U-CLL and high expression of CD38 and ZAP-70 and is associated with shorter treatment free survival [9]. 10-15% of patients have a mutation in this gene resulting in dysfunctional protein processing [8]. This mutation is associated with fludarabine therapy resistance. p53 is an important part in the cell cycle checkpoint. It is part of the DNA damage response. This mutation is associated with increased growth and resistance to chemotherapy [10].

Factors that are associated with high-risk CLL progression are the high expression of CD38, Beta2-microglobulin, and ZAP-70. CD38 is a cell surface protein on immune and CLL cells that

express CD38 are more responsive to BCR stimulation and exhibit enhanced migration and increased proliferation and chemotaxis via ZAP-70 and ERK 1/2 signaling [11]. Beta 2-microglobulin(b2-m) is a protein that is a component of the MHC class I complex. High b2-m is associated with advanced stage and high lymphocyte counts in CLL [12]. ZAP-70 is a protein expressed near the surface of T cells and has an important role in T cell signaling. Abnormal ZAP-70 expression is associated with increased BCR signaling and migration towards tumor microenvironment in CLL and shorter survival [13,14]. Patients with high expression of ZAP-70, CD38, and beta 2-microglobulin have a poorer prognosis.

Because CLL has a variety of factors that influence its progression and heterogeneity, it is important to identify how these factors alter B cell behavior on a systems level. Systems level approaches include genomics, transcriptomic, epigenetics, metabolomics, and proteomics. Although there is much insight provided by the various omics methods, proteins are the final product and the targets of many FDA approved therapiesand therefore may be more relevant to study. Furthermore, previous studies have reported low correlations between protein and gene expression. Hence, we should focus on exploring the post-translationally modified proteins to define and understand cancer biology. High throughput proteomics methods consist of multiplex immunoassays, phospho-specific flow cytometry, reverse phase protein arrays, quantitative immunohistochemistry, and mass spectrometry [15]. Phosphorylation specific flow cytometry is a high throughput method that quantifies phosphorylation levels of many cellular proteins [15]. Quantitative immunohistochemistry is a method that uses fluorescent labels to determine tumor and stroma localization, of proteins of interest [15]. Mass Spectrometry quantifies low and large abundance phospho-proteins. Multiplex immunoassays and Reverse Phase protein arrays allow the detection of proteins, with antibodies, from hundreds of analytes simultaneously and uses a small amount of sample [15]. The purpose of this review is to discuss proteomics methodologies used in CLL research and the insights that they have brought.

Outcomes of CLL Proteomics Research: Deciphering CLL Metabolism and Tumor Microenvironment Signaling

Deregulated cellular metabolism is an established hallmark of cancer [16]. Cancer cells derived from different tissues, have heterogeneous metabolic signaling. Proteomics methods have been ideal for quantifying metabolic signaling molecules to define cancer specific signatures. Mayer et al. Performed a study using mass spectrometry on 16 CLL and healthy donor samples from younger and older patients [17]. This study provided insight in the differences in the expression of proteins involved in the hallmarks of cancer. They observed that proteins involved in the inflammatory response, mitochondrial metabolism, DNA damage response, and mitochondrial stress and aging were deregulated in elderly patients versus the young patients. When assessing expression of proteins involved in the hallmarks of cancer, CLL cells had increased expression of proteins involved in "genome

instability and mutation", "avoiding immune destruction", "resisting cell death", and "deregulation of cellular energies". [17]

Thurgood et al. performed a study using a mass spectrometry analysis of PBMC(n=3) and healthy(n=6) or CLL(n=12) lymph node samples(n=12) and compared the results to healthy controls. [18] 12 and 3 CLL and healthy PBMC samples were included. 14 CLL and 6 healthy lymph nodes were examined. They observed that CLL cells had upregulation of proteins involved in ROS formation, the anti-oxidant response, and an auto-immune response that initiates hemolytic anemia compared to healthy controls. Differential expression analysis between the U-CLL and M-CLL samples revealed in 60 downregulated and 133 upregulated proteins. The upregulated proteins were involved in BCR stimulation and ROS production, consistent with previous report of increased BCR signaling in U-CLL. Pathways associated with the 189 differentially expressed proteins include migration, stress, PI3K, and glycolytic/ fatty acid signaling. Differential expression analysis of CLL and healthy lymph nodes revealed increased expression of CD99, STAT3, and anti-oxidant proteins. The authors also observed that there was a shift towards increased fatty acid and cholesterol synthesis in CLL cells.

CLL progression and survival is highly dependent on interactions within the tumor immune microenvironment [19]. The microenvironment consists of T, stromal, endothelial, and nurse-like cells. These cells provide essential signaling molecules such as chemokines, WNT, and interleukins for cells survival and proliferation. CLL cell dependency on the microenvironment has made it difficult to culture cells once they are removed from patients. The roles that each of these cell types play in CLL progression have not been defined. To identify the role of stromal cells in CLL progression, Vangapandu et al. [20] used RPPA on peripheral blood CLL cells that were co-cultured with stromal cells. CLL cells that were cultured with stromal cells have significant [20] increase in calveolin-1, STAT3, NFkB, PDGFR, and cyclin B1 versus CLL cells alone. Increased expression of NFkB and STAT3 are associated with aggressive CLL progression. The secretion of PDGF was previously shown to mediate the expansion of mesenchymal stem cells in the tumor microenvironment. This study established that the stromal cells aid in the malignant growth of CLL cells and increases interactions with cells in the tumor microenvironment.

A deregulated immune response is a key symptom of CLL. The origin of this deregulated immune response is currently unknown. Some hypothesize that CLL B cell expansion originated in response to a bacterial or viral infection or an auto-antigen. Griggio et al. identified cancer associated antigens in CLL that are recognized by patient antibodies.[21] They obtained these results from a serological proteome analysis (SERPA) of sera obtained from 35 patients who were untreated.

SERPA is a combination method that consists of gel electrophoresis, western blotting, and mass spectrometry. Proteins recognized by antibodies, in patient sera, include ENO1, G3P

dehydrogenase, pyruvate dehydrogenase E1 subunit, and an actin binding protein. Out of all the proteins recognized, ENO1 was the only protein that was significantly recognized. ENO1 was also found to be overexpressed in lymph node tissues, expressed intracellularly in peripheral blood derived CLL cells, and present on the surface of apoptotic B cells. The authors also noted that there was increased immunoreactivity in samples that had the high expression of ZAP70 and CD38. CLL cell recognition of these antigen promotes increased BCR signaling, hence increasing proliferation and survival, and establishing a chronic disease state.

Conclusion

Proteomic studies provide information on what proteins are actually being synthesized and post-translationally modified in normal and disease states. Examination of mutations in DNA are resourceful, but not if they do not have a consequence on the final protein product. Transcriptome information is also really good, but not all genes are translated into proteins. Proteins are the primary targets for cancer therapy. Proteomics studies have revealed new findings on the protein expression of CLL cells in the blood and lymph nodes along with young and old patients. These analyses have also brought new knowledge on the interactions between CLL cells and its tumor microenvironment and possible antigens that drive the chronic disease state. Although there have been many insights from past CLL proteomics studies, there is much left to do to define its pathogenic biology to find a cure. The aforementioned CLL protein studies were performed with mass spectrometry and small sample sizes, which is inefficient for proving a hypothesis. Also, the attributes examined in these studies did not reflect differences in patient protein expression reported based on their mutation, progression, and therapy status. Mutation, therapy, and progression status are prognostic factors that should be examined to establish signatures that can be used in a clinical setting. Including these factors will create a study that is truly representative of the heterogeneous patient population.

Recurrent mutation, metabolic, and gene signatures that have been identified that affect response,resistance, and survival status, but not protein signatures. It is important to identify protein signatures, since proteins are the actionable targets for FDA approved drugs. Resistance in CLL is typically attributed tomutations in BTK, PLCg, and BCL-2 proteins. Proteomics has the advantage identifying protein expression patterns associated with patients who have resistance status. For instance, Walliser et al. used proteomics to identify proteins associated with ibrutinib-resistant patients with mutations in PLCg. They discovered that PLCg is sensitive to RAC2 expression. Targeting RAC2 could be a novel therapy target for patients with the mutated PLCg, making their CLL cells more sensitive to ibrutinib therapy[22]. Information from proteomics studies has the potential to be used to identify novel therapy targets to improve treatment options. To validate previous findings and to gather more insight, a large patient cohort, that is truly representative of CLL heterogeneity, should be analyzed. Future CLL proteomics studies should also include more analyses of post translational modifications, extensive clinical phenotype associations, analysis of cells from the tumor immune microenvironment, and observe changes in protein expression over time.

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