Application of oncoproteomics to aberrant signalling networks in changing the treatment paradigm in acute lymphoblastic leukaemia

Elena López Villar a, *, Xiangdong Wang b, c, Luis Madero a, William C. Cho d, *

a Department of Oncohematology and Pediatrics, Hospital Infantil Universitario Niño Jesús, Universidad Autónoma de Madrid, Madrid, Spain
b Biomedical Research Centre, Fudan University Zhongshan Hospital, Shanghai, China
c Department of Respiratory Medicine, Zhongshan Hospital Fudan University School of Medicine, Shanghai Respiratory Research Institute, Shanghai, China
d Department of Clinical Oncology, Queen Elizabeth Hospital, Hong Kong

Abstract

Oncoproteomics is an important innovation in the early diagnosis, management and development of personalized treatment of acute lymphoblastic leukaemia (ALL). As inherent factors are not completely known – e.g. age or family history, radiation exposure, benzene chemical exposure, certain viral exposures such as infection with the human T-cell lymphoma/leukaemia virus-1, as well as some inherited syndromes may raise the risk of ALL – each ALL patient may modify the susceptibility of therapy. Indeed, we consider these unknown inherent factors could be explained via coupling cytogenetics plus proteomics, especially when proteins are the ones which play function within cells. Innovative proteomics to ALL therapy may help to understand the mechanism of drug resistance and toxicities, which in turn will provide some leads to improve ALL management. Most important of these are shotgun proteomic strategies to unravel ALL aberrant signalling networks. Some shotgun proteomic innovations and bioinformatic tools for ALL therapies will be discussed. As network proteins are distinctive characteristics for ALL patients, unrevealed by cytogenetics, those network proteins are currently an important source of novel therapeutic targets that emerge from shotgun proteomics. Indeed, ALL evolution can be studied for each individual patient via oncoproteomics.

Keywords: acute lymphoblastic leukaemia • personalized medicine • shotgun proteomics

Introduction

Early accurate diagnosis and personalized treatment are essential to treat complex and fatal diseases such as cancer. New paradigms are emerging for innovations on therapies, especially for chronic and malignant diseases such as acute lymphoblastic leukaemia (ALL) via shotgun proteomics [1].

ALL can be classified via cytogenetic and molecular subgroups for diagnosis, prognosis, and more importantly, for the application of the correct treatment. A remarkable but common in patients with ALL is the appearance of resistance against drugs/therapies. Mutations, genetic aberrations (Table 1) [2-5], cellular heterogeneity of ALL after initial response to therapy and deregulation of signalling networks (unknown for ALL patients with poor responses) play important roles in ALL resistance therapies [6].

Aberrant proteins and signalling networks of ALL evolution can be addressed directly from human body fluids via state of art shotgun oncoproteomics. This makes it possible to get the molecules which are not working well or are mutating (space, time) during ALL treatments of each patient. This allows detection of the molecular differences between patients with good response to ALL therapies and patients with poor response. ‘Omics’ shotgun oncoproteomic technologies coupled to cytogenetics are used today in integrated approaches to clarify ALL for future therapy innovations which will benefit ALL patients [7].
ALL treatments tend to be personalized via proteomics innovations

With the completion of the human genome project, standard ALL treatments may be adjusted by genetic mutations, including single nucleotide polymorphisms in predicting patient responses (Table 1). In broad sense, individualized medicine is not new, but the options and perspectives have been widely expanded within the last decade via omics innovations, including shotgun proteomics, especially when integrating hundreds or thousands of proteomic signatures from ALL patients’ evolution, bioinformatic tools offer different software to create an ALL ‘library’ to link biological discoveries for new drug designs [8].

Table 1 Common acute lymphoblastic leukaemia translocations and cytogenetic abnormalities (http://emedicine.medscape.com/article/207631-workup#a0756) [2–5]

| Mutation       | Related genes     | Survival after 2–3 years (%) |
|----------------|-------------------|------------------------------|
| t(10;14)(q24;q11) | HOX11/TCRA       | 75                           |
| 6q             | Unknown           | 47                           |
| 14q11          | TCRα/TCRD         | 42                           |
| 11q23          | MLL               | 18–26                        |
| 9p             | Unknown           | 22                           |
| 12             | TEL               | 20                           |
| t(1;19)(q23;p13) | PBX1/E2A         | 20                           |
| t(8;14)(q24;q32) | c-myc/IGH        | 17                           |
| t(2;8)(p12;q24)  | IGK/c-myc        | 80                           |
| t(8;22)(q24;q11) | c-myc/IGH        | 5–10                         |
| t(9;22)(q34;q11) | bcr-abl          | 66                           |
| t(4;11)(q21;q23) | AF4-MLL          | 0–10                         |

For integrating hundreds or thousands of proteomic signatures from ALL patients’ evolution, bioinformatic tools offer different software to create an ALL ‘library’ to link biological discoveries for new drug designs [8].

Proteomics-based studies for the improvements of ALL therapy

Accordi et al. [11] applied reverse phase protein microarrays to identify active-mutated proteins in 118 paediatric B-cell precursor (BCP)-ALL patients. In this study, 92 key signalling proteins have been shown to be activated for this pathology via phosphoproteomics. The involved pathways are related to cell proliferation in patients with poor prognosis. BCL-2 is hyperphosphorylated via AMPK activation in MLL-rearranged patients. AMPK could have an important role for...
Inhibition of apoptosis in these patients. This is an important issue for innovations in ALL therapy. In addition, Accordi et al. [11] realized that prednisone is up-modulating the LCK proto-oncogene. Src family tyrosine kinase (LCK) activity in ALL patients with poor response to this drug. LCK can also play important roles for developing new therapies for ALL patients resistant to prednisone. In addition, Cyclin E is highly expressed in patients suffering relapses at early-phases of the therapy. Thus, there is an important relationship between high level of Cyclin E and relapse incidence. This issue can be complemented via shotgun oncoproteomics with future research as it remains critical for improving therapy for ALL patients with relapses [15].

Spleen tyrosine kinase (SYK) is a key molecule controlling apoptosis related to the activation of PI3-K/AKT, NFκB and STAT3 anti-apoptotic signalling pathways in leukaemia type B. Uckun and Qazi [16] carried out a research study where they proposed that SYK might overcome the resistance of malignant B-lineage lymphoid cells to apoptosis providing the theory for more effective multi-modality treatment-therapy regimens for poor prognosis B-precursor ALL (BPL). Radiation by ionizing and different types of chemotherapeutic drugs used in BPL therapy produces DSB in nuclear DNA triggering apoptotic cell death. NFκB and PI3-K survival signalling pathways are activated by chemotherapeutic agents and contribute to drug resistance of leukaemic cells. In addition, NFκB and PI3-K signalling pathways are regulated by tyrosine kinase SYK. SYK phosphorylates SLP-65/BLNK (B-cell linker) are an integral part of effective pre-breaking-point cluster region (BCR) signalling in BCPs as well as BCR signalling in mature B lymphocytes. SYK plays a relevant regulatory function in early specification and maturation events during B-cell ontogeny. Inhibition of SYK blocks BCR and mTOR signalling pathways, leading to apoptotic death of leukaemic cells. In addition, SYK has another anti-apoptotic role related to leukaemic precursor B cells at the early stages of B-cell (human) ontogeny. Inhibition of SYK triggers apoptosis in primary leukaemic cells from BPL patients who are also resistant to therapy. They carried out studies using a liposomal nanoparticle formulation of a SYK substrate-binding site inhibitor called C61. C61 is coming from ‘nanomedicine’ potential candidate for poor prognosis cases and for relapses BPL cases. C61 was successfully tested in mice as it was able to induce apoptosis in radiation-resistant primary leukaemic cells coming from BPL patients. Uckun and Qazi [16] propose that C61 may be a useful strategy for innovations against ALL therapy refractory. When combining this important current ALL research study to proteomic strategies, signalling networks involved in ALL progression and affected by C61, could be discovered; thus, key signalling molecules ALL can be identified for good new specific target candidates [16].

When Notch signalling is abnormally activated/deactivated, this implies a relevant oncogenic mechanism for ALL T cells. ALL subtype T is commonly quite aggressive, especially for disease in children. Lin and coworkers [13] via proteomic assays identified DD5, an ATP-dependent DEAD-box RNA helicase, and MAML1 protein. DD5 has been shown to be associated with the endogenous NOTCH1 transcription activation complex in human T-ALL leukaemic cells. Thus, unraveling the molecular regulation of Notch signalling is crucial to identify new approaches to block aberrant Notch oncogenic activity during ALL progression. MAML1 transcriptional activator is critical for signalling activation of Notch. Indeed, MAML1 is the one involved in the regulation of Notch in leukaemic cells although its mechanism remains unknown. Lin et al. [17] also proved that lentivirus-mediated short-hairpin RNA knock-down of DD5 was because of low expression of Notch target genes, decreased cell proliferation and increased apoptosis in cultured human leukaemic cells together with signalling of Notch activation. This study demonstrates that DD5 is pushing for useful Notch-mediated transcription in leukaemic cells. Therefore, DD5 can be a future potential new target therapy for regulating Notch signalling in leukaemia [17].

PI3K/AKT pathway mutations have been found in T-cell ALL. Nevertheless, their relevance related to other genetic aberrations is not yet clear. PTEN mutations are proposed as secondary mutations which follow NOTCH1-activating mutations and later on, they can produce cellular resistance to γ-secretase inhibitors. Zuurbier et al. [18] investigated the role of PTEN, PI3K and AKT aberrations in paediatric T-cell leukaemia patient cohort (n = 146) treated on DCOG or COALL protocols. The authors discovered that both PTEN and AKT E17K aberrations appeared in around 13% and 2% of patients respectively. They realized that PTEN/AKT mutations appeared in a high percentage in TAL- or LMO-rear ranged leukaemia, although PTEN/AKT mutations did not appear in TLX3-rearranged patients (P = 0.03). The opposite data resulting was obtained for NOTCH1-activating mutations. In addition, Zuurbier et al. [14] detected that T-cell leukaemia patients without PTEN/AKT and NOTCH1-activating mutations fared well, with a cumulative incidence of relapse of only 8% versus 35% for PTEN/AKT and/or NOTCH1-activated patients (P = 0.005). This is critical and important information related to the significance of PTEN and AKT aberrations in paediatric T-cell ALL. Applying quantitative phosphoproteomics strategies to peripheral blood and bone marrow ALL, several signalling pathways (and networks) can be captured in a single experiment. Therefore, hundreds of phosphorylated protein kinases can be detected if they are working well during ALL or we can even get those which do not operate properly from each ALL patient evolution. This is key information for patients suffering relapses and resistance or toxicity to current ALL treatments [18].

Braoudaki et al. [19] developed proteomic studies to see the differential proteins expressed when comparing low- and high-risk patients suffering ALL. Cytogenetic assays were carried out in parallel to proteomics to get complementary data for clinical advances. Proteins were extracted from bone marrow and peripheral blood plasma of patients who belong to high- and low-risk ALL at diagnosis. They applied 2DE (2 Dimensional Electrophoresis) coupled to MALDI-MS/MS analysis and, later on, the differentially expressed proteins detected were validated via Western blot. Proteins Clus, Ceru, ApoE, ApoA4, ApoA1, Gels, S10A9, Ambp, Actb, Cata and Afam have an important role in leukaemia prognosis, mainly as distinctive signals for aggressive leukaemia cases. None of these identified biomarkers for ALL, as distinctive signals for aggressive cases, are currently evaluated routinely at hospitals. It could be interesting to check the previously resulting data of Braoudaki et al. [15] for each patient when he/she is diagnosed with ALL. If more patients could be checked for these biomarkers, more statistical and clinical value would be achieved, thus, ensuring true biomarkers useful for therapy improvements. We would change 2DE for ESI-LC-MS as it is faster and with higher accuracy despite the important ALL information provided [19].
Peripheral blood plasma was shown to be a good sample to predict clinical behaviour in ALL patients irrespective of the percentage of bone marrow blasts. Albitar et al. [20] analysed, via proteomics, this clinic sample type from 57 patients suffering ALL before initiation of therapy. They applied strong anion exchange coupled to protein chip arrays and surface-enhanced laser desorption/ionization. It was shown that recurrence prediction is independent to bone marrow blast count, cytogenetic assays and surface markers. Thus, evolution of responses of patients can be followed via proteomic tools to cover gaps which nowadays are not explained by genotype–phenotype assays (Table 1).

Also, proteomic research in paediatrics is important and most of the successes thus far are seen in research that utilize samples that require less invasive procedures and focus on prevailing childhood diseases such as ALL. Nevertheless, most of platelet proteome data obtained to date are derived from the adult population and the potential of platelet proteomic application in children has not yet been explored [21].

**Future perspectives**

Signalling networks do not operate in an independent way, as signalling cascades are connected. Thus, we defined a space (bone marrows and peripheral blood) and time-based strategies (at several states of the patients) to understand the ALL evolution of each patient and to capture the dynamics of phosphorylation events, from which useful targets can be discovered for treating ALL.

A shotgun oncoproteomic strategy, space and time-based, using sequential elution from immobilized metal affinity chromatography (SIMAC) [22] and isobaric tag for relative and absolute quantitation (iTRAQ) [23] coupled to liquid chromatography, electrospray ionization, tandem mass spectrometry (LC-ESI-MS/MS) [24, 25] is proposed to be applied in our research team by the Spanish Health System (SNS) in collaboration with international research teams to unravel signatures of ALL patients who do not respond well to treatments compared to those who do.

SIMAC coupled to iTRAQ and LC-ESI-MS/MS will allow us to identify the up- and down-regulated phosphorylated proteins via highly sensitive techniques. The resulting data should show us important clinical and biological information of signalling networks involved in ALL resistances and toxicities.

We expect that this research strategy will help to improve current ALL treatments, especially for ALL patients with poor prognoses (Figs 1 and 2). Flow-through routine analysis of blood for leukaemia diagnose via oncoproteomics and putative visualization of oncoproteomics ALL resulting data to several patients at different ALL states (diagnose, 14 days after treatment and at the end of the treatment) are detailed in both figures. Comparing different ALL states per each patient, ALL signatures evolution can be achieved, thus, unknown ALL molecules which are not working properly can be detected. In fact, when comparing the resulting data from ALL patients with good and poor prognosis, distinctive signatures involved in ALL progression can be identified. Therefore, therapy innovations can be achieved. As delegate of HUPO, for human proteome on children ALL studies at Hospital Universitario Niño Jesús, we are pursuing to...
support the human proteome in this context. We envision this will further benefit the understanding of the pathology of the disease and ultimately improve the diagnoses and personalized treatment. Selected/multiple reaction monitoring [26–28], Western blotting and ELISA assays can also help to validate identified signatures for each ALL patient from shotgun oncoproteomic strategies.

Fig. 2 Putative visualization of oncoproteomics ALL resulting data to several patients at different acute lymphoblastic leukaemia (ALL) states (diagnose, 14 days after treatment and at the end of the treatment). Comparing different ALL states per each patient, ALL signatures evolution can be achieved, thus, unknown ALL molecules which are not working properly can be detected. In addition, when comparing the resulting data from ALL patients with good and poor prognosis, distinctive signatures involved in ALL progression can be identified. Therefore, therapy innovations can be achieved.

Fig. 3 Schedule from clinical to oncoproteomics and bioinformatics analyses. Once the clinical goal and human body fluids have been selected from ALL patients, efficient shotgun oncoproteomics must be established and be coupled to bioinformatic tools, which can be applied for the potential development of personalized approaches, aimed at the concept of personalized ALL medicine. After long and tedious assays, new ALL targets can be discovered, and database mining from new binding sites from ALL candidates can be created. Also, verification of side effects and toxicities for the new ALL molecules have to be studied, to validate a new ALL drug candidate.
The resulting data per patient can be correlated and linked to other patients. Thus, a ‘library’ collection of ALL evolution for patients can be set-up, which will help to ultimata a reference, once a statistically significant number of ALL patients is studied.

Once the phosphorylation state of network proteins, constitutive or associated to ALL evolution is established by shotgun oncoproteomics, a range of bioinformatic methods permits deeper study of its properties and contacts. Using sequence analysis, sequence comparison, virtual approaches of protein–protein, protein–ligand interaction or molecular dynamics simulations, initial physical information can be applied for the potential development of personalized approaches, aimed at the concept of personalized ALL medicine. Bioinformatics covers a wide spectrum of techniques for the generation and use of beneficial information from structure, sequence or relationships among biological items (DNA, RNA, proteins, macromolecular complexes, etc.) [29, 30]. Those most useful in clinical ALL studies are: Ascore and PhosphoScore (statistical algorithms which measure the probability of correct phosphorylation site localization based on the presence and intensity of site-determining ions in MS2 spectra), and next-generation sequencing (NGS) was used in a detailed study of genes involved in colorectal cancer [31]. As a main conclusion of the study, the authors stated that sequencing of whole tumour exomes allowed prediction of the microsatellite status of trinucleotide CGC, facilitating, at the same time, the putative finding of relevant mutations. NGS can be applied to formalin-fixed and paraffin-embedded material, allowing the renewed study of all the ancient material stored in the pathology departments, sequence-to-sequence and sequence-to-structure comparisons (multiple sequence analysis) to obtain valuable information on the nature of the functional implications of the mutated residues in the protein ALL context. Homology modelling 3D structure of the active centre of a protein of interest (in absence of experimental crystal structures, the homology modelling methods), can develop a 3D model from a protein sequence based on the structures of a crystallized homologous protein. Information on the 3D structure of the active centre of a protein of interest and/or its natural ligands can be used as a basis for the design of effective drugs and the more sophisticated rational drug design and molecular dynamics techniques. Using shotgun oncoproteomics together with structural analysis of proteins and bioinformatic tools, important biological understanding of ALL evolution can be achieved. Prototypical shotgun oncoproteomic coupled to bioinformatics pipeline useful for clinical ALL research is illustrated [32, 33] (Fig. 3). In the schedule of Figure 3, it is illustrated in a simple manner that firstly, the clinical goal and human samples have to be established and selected from ALL patients; secondly, an efficient shotgun oncoproteomics must be designed and coupled to bioinformatic tools to get biomarkers for a given clinical issue, and to arrange the resulting data. From the resulting data, potential ALL new targets can be achieved, and therefore new ALL drug candidates can be designed and validated. In fact, verification of side effects and toxicities for the new ALL molecules have to be studied, to validate a new ALL drug candidate, aimed at the concept of personalized ALL medicine.

Conclusions

Network proteins with distinctive characteristics for ALL patients, unrevealed through cytogenetics, are currently an important source of novel therapeutic targets that emerge from shotgun proteomics. Shotgun oncoproteomics and bioinformatic strategies need to be combined to achieve the personalized medicine. Studies of reversible phosphorylation in proteins from ALL networks will allow the generation of models for protein–protein contacts at the molecular level taking into account each particular protein sequence. Molecular dynamic analysis of those contacts will allow the modification of the 3D computer models obtaining virtual structures tailored to individual patients. So, we should get, according to the resulting structure information, new drug candidates for adapting the therapies; and innovate new ALL treatments. In fact, the ALL evolution per each patient can be studied via shotgun oncoproteomics, thus ALL patients can be benefited.

Acknowledgements

Dr. E. López Villar is supported by Spanish Health System SNS ISCIII-BOE 2012. This work is supported by Project FIS PI13/02475. Special acknowledgement to Professor Juan Pablo Albar, RIP. His valuable suggestions, high professional and human level contributed greatly for the concession of this important scientific project. Dedicated to Professor Juan Pablo Albar: “Climb the mountains and get their good tidings. Nature’s peace will flow into you as sunshine flows into trees. The winds will blow their own freshness into you, and the storms their energy, while cares will drop away from you like the leaves of Autumn.” John Muir

Conflicts of interest

The authors confirm that there is no conflict of interest.

References

1. López Villar E, Wu D, Cho WC, et al. Proteomics-based discovery of biomarkers for paediatric acute lymphoblastic leukaemia: challenges and opportunities. J Cell Mol Med. 2014; 18: 1239–46.
2. Seiter K (Department of Internal Medicine, Division of Oncology/Hematology, New York Medical College, member of the following medical societies: American Association for Cancer Research, American College of Physicians, and American Society of Hematology). Disclosure: Novartis Honoraria Speaking and teaching; Novartis Consulting fee Speaking and teaching; Ariad Honoraria Speaking and teaching; Celgene
