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Phosphoprotein DIGE profiles reflect blast differentiation, cytogenetic risk stratification, FLT3/NPM1 mutations and therapy response in acute myeloid leukaemia

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ABSTRACT

Acute myeloid leukaemia (AML) is an aggressive blood cancer characterized by a distinct block in differentiation of myeloid progenitors, recurrent chromosomal translocations and gene mutations of which > 50% involve signal transduction through dysregulated kinases and phosphatases. In search for novel protein biomarkers for disease stratification we investigated the phosphoproteome in leukaemic cells from 62 AML patients at time of diagnosis using immobilized metal-affinity chromatography, protein separation by two-dimensional differential gel electrophoresis (2D-DIGE) and mass spectrometry before validation by selected reaction monitoring (SRM). Unsupervised clustering found 27 phosphoproteins significantly discriminating patients according to leukaemic cell differentiation (French-American-British (FAB) classification), cytogenetic and mutational (FLT3, NPM1) status or response to chemotherapy. Monocytic differentiation (FAB M4–M5) correlated with enrichment of proteins involved in apoptosis (MOES, ANXA5 and EFHD2). TALDO, a protein associated with thrombocytopenia if down-regulated, was elevated in patients with wild type NPM1 compared to patients with NPM1 mutation. This study demonstrates the potential of quantitative proteomics in AML classification and risk stratification.

Biological significance: Patients diagnosed with AML are currently categorized according to cellular morphology, cytogenetic alterations and mutations, although the majority of these cellular and genetic alterations have no or unsolved impact on therapy selection or prognosis. We therefore explored the phosphoproteome for abundance changes associated with traditional classifiers to unravel patterns that could stratify patients at the protein level. MOES, ANXA5 and EFHD2 were confirmed by SRM to be correlated to monocytic differentiation, whilst TALDO was elevated in NPM1 wild type patients.

1. Introduction

Acute myeloid leukaemia (AML) is a heterogeneous aggressive haematological malignancy characterized by accumulation of haematopoietic progenitor cells in the bone marrow. The patients are traditionally subdivided in eight French-American-British (FAB) classification groups (M0-M7) based on cellular morphology [1,2] however, somatic karyotype is currently the most important single parameter in prognostication [3–5]. Additional prognostic parameters are the response to the first chemotherapy cycle, peripheral blood blast count and genetic abnormalities detected by molecular analyses [6–8]. The most frequent and prognostically distinct molecular abnormalities are mutations in Fms-like tyrosine kinase 3 (FLT3) and Nucleophosmin-1 (NPM1) [9–12]. The FLT3 internal tandem duplication (ITD) mutation is found in approximately one third [13], and mutations in NPM1 are found in 35% of AML patients [14]. FLT3-ITD is associated with disease relapse and poor prognosis [15,16], whereas NPM1 mutations are associated with good prognosis for patients with wild type FLT3 [3,12,17,18].

Whole-genome sequencing has been used to detect additional prognostic mutations in AML-patients [19,20], and mutations in signal transduction pathways have been found in > 50% of all patients [21].

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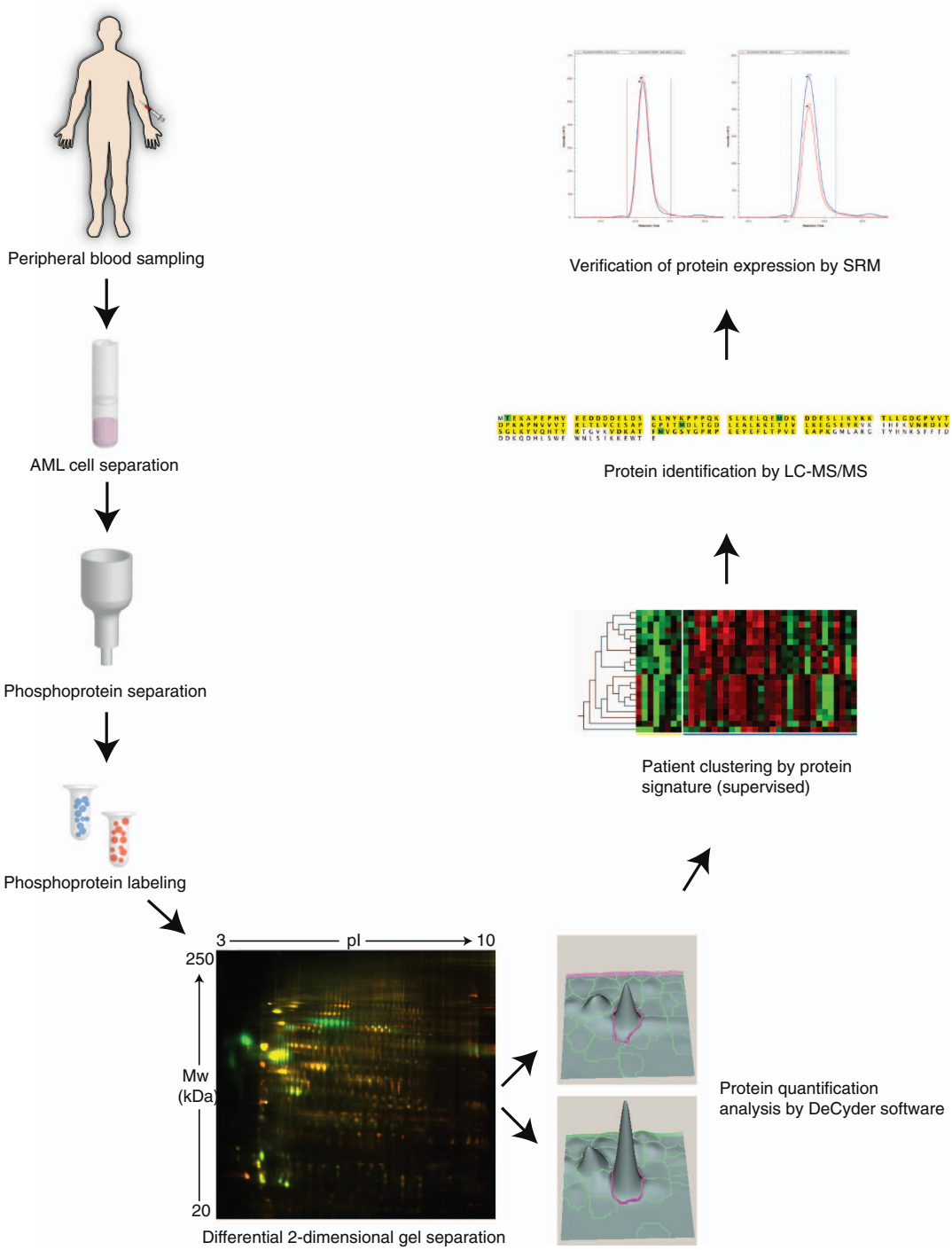

Fig. 1. Flow chart of experimental setup.
Peripheral blood samples were harvested from newly diagnosed AML patients prior to separating the leukaemic blasts by density gradient filtration. Phosphorylated proteins were collected by IMAC and covalently labelled with Cy5 or Cy3. Samples were pooled and separated by 2D-DIGE and patients were clustered according to protein signatures using Qlucore Omics Explorer. Proteins were subsequently identified by mass spectrometry, and verified using selected reaction monitoring.

Single cell profiling of intracellular phosphoproteins has successfully identified unique AML phosphoprotein network profiles that correlates both with genetics, prognosis and therapy response [22,23]. Proteomic analyses have been suggested for determination of leukaemic cell differentiation and for stratification of relapse risk [22–27], and a reverse phase protein array (RPPA) analysis identified 24 proteins that subdivided 256 newly diagnosed AML patients in their respective FAB groups as well as distinct protein abundance patterns for patients with FLT3-ITD [25]. Furthermore, a phospho-signature consisting of five phosphosites has been suggested to have predictive value for quizartinib treatment [28], a specific FLT3 inhibitor in clinical development. Together, phosphoproteomics may be feasible both for risk stratification and for identifying responders of targeted therapy in blood cancer.

In this study we investigated the immobilized metal-affinity chromatography (IMAC)-enriched phosphoproteome of AML cells by two-dimensional difference gel electrophoresis (2D-DIGE), and combined the biomarker discovery with validation using selected reaction monitoring (SRM) (Fig. 1). Our results showed that phosphoprotein profiles of AML reflected therapy response, differentiation, cytogenetics and the