Identifying protein expression changes in acute myeloid leukemia

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INTRODUCTION

Acute myeloid leukemia (AML) is a devastating cancer affecting the hematopoietic system. It is characterized by infiltration of abnormal proliferating immature myeloid cells originating in the bone marrow. Although this disease represents only 35% of diagnosed leukemias, it accounts for nearly 50% of leukemia-related deaths, making it the leading cause of leukemic-related mortality. Disease initiation often occurs through common recurrent genetic aberrations resulting in the formation of oncogenic fusion proteins. Two common mutations include mixed lineage leukemia (MLL) rearrangements and an inversion of chromosome 16, known as inv(16). Although many of these AML initiating mutations have been identified, the downstream effects leading to disease progression are still largely unknown. Previous research has relied on RNA sequencing and microarray techniques to study the downstream effects, providing data at the transcriptional level. While these studies have proven efficacious, they fail to capture the changes that occur at the proteomic level. To interrogate the effect of protein expression alterations in AML, we performed a quantitative mass spectrometric analysis using mouse models to compare three tumor types (inv(16), MLL-AF9, and MLL-ENL) to untransformed cells from the tumor-initiating population. In parallel, we performed RNA sequencing for the same populations. With these combined results, we identified 61 proteins whose expression was upregulated in AML tumors, but strikingly, were unaltered at the transcriptional level. These proteins are shown to be associated with mitochondrial function as well as RNA processing. In addition, analysis of patient expression data sets in adult and pediatric AML reveal that a number of the proteins differentially expressed have no significant RNA expression alterations. These studies identify a set of proteins that have not previously been associated with leukemia, and may ultimately serve as potential targets for therapeutic manipulation to hinder AML progression and help contribute to our understanding of this disease.

RESULTS

AML subtypes MLL-AF9 and MLL-ENL share similar protein expression, however show increased variability in RNA expression.

Differsences between expressed RNA and protein suggest additional translational regulation previously not associated with AML.

Combination of data at the transcriptional and proteomic levels allow for further analysis of the identified upregulated proteins and their roles in the dysregulation of AML cells.

AML patient data suggests no significant changes in RNA expression.

Proteins identified with TMT-labeled MS serve as potential therapeutic targets for drug discovery in AML studies.

CONCLUSIONS

Figure 1. TMT-labeled mass spectrometry in combination with RNA-seq analysis leads to identification of differentially expressed proteins. A Flow cytometry analysis of cKit, Gr1, and Mac1 identifies tumor population from each mouse sample. B Kaplan-Meier curve of mouse models depicts survival (n = 8). C Schematic of preparation for TMT MS and RNA-seq using spleen tumors isolated from mice expressing MLL-AF9 (n = 3), MLL-ENL (n = 3), Inv(16) (n = 2) and from bone marrow of WT mice (n = 2).

Figure 2. RNA-seq identifies 3,323 differentially expressed genes among AML subtypes inv(16), MLL-AF9, and MLL-ENL. A Schematic showing number of genes identified from RNA-seq analysis. B Principal component analysis showing clustering of WT dK+ cells compared to tumors from MLL-AF9, MLL-ENL, and inv(16) mice. C Venn diagrams showing differentially expressed genes <0.7 fold below WT and ≥1.3 fold over WT. D Volcano plots showing fold change of expressed genes from tumors compared to WT dK+ cells.

Figure 3. TMT-Labeled MS identifies 1,316 differentially expressed proteins among AML subtypes inv(16), MLL-AF9, and MLL-ENL. A Schematic showing number of peptides identified from MS analysis. B Principal component analysis showing clustering of WT dK+ cells compared to tumors from MLL-AF9, MLL-ENL, and inv(16) mice. C Venn diagrams showing differentially expressed proteins <0.7 fold below WT and ≥1.3 fold over WT. D Volcano plots showing fold change of expressed proteins from tumors compared to WT dK+ cells. E Heatmap with hierarchical clustering of the significantly upregulated (p < 0.05, ≥1.3 fold increase over WT) and downregulated (p < 0.05, ≤0.7 fold decrease from WT).

Figure 4. Comparison of RNA and protein expression reveals 114 differentially expressed proteins shared among data sets. A Combined MS and RNA-seq data reveals overlap between protein and RNA. B Gene ontology analysis showing pathways known to be associated with significantly up-regulated (p < 0.05, ≥1.3 fold change) proteins using DAVID software. C Abundance plots from MS analysis showing protein expression of ETFB and ETFAL in AML mouse tumors. D Abundance plots from RNA-seq analysis showing gene expression of ETFB and ETFAL in AML mouse tumors. (p < 0.05, **p < 0.01, ****p < 0.0001, ns = non-significant).

Figure 5. Electron transfer proteins ETFAL and ETFB are differentially expressed as proteins, but are unaltered in RNA-seq and RNA patient data. A qRT-PCR plot showing mRNA expression of ETFAL and ETFB in AML mouse tumors. B Western blot confirming ETFAL and ETFB overexpression in AML tumors and in patient-derived AML cell lines compared with cKit+ lysate. C and D Analyses of patient samples from the C MILE (Haferlach T, J Clin Oncol, 2010) and D TARGET (Farrar JE, Cancer Research, 2016) studies showing expression of ETFAL and ETFB proteins (p < 0.05, **p < 0.01, ****p < 0.0001, ns = non-significant).