LETTER TO THE EDITOR

Proteogenomic profiling uncovers differential therapeutic vulnerabilities between *TCF3::PBX1* and *TCF3::HLF* translocated B-cell acute lymphoblastic leukemia

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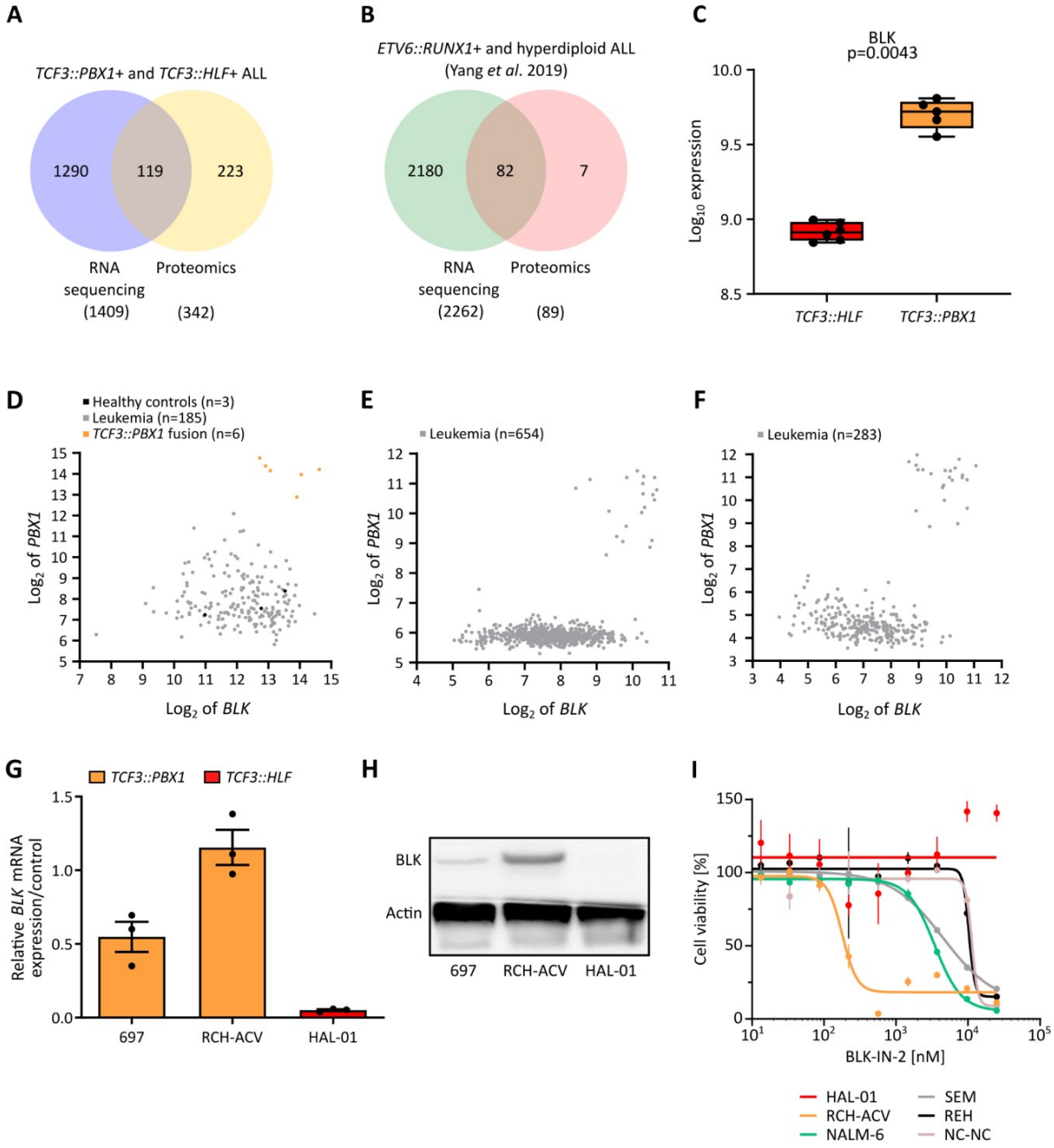
Supplement

Supplementary Tables (see excel files)

Supplementary Table 1: List of proteins detected upon proteomic profiling of *TCF3::HLF* and *TCF3::PBX1* positive leukemias.

Supplementary Table 2: Result of the high-throughput drug screening performed on *TCF3::PBX1* and *TCF3::HLF* positive leukemic cells. The drugs listed were purchased from MedChemExpress (Monmouth Junction, NJ, USA) as a customized library and were provided as 10 mM dimethylsulfoxide (DMSO) stock solutions. The area under the curve (AUC) serves as response parameter.

Supplementary Figure



Supplementary Figure 1: (A) Proteomic analysis for target prioritization in *TCF3*-rearranged ALL. Patient derived B cell acute lymphoblastic leukemia (B-ALL) xenografts (PDX) (n=5 *TCF3::PBX1*+ and n=6 *TCF3::HLF*+) were analyzed by RNA sequencing as previously reported by us.[1] 1409 significantly differentially expressed genes were detected within the two subtypes of B-ALL (9.5% of n=14,830 in total, cutoffs: p<0.05 and minimal log2 fold change of ±1) via RNA sequencing. Proteomic analysis carried out in the present study detected 342 differentially abundant proteins in the same PDX samples

using the same cutoffs (5.6% of n=6123 in total). 34.8% of differentially regulated proteins could be confirmed to be also differentially expressed on RNA level (n=119 of 342). In contrast, less than 10% of differentially regulated genes detected by RNA sequencing could be proven to have a significant impact on protein expression as detected by proteomics (n=119 of 1409, 8.45%) in our analyses. Therefore, 91.55% of the detected differentially expressed genes could be excluded as immediate targets for B-ALL subtype specific drug development by prioritization using proteomics. For proteomic analysis, liquid chromatography tandem mass spectrometry (LC-MS/MS) was performed. All peptide samples were separated and analyzed by coupling a RSLCnano system (Ultimate 3000, Thermo Scientific, Bremen, Germany) online to an Orbitrap Exploris 480 mass spectrometer (Thermo Scientific). Briefly, after LC-MS/MS and data Dependent Acquisition (DDA), data were searched using Sequest HT through proteome discoverer (version 2.4) and label free quantification (LFQ) was performed by using myProMS pre-processing to quantitatively profile changes in the proteome of *TCF3*-translocated samples. For data analysis, unsupervised consensus clustering approaches (e.g. NMF and SOM clustering) and supervised strategies (e.g. LIMMA and SAM) were used. Downstream pathway analyses were conducted using bioinformatics algorithms including Ingenuity Pathway Analysis (IPA, QIAGEN) and Gene Set Enrichment Analysis (GSEA). **(B) Overlap of significantly dysregulated genes detected by RNA sequencing and proteomics in primary samples of *ETV6::RUNX1*+ and high hyperdiploid ALL.** Previously published RNA sequencing and proteomic data of primary ALL samples (n=9 *ETV6::RUNX1*+ and n=18 high hyperdiploid)[2] were filtered for significance (cutoffs: $p<0.05$ and minimal log2 fold change of ±1). 2262 significantly differentially expressed genes were detected in the two subtypes of B-ALL using RNA sequencing, while proteomics detected 89 differentially expressed proteins in these samples using the same cutoffs. The majority of differentially regulated proteins was also found to be differentially expressed on RNA level (n=82 of 89, 92.13%). In contrast, only 3.63% of differentially regulated genes detected by RNA sequencing were identified as differentially expressed proteins by proteomics (n=82 of 2262). **(C) High BLK expression in**

***TCF3::PBX1*+ PDX samples.** BLK protein expression data of *TCF3::HLF*+ and *TCF3::PBX1*+ samples is derived from the proteomic data generated in this study. To determine differential expression, non-parametric Mann-Whitney t-test (two-tailed) was used. **(D-F) *BLK* is highly coexpressed with *PBX1*.** mRNA expression data of human patient samples is derived from data sets available at the R2: Genomics analysis and visualization platform (http://r2.amc.nl). Dot plots present *PBX1* and *BLK* expression in healthy controls (black), *TCF3::PBX1*+ (orange) and other unstratified leukemic samples (grey). Data sets shown are the B-ALL - Melnick - 194 - custom - gpl15088 study[3] (D), the Tumor ALL (BCP) Pediatric (combat) - Boer - 654 - MAS5.0 - u133p2 study[4] (E) and Tumor ALL (TARGET) - Roberts - 283 - MAS5.0 - u133p2 data set[5] (F). **(G,H) Validation of BLK expression on mRNA and protein level in *TCF3::PBX1*+ and *TCF3::HLF*+ leukemia cell lines.** (G) BLK mRNA expression is increased in *TCF3::PBX1*+ (orange) versus *TCF3::HLF*+ cells (red). mRNA expression was normalized to the housekeeping genes *glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*) and *β-actin* (*ACTB*). (H) Shown is the corresponding BLK protein expression analyzed by specific Western blot. Pan-actin was used as a loading control. **(I) BLK-IN-2 specifically targets *TCF3::PBX1*+ cells.** Dose-response curves for BLK-IN-2 showing differential responses of *TCF3::HLF*+ HAL-01 cells (red, IC_{50}=no response), *TCF3::PBX1*+ RCH-ACV cells (orange, IC_{50}=0.18 µM), *IGH::DUX4*+ NALM-6 cells (green, IC_{50}=3.4 µM), *KMT2A::AFF1*+ SEM cells (grey, IC_{50}=4.5 µM), *ETV6::RUNX1*+ REH cells (black, IC_{50}=10.3 µM) and the B lymphoblastoid cell line NC-NC (rose, IC_{50}=11.3 µM). Cell lines were treated with BLK-IN-2 (0.005 µM-25 µM) for 72 h. Representative drug response curves of two independent experiments are shown. Treatment was conducted in 384-well plates with technical triplicates. Data is depicted as the mean ±SEM. DMSO was used as a negative control. Cell viability was determined via CellTiter-Glo (Promega) measurement using a Tecan SPARK 10M reader.

Supplementary References

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