Transcriptional dysregulation of the multifunctional zinc finger factor 423 in acute lymphoblastic leukemia of childhood

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Abstract

Differentiation arrest is a hallmark of acute lymphoblastic leukemia (ALL). Among a variety of structural and chromosomal alterations, especially mutations in genes encoding for regulators of B cell differentiation are common. The objective of this study was a comprehensive assessment of transcriptional dysregulation and high-resolution genomic profiling of B cell differentiation factors. Here we provide extended materials and methods regarding transcriptome and genome-wide copy number variation analyses published by Harder et al. [1]. Our data provide a resource for the identification of yet undefined factors that play a putative functional role in leukemogenesis such as ZNF423, whose aberrant expression interferes with B-cell differentiation.

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Data in Brief

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Direct link to deposited data

Deposited data can be found here: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE42221 and http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE42056.

Experimental design, materials and methods

Patient samples

All primary human samples were obtained upon approval by Institutional Ethics Boards. Patients were recruited by the COALL multicenter clinical trial group (Germany) and enrolled in trials COALL 97 and 03.

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Fig. 1, samples are affected by slight degradation likely due to the elaborate cell selection and material isolation procedures followed by two amplification steps. In addition, a slight batch effect can be observed. Nevertheless, good sample comparability is given, because the degree of degradation is almost identical across all samples and the batch effect, which manifests as an overall higher signal intensity in one group, is readily eliminated during the normalization process.

Basic microarray analysis

The expression data of primary ALL at diagnosis (I1s, I2s, I3s, and I4; s, sorted cellular material) were independently filtered based on defined cutoff criteria such as present call, signal intensity (SI) $\geq 20$, increase (I) or decrease call (D), change P value $\leq 0.003$ for I/ $\geq 0.997$ for D and signal log ratio (SLR) $\geq 0.5849$ for I/ $\leq -0.415$ for D (equivalent to 1.5× up- or down-regulation), which arose from the comparison with the corresponding remission material (E1s, E2s, E3s, and E4s) as control. In all comparisons initial scaling was set to a target signal of 100. All genes meeting these cutoff criteria were considered to be differentially expressed, as depicted in the heatmap.

Quantitative real-time PCR

Ahead of high-resolution genomic profiling ZNF423 expression was evaluated by quantitative real time PCR (qPCR) in 200 primary B-precursor ALL samples. For this purpose RNA isolation was performed

Table 1
Patient characteristics. M, male; F, female; C-ALL, common ALL; NA, not available.

| ID   | Sex | Immuno-phenotype | BCR–ABL | MLL–AF4 | ETV6–RUNX1 | Hyper-diploidy | ZNF423 mRNA expression |
|------|-----|------------------|---------|---------|------------|----------------|-----------------------|
| 6646 | M   | C-ALL            | 0       | NA      | 0          | 1              | 36.23                 |
| 6787 | M   | preB-ALL         | NA      | NA      | 0          | 1              | 17.00                 |
| 6822 | M   | C-ALL            | 0       | 0       | 1          | 0              | 24.97                 |
| 6845 | M   | C-ALL            | 0       | NA      | 0          | NA             | 51.38                 |
| 6869 | M   | C-ALL            | 0       | 0       | 0          | 1              | 7.91                  |
| 6923 | M   | preB-ALL         | NA      | NA      | NA         | 0              | 22.20                 |
| 6924 | M   | C-ALL            | 0       | 0       | 0          | 0              | 9.79                  |
| 6965 | F   | C-ALL            | 0       | 0       | 1          | 0              | 19.08                 |
| 6992 | F   | preB-ALL         | 0       | 0       | 1          | NA             | 18.81                 |
| 7021 | M   | C-ALL            | 0       | 0       | 1          | 0              | 39.56                 |
| 7065 | M   | C-ALL            | 0       | 0       | 0          | 0              | 8.71                  |
| 7077 | M   | C-ALL            | 0       | 0       | 1          | NA             | 27.48                 |
| 7115 | M   | C-ALL            | 0       | 0       | 1          | 0              | 47.38                 |
| 7118 | M   | C-ALL            | 0       | 0       | 0          | 0              | 20.88                 |
| 7137 | F   | preB-ALL         | 0       | 0       | 0          | 0              | 13.54                 |
| 7191 | F   | C-ALL            | 0       | 0       | 1          | 0              | 69.07                 |
| 7293 | F   | C-ALL            | 0       | 0       | 1          | 0              | 6.52                  |
| 7360 | M   | C-ALL            | 0       | 0       | 1          | 0              | 17.42                 |
| 7503 | M   | C-ALL            | 0       | 0       | 1          | 0              | 27.27                 |
| 7523 | F   | C-ALL            | 0       | 0       | 1          | 0              | 26.80                 |

Fig. 1. Quality metrics for gene expression dataset GSE42221. (A) The rate of present calls (%), scaling factors (blue bars) as well as 3′ to 5′ ratios for $\beta$-ACTIN and GAPDH were calculated by the simpleaffy package. (B) The dissimilarity matrix of the arrays ahead of normalization shows a batch effect that is linked to hybridization date. (C) This effect is eliminated by the background and normalization procedure. The scores displayed in the dissimilarity matrices (blue: high similarity, yellow: low similarity) reflect the distance between each pair of arrays. They are computed as the mean absolute difference between the array data.
Table 2
Affymetrix SNP array quality metrics.

| GEO accession  | Sample name | Contrast QC | QC call rate |
|----------------|-------------|-------------|--------------|
| GSM1031509     | ALL 6890 initial | 1.00 | 94.04 |
| GSM1031510     | ALL 6845 initial | 1.43 | 94.01 |
| GSM1031511     | ALL 6646 initial | 1.77 | 93.33 |
| GSM1031512     | ALL 7021 initial | 1.24 | 91.99 |
| GSM1031513     | ALL 6923 initial | 2.14 | 95.43 |
| GSM1031514     | ALL 7077 initial | 2.29 | 95.2 |
| GSM1031515     | ALL 7191 initial | 2.04 | 96.13 |
| GSM1031516     | ALL 7115 initial | 1.21 | 91.53 |
| GSM1031517     | ALL 7360 initial | 2.02 | 97.15 |
| GSM1031518     | ALL 7065 initial | 1.93 | 94.97 |
| GSM1031519     | ALL 6965 initial | 1.67 | 95.96 |
| GSM1031520     | ALL 7303 initial | 2.14 | 95.47 |
| GSM1031521     | ALL 7523 initial | 2.18 | 96.03 |
| GSM1031522     | ALL 6992 initial | 2.02 | 96.03 |
| GSM1031523     | ALL 6787 initial | 2.32 | 97.25 |
| GSM1031524     | ALL 6924 initial | 1.68 | 93.28 |
| GSM1031525     | ALL 7137 initial | 2.49 | 97.88 |
| GSM1031526     | ALL 6822 initial | 2.79 | 95.57 |
| GSM1031527     | ALL 7118 initial | 4.26 | 95.96 |
| GSM1031528     | ALL 7293 initial | 2.07 | 97.29 |
| GSM1031529     | ALL 6869 remission | 1.75 | 93.81 |
| GSM1031530     | ALL 6845 remission | 1.21 | 94.57 |
| GSM1031531     | ALL 6646 remission | 1.66 | 94.71 |
| GSM1031532     | ALL 7021 remission | 1.87 | 94.11 |
| GSM1031533     | ALL 6923 remission | 1.95 | 96.23 |
| GSM1031534     | ALL 7077 remission | 2.29 | 95.43 |
| GSM1031535     | ALL 7191 remission | 1.72 | 94.08 |
| GSM1031536     | ALL 7115 remission | 1.98 | 94.34 |
| GSM1031537     | ALL 7360 remission | 0.0 | 93.58 |
| GSM1031538     | ALL 7065 remission | 2.62 | 97.58 |
| GSM1031539     | ALL 7293 remission | 1.57 | 94.57 |
| GSM1031540     | ALL 7503 remission | 1.7 | 94.61 |
| GSM1031541     | ALL 7523 remission | 1.97 | 95.6 |
| GSM1031542     | ALL 6992 remission | 1.99 | 95.93 |
| GSM1031543     | ALL 6787 remission | 2.12 | 97.32 |
| GSM1031544     | ALL 6924 remission | 2.17 | 96.29 |
| GSM1031545     | ALL 7137 remission | 2.18 | 96.62 |
| GSM1031546     | ALL 6822 remission | 2.41 | 95.9 |
| GSM1031547     | ALL 7118 remission | 1.91 | 92.55 |
| GSM1031548     | ALL 7293 remission | 1.42 | 94.28 |

Discussion
In this report we provide an extended description of materials and methods for two datasets deposited in the GEO database containing gene expression and high-resolution genomic profiling data. The corresponding biologic material was isolated from fluorescence activated sorted primary leukemic cells (except one case) and normal lymphoblasts (gene expression data) as well as from 20 initial ALL and intraindividually matched MNC from remission bone marrow (genomic data). Based on these data we identified a potentially causative role for ZNF423 in ALL by its interference with B-cell differentiation and modulation of Smad1–Smad4 dependent transcription [1]. We encourage the use of our deposited datasets for further investigation into mechanistic underpinnings of ALL. We will provide further information if needed.

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