A novel class of ZNF384 aberrations in acute leukemia

Marketa Zaliova,1,3 Lucie Winkowska,1,2 Jan Stuchly,1,2 Karel Fiser,1,2 Petr Triska,1,2 Martina Zwyrtkova,1,2 Ondrej Hrusak,1-3 Julia Starkova,1-3 Lucie Sramkova,2,3 Jan Stary,2,3 Jan Trka,1-3 and Jan Zuna1-3

1Childhood Leukaemia Investigation Prague, Prague, Czech Republic; 2Department of Paediatric Haematology and Oncology, Second Faculty of Medicine, Charles University, Prague, Czech Republic; and 3University Hospital Motol, Prague, Czech Republic

Fusion of the ZNF384 gene as the 3’ partner to several different 5’ partner genes occurs recurrently in B-cell precursor acute lymphoblastic and mixed phenotype B/myeloid leukemia. These canonical fusions (ZNF384r) contain the complete ZNF384 coding sequence and are associated with a specific gene expression signature. Cases with this signature, but without canonical ZNF384 fusions (ZNF384r-like cases), have been described previously. Although some have been shown to harbor ZNF362 fusions, the primary aberrations remain unknown in a major proportion. We studied 3 patients with the ZNF384r signature and unknown primary genetic background and identified a previously unknown class of genetic aberration affecting the last exon of ZNF384 and resulting in disruption of the C-terminal portion of the ZNF384 protein. Importantly, in 2 cases, the ZNF384 aberration, indel, was missed during the bioinformatic analysis but revealed by the manual, targeted reanalysis. Two cases with the novel aberrations had a mixed (B/myeloid) immunophenotype commonly associated with canonical ZNF384 fusions. In conclusion, we present leukemia cases with a novel class of ZNF384 aberrations that phenocopy leukemia with ZNF384r. Therefore, we show that part of the so-called ZNF384r-like cases represent the same genetic subtype as leukemia with canonical ZNF384 fusions.

Introduction

Insight into the genomic landscape of childhood B-cell precursor acute lymphoblastic leukemia (BCP-ALL) has significantly deepened over the past decade with the identification of novel genetic subtypes. This particularly applies to the “B-other” ALL subgroup (BCP-ALL negative for “classical” aberrations: hyper/hypo-diploidy, ETV6-RUNX1, TCF3-PBX1, BCR-ABL1 fusions, and KMT2A gene rearrangements [r]) comprising approximately one-fourth of childhood BCP-ALL.

The ZNF384 gene encoding transcription factor zinc-finger protein 384 is rearranged in 5% to 10% of B-other leukemias in the form of fusion to 10 different partners (most frequently TCF3, EP300, TAF15, or CREBBP).1-7 Within these “canonical” fusions, the entire ZNF384 coding region is fused in-frame to the partner coding sequence and forms the 3’ part of the fusion gene/C-terminus of the resulting fusion protein. The ZNF384r leukemias have a predominant BCP immunophenotype, although typical immature B-lineage marker CD10 is only weakly expressed in some patients, and myeloid markers (CD13/CD33) are often present.2,4,5,7,8 The immunophenotype of ZNF384r leukemia often fulfills the criteria for mixed phenotype acute leukemia (MPAL), and the ZNF384r leukemias comprise approximately half of all childhood B/myeloid MPAL cases.5,9
The unique gene expression profile (GEP) of ZNF384r leukemias is enriched for hematopoietic stem cell and immature myeloid lineage features and reflects upregulation of the JAK-STAT signaling pathway. In studies using the GEP to cluster B-other ALL, the ZNF384r cluster has often included cases without detectable ZNF384 rearrangement. In some of these "ZNF384r-like" leukemias, rearrangement of ZNF362 (paralog of ZNF384) was identified. However, in some "ZNF384r-like" cases, no established primary aberration has been found.

Here, we describe leukemias with the "ZNF384r-like" GEP and previously undiscovered ZNF384 aberrations disrupting the C-terminal portion of the encoded protein.

Methods

This study is based on a patient cohort reported in our previous paper and enlarged by recently diagnosed consecutive B-other patients. A total of 643 children were diagnosed with BCP-ALL or B/myeloid...
MPAL in the Czech Republic between December 2010 and December 2020, including 158 classified as B-other ALL. Hierarchical clustering analysis (HCA) was performed for 110 cases from this cohort that were diagnosed from December 2010 to December 2017, as published previously, as well as patients with the ZNF384 aberration diagnosed before (n = 3) and after (n = 4) this period. Analyses of the biological and clinical features of ZNF384 leukemias also included another 4 patients diagnosed before December 2010 using RT-PCR.

Routine diagnostics, whole-transcriptome sequencing (RNA-seq), including GEP and HCA, whole-exome sequencing, and single-nucleotide polymorphism array analyses were performed as described previously. Variant calling was performed using VarScan and Samtools (http://samtools.sourceforge.net/). Fusion calling was performed using TopHat, deFuse, and Cicero. Analyses of the biological and clinical features of ZNF384 leukemias also included another 4 patients diagnosed before December 2010 using RT-PCR.

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Results and discussion

Unsupervised HCA using the RNA-seq–based GEP data identified 3 patients that coclustered with ZNF384r leukemias but lacked canonical ZNF384 fusion (Figure 1A).

Routine analysis of RNA-seq data using in-house bioinformatic pipelines for single-nucleotide variants, indels, and fusion gene detection revealed noncanonical ZNF384 fusion (ZNF384 as the 5' partner) in 1 patient, but no aberration in ZNF384 or ZNF362 was found in the remaining 2 patients. We manually reanalyzed the reads mapping to ZNF384 in all remaining B-other cases included in our HCA (n = 105), but no further ZNF384 aberration was found. Combined with the high-allele frequency of ZNF384 indels, the findings suggest that the novel ZNF384 aberrations represent primary genetic hits, mutually exclusive with other known primary aberrations.

In accordance with the immunophenotypic features of ZNF384r leukemias, all 3 patients expressed myeloid markers, and CD10

### Table 1. Immunophenotypes of leukemia cases with canonical ZNF384 fusions and novel ZNF384 aberrations

| Patient ID | Type of ZNF384 aberration | Immunophenotypic leukemia classification | CD45 | HLA-DR | CD34 | CD10 | CD19 | CD20 | CD32 | IGM | CD33 | CD13 | CD15 | CD117 | MPO | CD2 | CD5 | CD66c |
|------------|---------------------------|----------------------------------------|------|--------|------|------|------|------|------|-----|------|------|------|-------|-----|-----|-----|-------|
| ZNF384r-01 | TCF3/ZNF384                | MPAL                                   | 100  | 74     | 29    | 20   | 96   | 9    | 49   | 3   | 61   | 37   | 71   | 55    | 1   | 1   | 2   | 26    |
| ZNF384r-02 | TCF3/ZNF384                | MPAL                                   | 97   | 87     | 82    | 31   | 87   | 7    | 73   | 6   | 6    | 63   | 9    | 84    | 22  | 11  | 8   | 16    |
| ZNF384r-03 | TCF3/ZNF384                | BCP-ALL                                | 87   | 54     | 11    | 56   | 69   | 7    | 60   | 27  | 0    | 32   | 15   | 52    | 5   | 20  | 20  | 20    |
| ZNF384r-04 | TCF3/ZNF384                | BCP-ALL                                | 98   | 99     | 94    | 93   | 95   | 7    | 55   | 20  | 19   | 91   | 3    | 2    | 2   | 28   | 5    | 3    | 2    |
| ZNF384r-05 | TCF3/ZNF384                | BCP-ALL                                | 79   | 86     | 82    | 25   | 91   | 11   | 79   | 1   | 78   | 53   | 5    | 6    | 2   | 28   | 45   | 43   |
| ZNF384r-06 | TAF15/ZNF384               | BCP-ALL                                | 100  | 98     | 96    | 34   | 95   | 16   | 55   | 2   | 5    | 9    | 67   | 57    | 2   | 2   | 1   | 0     |
| ZNF384r-07 | EP300/ZNF384               | MPAL*                                  | 100  | 99     | 98    | 3    | 100  | 36   | 98   | 8   | 49   | 21   | 22   | 1    | 4   | 9   | 3    | 4     |
| ZNF384r-08 | EP300/ZNF384               | BCP-ALL                                | 89   | 86     | 84    | 39   | 84   | 9    | 71   | 9   | 72   | 29   | 21   | na    | 6   | 5   | 7    | 5     |
| ZNF384r-09 | EP300/ZNF384               | MPAL*                                  | 72   | 89     | 90    | 21   | 89   | 93   | 73   | 4   | 51   | 22   | 34   | 3    | 0   | 8   | 5    | 6     |
| ZNF384r-10 | EP300/ZNF384               | MPAL*                                  | 72   | 89     | 90    | 21   | 89   | 93   | 73   | 4   | 51   | 22   | 34   | 3    | 0   | 8   | 5    | 6     |
| ZNF384r-11 | EP300/ZNF384               | BCP-ALL                                | 90   | 85     | 80    | 17   | 80   | 4    | 83   | 1   | 67   | 57   | 7    | 7    | 1   | 3   | 7    | 7     |
| ZNF384r-12 | EP300/ZNF384               | BCP-ALL                                | 94   | 93     | 95    | 28   | 98   | 1    | 87   | 1   | 42   | 6    | 9    | 7    | 0   | 2   | 3    | 2     |
| ZNF384r-13 | EP300/ZNF384               | BCP-ALL                                | 96   | 85     | 95    | 12   | 98   | 1    | 81   | 1   | 85   | 35   | 73   | 31    | 6   | 2   | 2    | 2     |
| ZNF384r-14 | EP300/ZNF384               | BCP-ALL                                | 99   | 90     | 99    | 3    | 99   | 16   | 81   | 3   | 24   | 45   | 57   | 7    | 0   | 2   | 1    | 2     |
| ZNF384r-15 | EP300/ZNF384               | BCP-ALL                                | 99   | 100    | 99    | 66   | 99   | 19   | 97   | 28   | 99   | 31   | 3    | 0    | 1   | 1    | 1    | 0     |
| ZNF384r-16 | EP300/ZNF384               | BCP-ALL                                | 100  | 99     | 99    | 2    | 99   | 28   | 95   | 2   | 89   | 62   | 12   | 5    | 1   | 1    | 1    | 3     |
| Patient 1  | ZNF384r-TEKX1              | BCP-ALL                                | 94   | 93     | 90    | 37   | 90   | 13   | 79   | 2   | 92   | 6    | 8    | 1    | 0   | 1    | 1    | 1     |
| Patient 2  | ZNF384r indel              | BCP-ALL                                | 100  | 99     | 99    | na   | 69   | 6    | 44   | 1   | 12   | na   | 32   | 6    | 3    | 20   | 4     |
| Patient 3  | ZNF384r indel              | BCP-ALL                                | 76   | 88     | 80    | 50   | 86   | 5    | 80   | 3   | 40   | 40   | 65   | 35    | 17  | 18  | 8    | 10    |

Percent positive cells are shown for each antigen. BCP-ALL, B-cell precursor acute lymphoblastic leukemia; MPAL, mixed phenotype acute lymphoblastic leukemia; na, not analysed; * scored MPAL according to EGIL criteria (WHO criteria for mixed phenotype not fulfilled); minimal to maximal antigen expression is depicted in green to red gradient ("heatmap" style) color scale.
ZNF384r leukemias represent 6% (10/158) of B-other ALL and 1.6% (10/643) of BCP-ALL cases in the cohort; when combining these with the novel ZNF384 aberrations, the frequency is 8% (12/158) and 1.9% (12/643), respectively. The leukemias with novel ZNF384 aberrations comprise 17% (2/12) of consecutively diagnosed cases with ZNF384 aberrations. The basic characteristics of the cases with ZNF384 aberrations are given in supplemental Table 1.

The chimeric ZNF384 oncoproteins studied thus far exhibit perturbed DNA binding and drive transcriptional deregulation.2,9 DNA binding is likely preserved in ZNF384 with novel aberrations, as the 6 zinc fingers are mostly intact. However, as the more distal C-terminal portion of the protein affected by the novel aberrations lacks well-defined functional domains, functional studies would be necessary to directly demonstrate the biological consequences of these aberrations and to compare them with canonical ZNF384 fusions.

Despite indisputable progress in the analysis of genomic data, some “blind spots” insufficiently covered by widely used sequencing/analytical approaches may still exist in the leukemia mutational landscape. In our experience, particularly the detection of larger or more complex indels remains challenging when using “standard” variant callers. We cannot exclude that some other variant callers, which we did not test, may have found these mutations. However, the fact that similar ZNF384 mutations were not reported in any genomic study published thus far, or in publicly available databases, supports our belief that this type of mutation is easily missed in sequencing data by most automated approaches.

In conclusion, we presented childhood ALL/MPAL patients with a novel class of ZNF384 aberrations that phenocopy leukemias defined by canonical ZNF384 fusions and, thus, represent the same biological subtype. We think that the novel ZNF384 aberrations can recur enough to not be neglected in future leukemia diagnostics. Therefore, targeted reanalysis of available sequencing data in samples with the ZNF384r ALL gene expression signature and seemingly unaffected ZNF384/ZNF362 is highly encouraged in order to further assess the frequency of ZNF384 aberrations other than canonical fusion, and to provide additional data on the biological and clinical features that could aid in understanding the genomic landscape of childhood leukemia.

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**Authorship**

Contribution: M. Zaliova and J.T. designed the study; M. Zwyrtkova performed NGS; M. Zaliova, L.W., J. Stuchly, K.F., and P.T. analyzed transcriptomic and genomic data; O.H., J. Starkova, L.S., J. Stary, J.T., and J.Z. provided diagnostic and clinical data; all authors participated on data integration, interpretation, and presentation; M. Zaliova and J.Z. wrote the draft; and all authors revised the draft and contributed to the final manuscript.

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ORCID profiles: M. Zaliova, 0000-0002-1639-7124; K.F., 0000-0002-7265-3268; M. Zwyrtkova, 0000-0003-1704-2154; O.H., 0000-0002-7611-1335.

Correspondence: Marketa Zaliova, Department of Paediatric Haematology and Oncology, Second Faculty of Medicine, Charles University and University Hospital Motol, V Uvalu 84, 150 06 Prague, Czech Republic; e-mail: marketa.zaliova@lfmotol.cuni.cz.

**References**

1. Gocho Y, Kiyokawa N, Ichikawa H, et al; Tokyo Children’s Cancer Study Group. A novel recurrent EP300-ZNF384 gene fusion in B-cell precursor acute lymphoblastic leukemia. *Leukemia*. 2015;29(12):2445-2448.
2. Qian M, Zhang H, Kham SK, et al. Whole-transcriptome sequencing identifies a distinct subtype of acute lymphoblastic leukemia with predominant genomic abnormalities of EP300 and CREBBP. *Genome Res.* 2017;27(2):185-195.
3. Gu Z, Churchman M, Roberts K, et al. Genomic analyses identify recurrent MEF2D fusions in acute lymphoblastic leukaemia. *Nat Commun.* 2016;7:13331.
4. Hirabayashi S, Ohki K, Nakabayashi K, et al; Tokyo Children’s Cancer Study Group (TCCSG). ZNF384-related fusion genes define a subgroup of childhood B-cell precursor acute lymphoblastic leukemia with a characteristic immunotype. *Haematologica*. 2017;102(1):118-129.
5. Liu YF, Wang BY, Zhang WN, et al. Genomic profiling of adult and pediatric B-cell acute lymphoblastic leukemia. *EBioMedicine*. 2016;8:173-183.
6. Zaliova M, Stuchly J, Winkowska L, et al. Genomic landscape of pediatric B-other acute lymphoblastic leukemia in a consecutive European cohort. *Haematologica*. 2019;104(7):1396-1406.
7. Hirabayashi S, Butler ER, Ohki K, et al. Clinical characteristics and outcomes of B-ALL with ZNF384 rearrangements: a retrospective analysis by the Ponte di Legno Childhood ALL Working Group [published online ahead of print 10 March 2021]. *Leukemia*. 2021.
8. Novakova M, Zaliova M, Fiser K, et al. DUX4r, ZNF384r and PAX5-P80R mutated B-cell precursor acute lymphoblastic leukemia frequently undergo monocytic switch. *Haematologica*. 2021;106(8):2066-2075. haematol.2020.250423.
9. Alexander TB, Gu Z, Iacobucci I, et al. The genetic basis and cell of origin of mixed phenotype acute leukaemia. *Nature*. 2018;562(7727):373-379.
10. Li JF, Dai YT, Lilljebjörn H, et al. Transcriptional landscape of B cell precursor acute lymphoblastic leukemia based on an international study of 1,223 cases. Proc Natl Acad Sci USA. 2018;115(50):E11711-E11720.

11. Gu Z, Churchman ML, Roberts KG, et al. PAX5-driven subtypes of B-progenitor acute lymphoblastic leukemia. Nat Genet. 2019;51(2):296-307.

12. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics. 2010;26(5):589-595.

13. Dobin A, Davis CA, Schlesinger F, et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics. 2013;29(1):15-21.

14. Koboldt DC, Zhang Q, Larson DE, et al. VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res. 2012;22(3):568-576.

15. Kim D, Salzberg SL. TopHat-Fusion: an algorithm for discovery of novel fusion transcripts. Genome Biol. 2011;12(8):R72.

16. McPherson A, Hormozdiari F, Zayed A, et al. deFuse: an algorithm for gene fusion discovery in tumor RNA-Seq data. PLOS Comput Biol. 2011;7(5): e1001138.

17. Roberts KG, Li Y, Payne-Turner D, et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. N Engl J Med. 2014;371(11):1005-1015.