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blood advances

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 Zuna[1-3]

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

Key Points

- A novel class of ZNF384 aberrations affects the last exon of ZNF384 and results in disruption of the C-terminal portion of ZNF384 protein.
- Leukemias with the novel class of ZNF384 aberrations phenocopy leukemias with the canonical ZNF384 fusions.

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.[6,9]

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For data sharing, contact the corresponding author: marketa.zaliova@lfmotol.cuni.cz.

The full-text version of this article contains a data supplement.

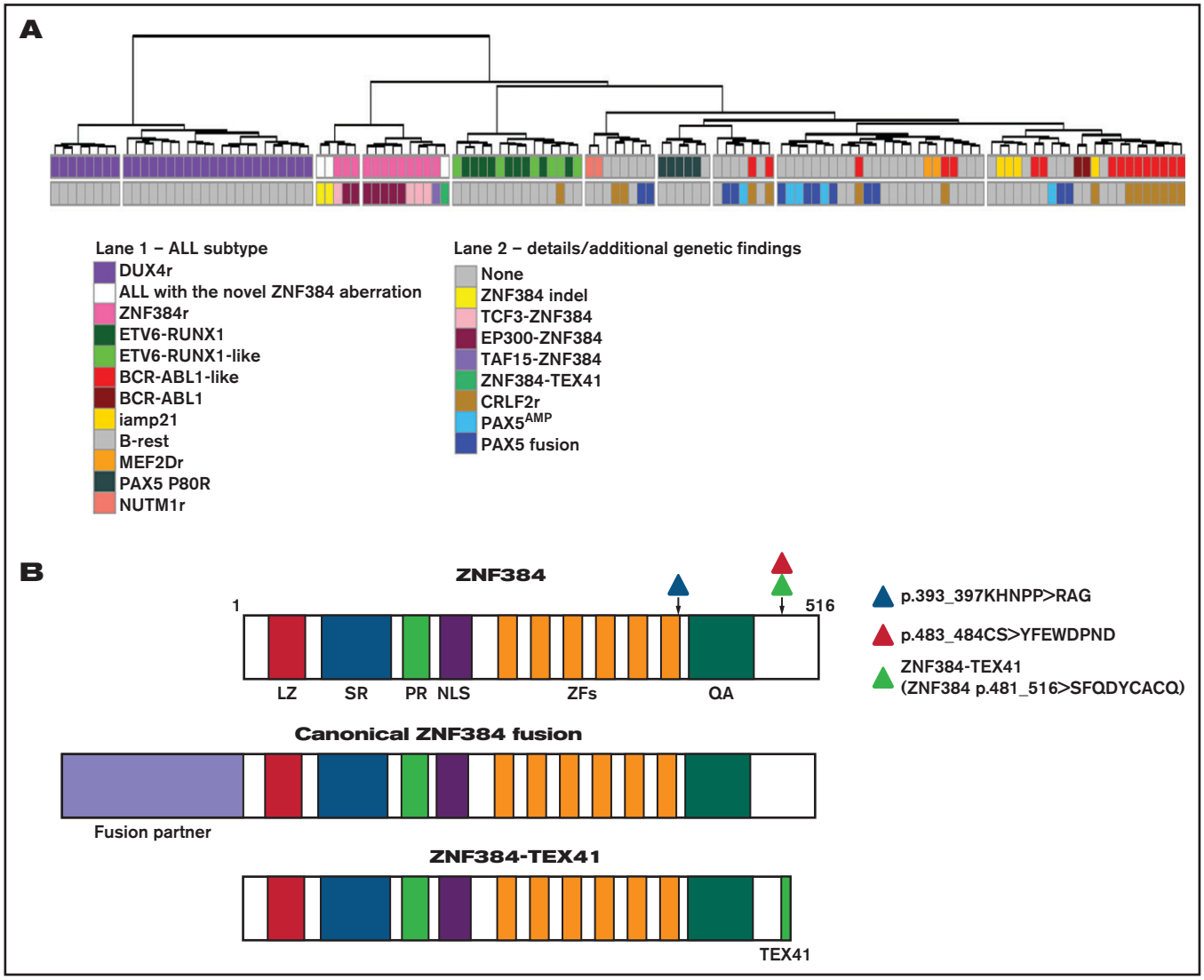

Figure 1. Hierarchical clustering and schematic representation of ZNF384 proteins. (A) Hierarchical clustering based on RNA-seq gene-expression data. A total of 117 B-other, 9 ETV6-RUNX1–positive, and 2 BCR-ABL1–positive ALL/MPAL cases were clustered hierarchically (vst normalization, ward.D method, and Euclidean distance linkage for hierarchical clustering) based on the expression of the most variably expressed transcripts (transcripts with standard deviation of expression ≥35% of the maximal standard deviation; n = 391). The resulting dendrogram is shown. ALL was classified as BCR-ABL1-like and ETV6-RUNX1-like based on coclustering with BCR-ABL1–positive and ETV6-RUNX1–positive ALL, respectively, in supervised HCA.[6] Classification into genetically defined subtypes was based on the identification of defining genetic lesions. The cohort consists of 110 patients as reported previously,[6] complemented by 7 additional patients with ZNF384 gene aberrations and available RNA-seq data. B-rest, not classified into any established subtype; iamp, intrachromosomal amplification; AMP, amplification. (B) Schematic representation of the wild-type ZNF384 protein with the positions of 3 novel aberrations and chimeric proteins encoded by canonical ZNF384 fusion and the novel ZNF384-TEX41 fusion. LZ, leucine-rich domain; NLS, nuclear localization signal; PR, proline-rich domain; QA, Gln-Ala repeat; SR, serine-rich domain; ZFs, Kruppel-type C2H2 zinc-finger domains. Adapted from Liu et al[5] with permission.

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.[4,5,10] In studies using the GEP to cluster B-other ALL, the ZNF384r cluster has often included cases without detectable ZNF384 rearrangement.[6,10,11] In some of these "ZNF384r-like" leukemias, rearrangement of ZNF362 (paralog of ZNF384) was identified.[10] 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[6] 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,[6] 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.[6,12,13] Variant calling was performed using VarScan[14] and Samtools (http://samtools.sourceforge.net/). Fusion calling was performed using TopHat,[15] deFuse,[16] and Cicero.[17]

For more detailed methods, see the supplemental material.

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 the respective genes in these 2 patients and identified in-frame indels in the last exon of ZNF384 in both. Therefore, unlike canonical ZNF384 fusion, all the novel aberrations resulted in alteration of the C-terminal portion of the ZNF384 protein.

Patient 1 harbored a novel ZNF384-TEX41 fusion consisting of the large 5' portion of ZNF384 (including the coding sequence for all 6 zinc fingers) fused to the 3' portion of TEX41, a gene not shown to be recurrently involved in leukemia (see supplemental material). The out-of-frame fusion led to an early stop codon with only 9 amino acids (AAs) encoded by TEX41 intron 1 (ZNF384 p. 481_516 > SFQDYCACQ). In patients 2 and 3, the indels led to replacement of 5 and 2 AAs by 3 and 8 new AAs, respectively, with the remaining 119 and 32 C-terminal AAs maintained from the wild-type ZNF384. In patient 2, the indel (c.1179_1192del14insGGGCAGGG; ZNF384 p.393_397KHNPP>RAG) had an allelic frequency 47% in RNAseq and was located just at the end of the last zinc finger. In patient 3, the indel (c.1448_1451del4insATTTTGAGTGGGACCCGAACGA; ZNF384 p.483_484CS>YFEWDPND) had an allelic frequency 52% and 43% in RNAseq and whole-exome sequencing, respectively (Figure 1B). More detailed results of the molecular analyses are provided in the supplemental material.

To exclude the presence of similar mutations (missed by variant or fusion callers employed in our bioinformatic pipeline) in other 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	CD22	cIGM	CD33	CD13	CD15	CD117	iMPO	CD2	CD3	CD5	CD7	CD66c
ZNF384r_01	TCF3/ZNF384	MPAL	100	74	29	20	96	9	49	3	61	37	71	1	55	1	1	2	2	26
ZNF384r_02	TCF3/ZNF384	MPAL	97	87	82	31	87	7	73	6	5	63	9	84	22	11	8	9	16	4
ZNF384r_03	TCF3/ZNF384	BCP-ALL	87	54	11	56	69	7	60	27	0	32	15	52	0	20	20	20	20	1
ZNF384r_04	TCF3/ZNF384	BCP-ALL	98	99	94	93	95	7	55	20	19	31	3	2	0	28	9	3	2	2
ZNF384r_05	TCF3/ZNF384	BCP-ALL	79	85	82	25	81	11	79	1	79	53	6	1	3	8	8	8	8	1
ZNF384r_06	TAF15/ZNF384	BCP-ALL	100	98	94	34	95	16	55	2	4	5	2	67	2	7	2	1	0	1
ZNF384r_07	EP300/ZNF384	MPAL*	100	99	98	3	100	36	98	8	49	21	22	1	4	9	3	3	4	3
ZNF384r_08	EP300/ZNF384	BCP-ALL	89	86	84	39	84	9	71	9	72	29	21	1	na	6	5	7	5	2
ZNF384r_09	EP300/ZNF384	MPAL	72	89	90	21	89	3	73	4	51	22	34	3	0	8	5	6	5	3
ZNF384r_10	EP300/ZNF384	MPAL*	72	89	90	21	89	3	73	4	51	22	23	0	18	8	5	6	5	3
ZNF384r_11	EP300/ZNF384	BCP-ALL	90	85	80	17	80	1	83	1	67	57	7	1	1	7	6	7	7	0
ZNF384r_12	EP300/ZNF384	BCP-ALL	94	93	99	28	98	1	87	1	42	6	9	7	0	2	3	2	2	9
ZNF384r_13	EP300/ZNF384	MPAL*	96	85	95	12	98	1	91	1	85	38	73	31	5	2	2	2	3	4
ZNF384r_14	EP300/ZNF384	MPAL	99	90	99	0	99	16	81	2	28	45	57	0	1	1	1	1	0	1
ZNF384r_15	EP300/ZNF384	BCP-ALL	99	100	99	66	99	19	97	28	99	31	3	0	2	1	1	0	0	2
ZNF384r_16	EP300/ZNF384	MPAL*	100	99	99	2	99	28	95	2	89	62	12	5	1	1	2	1	3	1
Patient 1	ZNF384-TEX41	BCP-ALL	94	93	90	37	90	13	79	2	92	6	3	1	2	1	1	1	1	30
Patient 2	ZNF384 indel	MPAL	100	99	0	98	96	na	69	6	44	1	12	na	32	6	3	20	4	55
Patient 3	ZNF384 indel	MPAL	76	88	80	50	86	5	82	3	40	40	62	1	17	18	8	10	7	55

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 critera for mixed phenotype not fulfilled); minimal to maximal antigen expression is depicted in green to red gradient ("heatmap" style) color scale.

expression was weak in 2. Two of the 3 patients were classified as MPAL. Notably, besides other myeloid markers, the 3 patients with novel ZNF384 aberrations expressed CD66c, which is rarely expressed in ZNF384r ALL (Table 1).[8]

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.

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