Abstract

Background: SET-NUP214 fusion resulting from a recurrent cryptic deletion, del(9)(q34.11q34.13) has recently been described in T-cell acute lymphoblastic leukemia (T-ALL) and in one case of acute myeloid leukemia (AML). The fusion protein appears to promote elevated expression of HOXA cluster genes in T-ALL and may contribute to the pathogenesis of the disease. We screened a panel of ALL and AML cell lines for SET-NUP214 expression to find model systems that might help to elucidate the cellular function of this fusion gene.

Results: Of 141 human leukemia/lymphoma cell lines tested, only the T-ALL cell line LOUCY and the AML cell line MEGAL expressed the SET(TAF-Iβ)-NUP214 fusion gene transcript. RT-PCR analysis specifically recognizing the alternative first exons of the two TAF-I isoforms revealed that the cell lines also expressed TAF-Iα-NUP214 mRNA. Results of fluorescence in situ hybridization (FISH) and array-based copy number analysis were both consistent with del(9)(q34.11q34.13) as described. Quantitative genomic PCR also confirmed loss of genomic material between SET and NUP214 in both cell lines. Genomic sequencing localized the breakpoints of the SET gene to regions downstream of the stop codon and to NUP214 intron 17/18 in both LOUCY and MEGAL cells. Both cell lines expressed the 140 kDa SET-NUP214 fusion protein.

Conclusion: Cell lines LOUCY and MEGAL express the recently described SET-NUP214 fusion gene. Of special note is that the formation of the SET exon 7/NUP214 exon 18 gene transcript requires alternative splicing as the SET breakpoint is located downstream of the stop codon in exon 8. The cell lines are promising model systems for SET-NUP214 studies and should facilitate investigating cellular functions of the the SET-NUP214 protein.

Background

Leukemia subtypes are often associated with specific recurrent chromosome translocations. Translocations may function by constitutively activating proto-oncogenes or they may create new oncogenes by fusing two formerly independent genes. The SET-NUP214 (TAF-1/CAN) gene fusion has previously been described as result of a chromosomal translocation t(9;9)(q34;q34) in a case of acute undifferentiated leukemia [1]. The fusion gene appears to inhibit differentiation, while secondary chromosomal aberrations are necessary to induce tumorigenesis [2,3]. Recent studies have shown that the SET-NUP214...
fusion can also result from a recurrent deletion, del(9)(q34.11q34.13) in patients with T-cell acute lymphoblastic leukemia (T-ALL) [4]. It has also been reported in a single case of acute myeloid leukemia (AML) [5]. SET-NUP214 positive T-ALL patients exhibited high expression levels of HOXA cluster genes [4]. Downregulation of the fusion gene repressed HOX gene expression and induced differentiation in the SET-NUP214 positive cells confirming that SET-NUP214 keeps hematopoetic cells in an undifferentiated stage [4].

We screened a panel of 141 human cell lines to investigate the occurrence of the SET-NUP214 fusion in different hematologic malignant contexts.

**Results and discussion**

Cell lines are useful model systems to elucidate the cellular function of oncogenes. Therefore, we performed a reverse transcriptase (RT)-PCR based screening of 141 leukemia/lymphoma cell lines of T-, B- and myeloid cell origin to detect SET-NUP214 positive examples. A T-ALL cell line LOUCY (1/43 T cell lines tested) and an AML cell line MEGAL (1/53 myeloid cell lines tested) were the only cell lines expressing the fusion gene. Both cell lines expressed SET exon 7/NUP214 exon 18 fusion mRNA (Fig. 1). SET is the β isoform of TAF-I, differing from TAF-Iα by alternative first exons. RT-PCR with primers recognizing the isoform-specific exons revealed that both cell lines expressed TAF-Iα-NUP214 and TAF-Iβ(SET)-NUP214. Fluorescence in situ hybridization (FISH) analysis with tilepath BAC and fosmid clones (Fig. 2) and array-based copy number analysis revealed del(9) (q34.11q34.13) for LOUCY and MEGAL cells (data not shown). Quantitative genomic PCR confirmed loss of genomic material between SET and NUP214 for both cell lines as indicated by FISH (Fig. 3). Genomic sequencing allocated the centromeric fusion to the untranslated region of SET exon 8 in LOUCY, and to the 3’ region of SET in MEGAL, and telomerically to NUP214 intron 17/18 in both cell lines (Fig. 4). Expression of the SET exon 7/NUP214 exon 18 fusion transcript requires alternative splicing; otherwise, full-length SET would be transcribed at the expense of the fusion gene. Alternative splicing as mechanism for SET/NUP214 expression had already been postulated for the first reported case of this fusion gene [6]. Thus, one might speculate that alternative splicing is an obligatory step for SET-NUP214 expression besides the chromosomal aberration itself.

As previously reported for LOUCY, also cell line MEGAL expressed the SET-NUP214 fusion protein with a molecular weight of about 140 kDa (Fig. 5) [4].

**Figure 1**

**SET-NUP214 screening in cell lines.** SET-NUP214 expression screening performed with a SET exon 7 forward primer and a NUP214 exon 18 reverse primer. Cell lines LOUCY and MEGAL were the only SET-NUP214 positive cell lines from 141 cell lines tested. Identity of the SET Ex7/NUP214 Ex18 PCR product was confirmed by sequencing.

**Figure 2**

**Deletion del(9)(q34.11q34.13) in cell lines LOUCY and MEGAL.** FISH analysis with BAC clones showed loss of the central (green) signal containing ABL1 and the 5’part of NUP214 in one chromosome 9 homolog in both cell lines. Note that cell line MEGAL carries three copies of chromosome 9.

**Figure 5**

**HOXA cluster genes are described as targets of the SET-NUP214 fusion protein [4].** Accordingly, downregulation of SET-NUP214 expression decreases HOX gene expression and inhibits proliferation in the SET-NUP214 positive T-ALL cell line LOUCY [4]. We performed quantitative RT-PCR to verify whether cell lines with high expression levels of SET-NUP214 also expressed above average levels of HOXA9. Confirming a positive correla-
tion between SET-NUP214 and HOX gene expression, quantitative real-time PCR revealed more than 1000× higher HOXA9 levels in the SET-NUP214 positive cell line LOUCY than in six other T-ALL cell lines tested (data not shown). HOXA9 expression levels were also high in cell line MEGAL, but not above many SET-NUP214 negative AML cell lines (data not shown) which may be due to the fact that HOXA cluster genes are often highly expressed in myeloid leukemias [7,8].

Conclusion
We demonstrated the presence of the SET-NUP214 gene in the T-ALL cell line LOUCY and in the AML cell line MEGAL by genomic sequencing. In both cell lines, the centromeric fusion is located downstream to the stop codon of SET. Therefore, alternative splicing might turn out to be obligatory for expression of SET-NUP214 mRNA.

Methods
Human cell lines
The 141 continuous cell lines investigated in this study were either taken from the stock of the cell bank (DSMZ – German Collection of Microorganisms and Cell Cultures) or were generously provided by the original investigators. Detailed references and cultivation protocols have been described previously [9].

SET-NUP214 screening and breakpoint determination
Screening of cell lines for SET/NUP214 mRNA expression was performed applying RT-PCR. RNA was prepared using the Trizol reagent (Invitrogen, Karlsruhe, Germany). For mRNA quantification, reverse transcription was performed using the SuperScript II reverse transcriptase kit (Invitrogen, Karlsruhe, Germany). Previous studies identified SET exon 7/NUP214 exon 17 and SET exon 7/ NUP214 exon 18 fusions in T-ALL and AML patients [4,5,10]. We applied primers from SET exon 6 and NUP214 exon 20 for SET-NUP214 expression screening. Analyses were repeated with previously described primers from SET exon 7 and NUP214 exon 18 [10]: SET exon 6 forward: 5’-GAA GAG GCA GCA TGA GGA AC-3’; NUP214 exon 20 reverse: 5’-TAC TTT GGG CAA GGA TTT G-3’. Detailed references and cultivation protocols have been described previously [9].

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Figure 3
Deletion del(9)(q34.11q34.13) in cell lines LOUCY and MEGAL. Quantitative genomic PCR confirmed loss of the genes ABL1 and CRAT, located between SET and NUP214. SET primers were chosen from the intron 1, primers of NUP214 were located in intron 33.
AC-3'. For the determination of genomic SET and NUP214 breakpoints in cell lines LOUCY and MEGAL, genomic PCR was performed with the following sets of primers: (i) SET exon 7 forward: 5'-TGA CGA AGA ACG GGA TGA GGA T-3'; NUP214 exon 18 reverse: 5'-ATC ATT CAC ATC TTG GAC AGC A-3'; (ii) SET intron 8/exon 8 forward: 5'-ATC ATT CCA GCA GAG AGG TGG TA-3'; NUP214 intron 17/18 reverse: 5'-GAG GTG GCA GAG AGG TGG TA-3'; (iii) SET exon 8 forward: 5'-CTG CCA CTC AAT GGG AGA AT-3'; NUP214 intron 17/18 reverse: 5'-AGA TGT GCA GAG AGG TGG TA-3'.

Quantitative PCR analysis

Quantitative PCR was carried out using a 7500 Applied Biosystems real-time PCR system following the manufacturer's protocol (Darmstadt, Germany). TaqMan probes (Applied Biosystems) were used to quantify human HOXA9 (Hs00365956_m1) expression levels with TBP as endogenous control. For copy number analysis of genomic DNA, we performed relative quantitative PCR with the following oligonucleotides: ABL1 forward: 5'-CAC CGT TAA TTA GTG GGA TGT G-3'; ABL1 reverse: 5'-AAT GGT AGA GTG GTG CTC CTT G-3'; NUP214 forward: 5'-TAG ACA GCG CCT AGC ACA TC-3'; NUP214 reverse: 5'-GGC CAG GTT GGA TTT CAT AC-3'; PCR was performed in a total volume of 50 μl with a DNA thermal cycler (Perkin Elmer Cetus, Heidelberg, Germany) for 35 cycles under standard conditions. Products were electrophoresed in 1.2% agarose gels and observed under UV light. PCR products were ligated into the pGEM-T Easy Vector System (Promega, Mannheim, Germany) and sequenced (Eurofins MWG Operon, Martinsried, Germany).

Western blot analysis

Analysis of SET-NUP214 protein expression was performed as follows: 1 × 10⁶ cells were pelleted and washed with ice-cold phosphate-buffered saline (PBS), resuspended and boiled for 10 min in 25 μl SDS sample buffer containing 15% glycerol, 125 mM Tris-HCl pH 6.8, 5 mM EDTA, 2% SDS, 0.1% bromophenol blue and 1% β-mercaptoethanol. The samples were separated on 7% or 12% gels depending on the size of the wild-type proteins to be detected. Blotting and staining conditions were as described previously [12]. The anti human SET Ab react-
ing with amino acids 3–18 was purchased from Abcam (Cambridge, UK), the anti human NUP214 Ab directed against the C-terminal part of the protein, was obtained from antibodies-online (Aachen, Germany).

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
HQ designed the study and wrote the paper. BS developed and performed the genomic quantitative PCR. SR co-wrote the manuscript. JR performed Western blot analyses. MZ carried out PCR analyses. RML performed the cytogenetic part of the study. HGD provided and cultivated cell lines and critically read the manuscript. All authors read and approved the manuscript.

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