LETTER TO THE EDITOR

Chromosome 11q23 aberrations activating FOXR1 in B-cell lymphoma

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Recurrent chromosome 11q23 abnormalities, including focal gains and losses have been described in mantle cell lymphoma, diffuse large B-cell lymphoma (DLBCL) and in a subset of high-grade B-cell lymphomas lacking MYC rearrangements.1-3 We describe a novel fusion of FOXR1 forchhead box gene, located at 11q23, with a neighboring gene in B-cell lymphoma.

RNaseq and sequencing of cloned PCR products revealed fusion transcripts of S1 Ribosomal Protein S25 (RPS25) with FOXR1 in the DLBCL cell line U-2932, both genes located at the amplified chromosomal region 11q23 (Figure 1a, Supplementary Figure S1A). Genomic cloning localized the breakpoint to intron 2 of RPS25 and to the promoter region of FOXR1 (bp – 3532).

Cell line U-2932 comprises two distinct clones traceable to subclones present in the patient’s tumor.4 These differences also affected 11q32, FOXR1 and RPS25 being tetraploid in subclone R1, and triploid in subclone R2 (3n) (Supplementary Figure S1A). In accordance with the genomic data, the RPS25/FOXOR1 fusion was detected in subclone R1 but not in R2 (Figure 1b). RPS25/FOXOR1 was also verified in the patient’s DNA, which collectively suggested that the fusion had occurred at some later stages of tumor development (Figure 1b).

Physiological FOXR1 (formerly FOXN5) expression is restricted to the early stages of embryogenesis.4-6 Ectopic expression as result of 11q23 intrachromosomal deletion-fusion has hitherto been described in neuroblastoma only.7 In-frame fusions with the S’ MLL or PAFAH1B2 genes led to overexpression of FOXR1.7 In accordance with the notion that a constitutively expressed S’ gene (RPS25) might be responsible for the ectopic expression of FOXR1 in B-cell lymphoma also, FOXR1 levels were 1000× higher in the fusion-positive than in the fusion-negative U-2932 subclone. Expression array analyses showed that the RPS25/FOXOR1-positive U-2932 subclone had the highest FOXR1 expression level of 55 B lymphoma cell lines tested, three log-scales higher than average (Figure 2a). Quantitative PCR analysis conducted to verify the expression arrays included 17 additional B lymphoma cell lines, revealing that the primary effusion lymphoma cell line CRO-AP3

Figure 1. FOXR1 aberration in B lymphoma cell lines. (a) RPS25 exon 2 / FOXR1 exon 2 fusion expressed in U-2932 subclone R1. Two additional transcripts targeting RPS25 exon 2 with FOXR1 S’ sequences were also detected. (b) RPS25/FOXOR1 fusion in patient’s DNA and in one of two subclones of patient-derived cell line. Cell lines OCI-LY3 and HL-60 were used as negative controls. Size of PCR product: 294 bp. (c) FOXR1 was amplified (4n) in CRO-AP3 according to Cytoscan HD Array analysis (Affymetrix, Santa Clara, CA, USA). NTC, nil template control.

Figure 2. FOXR1 expression in B lymphoma. (a) According to expression array analysis, RPS25 is constitutively expressed in 55 B lymphoma cell lines, FOXR1 is highest in the RPS25/FOXOR1-positive U-2932 subclone (red dot). (b) Quantitative reverse-transcriptase PCR-verified ectopic expression in U-2932 subclone R1 and in the PEL cell line CRO-AP3. Cell lines NAMALWA (Burkitt’s lymphoma), CARNAVAL, OCI-LY7 and HT (all DLBCL) do not express FOXR1. (c) Reanalysis of previously published normalized expression profiling data showing ectopic FOXR1 expression in primary DLBCL and chronic lymphocytic leukemia (processed data from GEO).8-10 Red dots indicate FOXR1-high outliers.
expressed FOXR1 at a level similar to the DLBCL cell line U-2932 (Figure 2b). High-density genomic array analysis demonstrated copy-number transition from 3n to 4n in CRO-AP3, occurring 5' of FOXR1 (Figure 1c, Supplementary Figure S1B). Quantitative genomic PCR localized the site of amplification to the first 170 bases of exon 1. 5'-RACE, performed to identify potential 5'-mRNA partners in the two FOXR1 expressing cell lines, confirmed RPS25 as fusion partner of FOXR1 in U-2932. In CRO-AP3, the 5'-RACE PCR product terminated inside the amplified region of FOXR1 exon 1, upstream of the open reading frame. These results suggested that in CRO-AP3, FOXR1 overexpression was the result of gene amplification without fusion mRNA formation. Fluorescence in situ hybridization using a fosmid clone covering FOXR1 (G248P85736G6) yielded wild-type signals only restricted to chromosome 11 (not shown), leaving the putative 5' regulatory gene elusive.

Santo et al.7 reported that FOXR1 acts as negative regulator of forkhead box factor-mediated transcription and suggested a possible role in tumorigenesis. We found ectopic expression of FOXR1 in 2/72 (2.8%) B lymphoma cell lines. Both cell lines showed amplification of the FOXR1 gene. In one cell line, FOXR1 was fused to a constitutively expressed gene on 11q23, suggesting that the interstitial deletion was responsible for activation of FOXR1. Bioinformatic analyses document that the aberrant expression of FOXR1 is rare, but recurrent in B-cell lymphoma (Figure 2c).8–10

In conclusion, we show for the first time that FOXR1 fusions, described as candidate oncogenes in neuroblastoma, also occur in B-cell lymphoma. Cell lines U-2932 and CRO-AP3 are presented as models for the functional analysis of FOXR1-mediated cellular events.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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