LYMPHOID NEOPLASIA

Brief report

**TBL1XR1/TP63**: a novel recurrent gene fusion in B-cell non-Hodgkin lymphoma

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Recently, the landscape of single base mutations in diffuse large B-cell lymphoma (DLBCL) was described. Here we report the discovery of a gene fusion between TBL1XR1 and TP63, the only recurrent somatic novel gene fusion identified in our analysis of transcriptome data from 96 DLBCL cases. Based on this cohort and a further 157 DLBCL cases analyzed by FISH, the incidence in de novo germinal center B cell-like (GCB) DLBCL is 5% (6 of 115). The fusion appears exclusive to GCB and was not seen in 138 non-GCB cases examined (P = .008, Fisher exact test) but was present at low incidence in follicular lymphoma (1 of 81). In all 7 cases identified, the 3’ end of the fusion consists of exons 4 and onwards of TP63. The recurrence, subtype enrichment, and the remarkably conserved nature of the TP63 portion of the fusion suggest an important functional role in the lymphomas that harbor this event. (Blood. 2012;119(21):4949-4952)

Introduction

Chromosomal rearrangements are a hallmark of hematologic malignancy. The 3 most recurrent translocations in diffuse large B-cell lymphoma (DLBCL) were discovered by examination of karyotypes, with the partner genes identified in the 1980s and 1990s.1 Translocations involving BCL6, BCL2, and MYC are reported in 30%, 20% to 30%, and up to 10% of de novo DLBCLs, respectively.2 Recently, we and others have described the mutational landscape of DLBCL, focusing on single nucleotide variations and small insertion/deletions.3-5 Analysis of transcriptomes also offers the opportunity to identify novel fusion gene transcripts resulting from cryptic chromosomal rearrangements hitherto unsuspected from karyotype analysis, which is limited by resolution and the complexity of the chromosomal events.6 TP63 is a paralog of the tumor-suppressive transcription factor TP53.7 In contrast to TP53, TP63 is rarely mutated in malignancy.4-10 However, overexpression of ΔN-TP63, a set of TP63 isoforms lacking the transactivation (TA) domain, has been associated with malignancies of epithelial origin.11,12 TBL1XR1 encodes a protein that is part of the NCoR/SMRT transcription repressor complex.13 Recently, deletion of the TBL1XR1 gene locus has been described in DLBCL4 and primary CNS lymphoma.14 Here, we describe a novel recurrent gene fusion involving TP63 and TBL1XR1 discovered during analysis of DLBCL transcriptomes.

Methods

To identify candidate gene fusion transcripts, Trans-ABYSS15 and deFuse16 were applied to the previously described1 transcriptome data generated from 96 DLBCL and 13 follicular lymphoma (FL) cases. Validation of candidate fusion transcripts was achieved using Sanger sequencing of amplicons produced by RT-PCR with primers specific for the predicted gene fusion (supplemental Table 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online article). Cell of origin was assigned using gene expression profiling data when available. The tally of breakpoints in the genomic DNA were identified in all cases by Sanger sequencing of amplicons generated using RT-PCR. The existence of the TBL1XR1/TP63 fusion (supplemental Table 4). Parallel RNA sequencing (RNA-seq) of pretreatment de novo DLBCL biopsies. A novel gene fusion involving TBL1XR1 and TP63 was seen in the tumors from 4 patients (Figure 1). The existence of the TBL1XR1/TP63 fusion was validated in all 4 cases by Sanger sequencing of amplicons generated using RT-PCR. The breakpoints in the genomic DNA were identified in all cases (Figure 1B), and the genetic rearrangement was shown to be somatic by PCR and by whole genome shotgun sequencing of the constitutional DNA of one case. The TBL1XR1/TP63 fusion was the only recurrent novel somatic gene fusion observed.

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**TBL1XR1** and **TP63** are located 12 Mb apart on the long arm of chromosome 3, flanking the **BCL6** locus. To determine the incidence of this genetic rearrangement, FISH was performed on tissue microarrays (TMAs) comprising cores of pretreatment de novo DLBCL biopsies of 187 patients. The 30-patient overlap between the RNA-seq cohort and the TMA showed that RNA-seq and FISH had 100% concordance in detecting the **TBL1XR1**/**TP63** fusion in these samples (Figure 2A). The TMA analysis also revealed 2 new fusion-containing cases that displayed breakapart of both **TBL1XR1** and **TP63** loci. Sanger sequencing of amplicons generated using RT-PCR from RNA extracted from the formalin-fixed, paraffin-embedded biopsy (Figure 2A) confirmed the presence of the gene fusion in both cases. In aggregate, the incidence of the **TBL1XR1**/**TP63** fusion was 6 of 115 (5%) cases of germinal center B cell–like (GCB) DLBCL. The fusion appeared to be exclusive to GCB DLBCL with no **TBL1XR1**/**TP63** fusions seen in 138 cases of non-GCB DLBCL (P = .008, Fisher exact; Figure 2B).

The shared mutational landscape between GCB DLBCL and FL led us to determine the incidence of the fusion in FL. The **TBL1XR1**/**TP63** fusion was predicted and confirmed using RNA-seq data in one of 13 FLs. FISH was performed on a TMA comprising cores of FL biopsies of 68 patients. The results, with no cases identified where both **TBL1XR1** and **TP63** loci displayed breakapart, indicate that the fusion is a rare event in FL.

With **TBL1XR1** encoded from the negative DNA strand and **TP63** from the positive DNA strand, the chromosomal rearrangement event(s) include an inversion that produces gene fusions encoded on the same strand (Figure 1B). In all cases, the identified junctions between the **TBL1XR1**/**TP63** exons preserve the distal reading frame. Suggestive of functional significance, the **TP63**
portion of the gene fusion is conserved across all 7 cases, encoding exons 4 onwards. In 5 of the DLBCL cases, the 5’ junction point is the 3’ end of exon 7 of TBL1XR1, with exon 14 in the remaining DLBCL case and exon 4 in the FL case. In contrast, the predicted corollary, TP63/TBL1XR1, is disrupted in 3 of the 4 DLBCL cases examined. The mechanisms include a subsequent chromosomal translocation, deletion of the 3’ TBL1XR1 portion, and skipping of exon 8 of TBL1XR1 producing a frameshift resulting in a truncated protein.

The observation that the expression of the TBL1XR1/TP63 fusion was 5.2-±0.5-fold (mean ± SEM) higher than the wild-type TBL1XR1 mRNA, using quantitative RT-PCR on 3 of the DLBCL cases, suggests that there is a contextual change in the control of the TBL1XR1 promoter, which is driving the expression of this fusion.

The recurrent nature of the TBL1XR1/TP63 gene fusion, the conserved nature of the TP63 portion of the fusion, and its exclusive detection in the GCB subtype suggest a role in the pathogenesis of lymphomas harboring this event. Concomitant alterations of other genes known to be involved in the pathogenesis of DLBCL are given in supplemental Table 5. TP63 has 2 major sets of isoforms, distinguished at the N-terminus by the presence (TA-TP63) or lack (AN-TP63) of the TA domain, encoded by the first 3 exons. The TA-TP63 isoform has overlapping function with TP53 regarding induction of apoptosis in response to genotoxic stress. The ΔN-TP63 isoforms have a distinct function in the development and maintenance of stratified epithelial structures by contributing to self-renewal of basal epithelial cells. Lacking the TA domain, the TBL1XR1/TP63 protein may function similarly to ΔN-TP63, antagonizing the action of TP53, TA-TP63, and TA-TP73. We suspect that this may not only provide a proliferative advantage but also resistance to the genotoxic stress induced by chemotherapy. This is supported by the observation that 3 of the patients with DLBCL had primary refractory disease when treated with R-CHOP (supplemental Table 6). With the observation of the disruption of the TBL1XR1 locus in DLBCL and CNS lymphoma, the disruption of TBL1XR1 function represents another possible mechanism of action. On activation, the E3 ligase activity of TBL1XR1 polyubiquitinates the NCoR/SMRT complex, thus targeting it for degradation, releasing transcriptional repression. Similarly, TBL1XR1 is involved in the polyubiquitination and degradation of the tumor oncoprotein BCL3.

The TBL1XR1/TP63 gene fusion is predicted to give rise to a unique chimeric protein, in contrast to the deregulated expression of wild-type BCL6, BCL2, and MYC that result from the other recurrent chromosomal rearrangements in DLBCL. Although characterization of the function of this fusion protein is awaited, it
raises the possibility that this protein may be a novel target for therapeutic intervention.

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Authorship

Contribution: D.W.S. designed and performed the research, analyzed and interpreted data, and wrote the paper; K.L.M., S.R., R.D.M., F.C.C., and R.S.L. analyzed the RNA-seq data; S.B.-N. performed experiments and analyzed data; G.W.S. and K.L.T. reviewed pathology; J.M.C. curated the lymphoma database, participated in the original design of the project, reviewed the manuscript, and provided editorial input; M.A.M. and A.J.M. participated in the design of the original project and oversaw data collection and analysis; C.S. designed experiments and wrote the paper; and R.D.G. participated in the design of the original project, designed experiments, and wrote the paper.

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