LETTER TO THE EDITOR

Inactivation of BANK1 in a novel IGH-associated translocation t(4;14)(q24;q32) suggests a tumor suppressor role in B-cell lymphoma

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Immunoglobulin heavy chain (IGH) gene-associated translocations occur frequently in different subtypes of B-cell lymphomas.1 These translocations result in deregulated expression of partner genes and play pivotal roles in the pathogenesis of lymphomas. Molecular characterization of IGH-associated translocation breakpoints has been instrumental in the identification of genes important to the development of normal and malignant B cells.

We identified a novel balanced reciprocal translocation, t(4;14)(q24;q32), by conventional karyotyping (Figure 1a) in a case of post-transplant lymphoproliferative disorder (PTLD). The patient underwent stem cell transplantation for classical Hodgkin’s lymphoma and developed a gastric mass that was diagnosed as Epstein–Barr virus (EBV)-positive polymorphic PTLD with monomorphic areas. Fluorescence in situ hybridization (FISH) with IGH dual-color probe demonstrated that the translocation involved the IGH locus (Figure 1b). The breakpoint sequence was cloned by long-distance inverse PCR2-4 and sequence analysis identified the partner gene as BANK1 (B-cell scaffold protein with ankyrin repeats 1) (Figure 1c). The breakpoints were located in the switch region (S2) proximal to the Cx2 gene segment near the 3‘ end of IGH, and in intron 1 of the BANK1 gene ~830 bp telomeric to the 3‘ end of exon 1a. The juxtaposed IGH and BANK1 have opposite transcription orientations with a head-to-head configuration.

IGH/BANK1 translocation was further confirmed by FISH using double-color break-apart assay with two BAC probes, RP11-96J17 and RP11-138I19, performed on cells isolated from the PTLD case (Figure 1d). We screened an additional 68 diffuse large B-cell lymphomas (DLBCLs) and 15 PTLDs by FISH using the same probes. None of them were found to harbor any translocations (Figure 1d). We screened an additional 68 diffuse large B-cell lymphomas (DLBCLs) and 15 PTLDs by FISH using the same probes. None of them were found to harbor any translocations (Figure 1d).

BANK1 acts as an important adaptor molecule to link B-cell receptor (BCR)-mediated signaling to the generation of intracellular secondary messengers in B cells. Phosphorylated BANK1 enhanced BCR-induced calcium mobilization by binding to LYN, thus promoting LYN-mediated tyrosine phosphorylation of 1,4,5-triphosphate receptor (IP3R) that leads to release of calcium from intracellular stores.5 BANK1 has been shown to physically interact with B-cell lymphoid kinase (BLK),6 another B-cell specific Src family kinase, and phospholipase C γ2 (PLCg2),7 a major molecular switch in B-cell signal transduction, upon BCR engagement. In addition to an enhancing function in BCR signaling, BANK1 may have an inhibitory role in the CD40 signaling pathway. Studies on BANK1-deficient mice suggested that BANK1 attenuates CD40-mediated AKT activation to prevent hyperactive B-cell responses.8 BANK1 single-nucleotide polymorphic variants have also been implicated in autoimmune diseases such as systemic lupus erythematosus.9 Although evidence of an important function of BANK1 in B-cell physiology is emerging, a role for BANK1 in lymphoma development has not been reported previously.

BANK1 encodes three RNA isoforms, 1a, 1b and Δ2, with 1a being the major isoform.5,9 The two full-length isoforms, 1a and 1b, are derived from alternative promoter usage that results in the generation of exon 1a or 1b. The Δ2 isoform contains an in-frame deletion of exon 2. BANK1 is mainly expressed in immature and mature B cells with very low expression in pro B cells and absent expression in T cells. To determine whether BANK1 expression is differentially expressed during B-cell differentiation, B-cell subsets including naive, germinal center (GC) and memory B cells were isolated and BANK1 mRNA expression was determined by real-time quantitative reverse transcriptase-PCR (qRT-PCR). ALL BANK1 isoforms were expressed at higher levels (2.5–8 fold) in the naive and memory B cells compared with the GC B cells (Figure 2A). In line with this, immunohistochemistry (IHC) on reactive tonsils showed a distinctly lower level of expression in the GCs and higher levels in the mantle zones (Figure 2A). These findings imply a physiological downregulation of BANK1 expression during GC transit.

To determine the effect of the t(4;14) translocation on BANK1 expression, real-time qRT-PCR was performed to quantify total BANK1, total full-length BANK1 (that is, 1a plus 1b) and the three individual BANK1 isoforms (1a, 1b and Δ2) mRNA levels. Interestingly, we did not detect evidence of activation of BANK1 expression by the IGH translocation. Instead, we observed downregulation of BANK1 expression as a result of the translocation. The expression for total and full-length BANK1 mRNAs was greatly reduced (>90%) in the PTLD case compared with GC B cells (Figure 2B). BANK1 1a and Δ2 transcript isoforms, both initiated from the exon 1a promoter, were only barely detectable. The level of BANK1 1b transcript isoform was ~40% of the normal GC B cells. These results are consistent with the loss of expression of the major BANK1 1a isoform because of dissociation of exon 1 from the rest of the BANK1 gene, and concurrent silencing of the nontranslocated BANK1 allele. The latter allele is grossly intact, as implied by FISH, and we have excluded the possibility of hypermethylation (data now shown), suggesting other unknown mechanisms are responsible for repressing BANK1 transcription from the nontranslocated allele. It is noteworthy that although the translocated IGH locus is in a favorable position to do so, we did not observe activation of transcription initiated from the adjacent alternative BANK1 exon 1b promoter or from an intronic cryptic promoter. IHC confirmed the lack of BANK1 in the tumor cells (Figure 2B). In contrast, we detected variable BANK1 expression by IHC in other (8 of 8) PTLDs, 4 of 4 EBV-positive DLBCLs of the elderly and 48 of 54 DLBCL, not otherwise specified (DLBCL-NOS), all of which did not harbor BANK1 rearrangements (Supplementary Table 1 and Figure 2C). Thus, we believe that the main consequence of IGH translocation to the BANK1 locus is most likely downregulation of BANK1 through dissociation of the major promoter. Of the 54 DLBCLs, 6 also lacked BANK1 expression, suggesting the presence of alternative mechanism(s) of BANK1 downregulation in B-cell lymphomas.

Target gene downregulation is a highly unusual consequence of IGH translocations, as they have been shown to lead to overexpression of the target gene in almost all circumstances.
IGH-mediated gene inactivation has been implicated only once before in hematopoietic malignancies for the t(14;16)(q32;q23) translocation that is found in <5% of plasma cell myelomas. All the breakpoints of this translocation are located in the last intron of the tumor suppressor gene WWOX (WW domain containing oxidoreductase). This intron is nearly 1-Mb long and contains the fragile site FRA16D. IGH in this translocation is thought to have a dual pathogenetic function. It is in a position to disrupt the WWOX gene and prevent the generation of a full-length protein and, at the same time, causes overexpression of the neighboring MAF proto-oncogene located telomeric to WWOX via the IGH enhancer.

We have also considered the remote possibility that the IGH translocation activates a gene in the vicinity of BANK1. Three coding genes, PPP3CA, MIR1255A and FLJ20021, reside ~443 kb centromeric to the BANK1 breakpoint, and one coding gene, SLC39A8, is located ~460 kb telomeric to the BANK1 breakpoint (http://www.ncbi.nlm.nih.gov/gene/55024) (Figure 1b). PPP3CA, MIR1255A and SLC39A8 have the same orientation as the translocated IGH, whereas FLJ20021 has the opposite orientation to the translocated IGH. It is unlikely that the translocated IGH on der(4) drives expression of any of these three genes using the germline I transcript promoter because of their considerable distance from the IgH and/or incompatible relative transcriptional orientation. Furthermore, the configurations of the translocated IGH relative to these genes, either upstream/same orientations for PPP3CA and MIR1255A or downstream/opposite orientations for FLJ20021, are not considered favorable for an enhancer activation mechanism. Although IGH is located downstream of SLC39A8 in the same orientation and can conceivably activate the latter through its 3' enhancer on the der(14) chromosome, SLC39A8 mRNA level in this case was not significantly higher compared with EBV-positive B-cell lymphomas without the IGH/BANK1 translocation (data not shown). Thus, there is no definitive evidence for activation of SLC39A8 by the translocated IGH locus.

The inactivation of BANK1 in a IGH-associated translocation suggests that abnormal downregulation of BANK1 can promote lymphoma development. To show directly that BANK1 has a negative inhibitory effect on lymphomagenesis, we transfected BC3 primary effusion lymphoma cells with an all-in-one doxycycline-inducible expression vector pRTS-1, carrying BANK1 complementary DNA to establish an inducible BANK1-expressing cell line. The BC3 cell line was chosen because it has no detectable endogenous BANK1. Addition of doxycycline resulted in efficient induction of BANK1 and a significant retardation in the rate of cell number increase compared with the control transfectants.
Bromodeoxyuridine staining demonstrated a decreased incorporation from 31.1 to 15.6% upon BANK1 expression, implying a negative effect of BANK1 on cell cycle (Figure 2Di).

This study identified BANK1 as a novel IGH translocation partner and demonstrated a rare mechanism of gene inactivation by IGH through promoter dissociation. Our initial functional studies demonstrating a negative effect of BANK1 on cell proliferation suggest that BANK1 inactivation may contribute to lymphoma by facilitating cell proliferation. Unlike the myeloma-associated t(14;16)(q32;q23), which has a dual impact on two neighboring genes (WWOX and MAF), the impact of (4;14) translocation described here appears to be restricted to BANK1. Our study underscores the utility of cloning IGH-associated translocations, even the rare ones, to identify genes with important functions in normal and neoplastic B-cell biology. We provide, for the first time, genetic and functional data suggesting a possible tumor suppressor role of BANK1 in B-cell lymphomagenesis. Further studies are warranted to further investigate the involvement of BANK1 in mature B-cell malignancies.

CONFLICT OF INTEREST
The authors declare no conflict of interest.
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