To the editor:

Convergent *BCL6* and lncRNA promoters demarcate the major breakpoint region for *BCL6* translocations

We recently published a study showing that *BCL6* translocations occur at distinct sequence motifs within intron 1 of the *BCL6* gene that are recognized and deaminated by activation-induced cytidine deaminase (AID).[1] It has been unclear, however, why these breakpoints cluster in an ~2-kb major breakpoint region (MBR) within this 11-kb-long intron. Recent studies in mouse models of AID-dependent translocations suggest that AID-induced breaks may target intragenic regions of convergent transcription and/or H3K27Ac-defined super-enhancers.[2,3] Indeed, analysis of the human *BCL6* gene identified regions of convergent transcription, as defined by global run-on sequencing (GRO-seq) (a measure of global transcription) and/or super-enhancers overlapping with *BCL6* intron 1, but these regions are at least threefold larger than the ~2-kb breakpoint region identified in actual human lymphoma samples (compare lines 3-5 with lines 6-8 in Figure 1). Looking in the LNCipedia long noncoding RNA (lncRNA) database,[4] we found that the lncRNA gene lnc-RP11-211G3.3.1-1 (transcribed from the positive strand, which is opposite to the transcription direction of the *BCL6* gene) is located where >87% of the translocations occur. Remarkably, the boundaries of this lncRNA match the boundaries of the *BCL6* translocation zone quite precisely (compare line 2 with lines 3-5 in the Figure 1). Moreover, this lncRNA is a natural transcript in human diffuse large B-cell lymphoma (DLBCL); expression of lnc-RP11-211G3.3.1-1 was detected in the germinal cell B-cell type of DLBCL and at a lower level in the activated B-cell type of DLBCL.[5] Other than its expression and precise correlation with the *BCL6* translocation zone as described in this letter, there are no currently published data about this novel lncRNA.

The correlation of lnc-RP11-211G3.3.1-1 with the *BCL6* translocation zone supports the view that convergent transcription is an important factor for defining some translocation fragile zones.[2] Interestingly, a convergent arrangement where 2 promoters are close together will eventually become divergent when the 2 RNA polymerases progress away from the zone of overlap. Divergent configurations result in increased DNA melting and single-stranded DNA,[6] which are necessary for AID action.[7] Our initial report described the AID motifs in the human *BCL6*, providing evidence that AID is responsible for the breaks at the human *BCL6* gene.[1] This additional note adds that the fragile zone in which AID causes chromosome breaks may require the transcription on both strands that may then permit not simply AID mutation (involving only 1 strand of the DNA), but AID action on both DNA strands to permit a double-strand DNA break.

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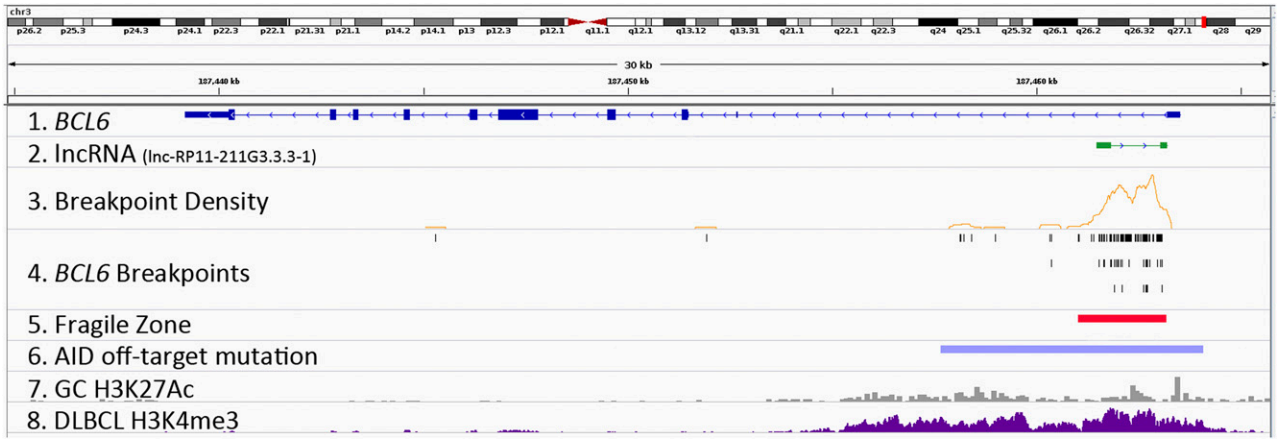

Figure 1. *BCL6* translocation fragile zone overlaps with lncRNA transcript. The tracks in this Integrative Genomics Viewer snapshot show: *Line 1*, the *BCL6* gene (blue, transcribed from negative strand); *Line 2*, the lncRNA (shown in green and transcribed from positive strand; gene called lnc-RP11-211G3.3.3-1); *Line 3*, the density of patient *BCL6* translocation breakpoints adapted from previous publication[1] (tan line graph illustrating the number of *BCL6* breakpoints counted in 500-bp sliding window with steps of 50 bp; the y-axis range of this graph is 0-48); *Line 4*, the actual location of *BCL6* breakpoints described in line 3 (black vertical lines are distributed over 3 layers for clarity); *Line 5*, the fragile zone of 2156 bp that encompasses 90% of the breakpoints (red bar); *Line 6*, AID off-target mutation zone in the Ramos cell line (light purple bar)[5]; *Line 7*, the H3K27Ac chromatin immunoprecipitation–seq read density in human tonsil germinal center B cells (gray, GSM1519135)[5]; *Line 8*, the H3K4me3 chromatin immunoprecipitation–seq read density in the human lymphoma (DLBCL) cell line, OCI-Ly1 (dark purple); the y-axis range of this graph is 0 to 60.[1] The lncRNA gene overlaps with >87% of the *BCL6* breakpoints.

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To the editor:

NOTCH2 missplicing can occur in relation to apoptosis

In a recent study in *Blood*, Adamia et al have identified from an analysis on RNA sequencing and exon array data, that *NOTCH2* and *FLT3* gene missplicings are common events and potential targets in acute myeloid leukemia (AML).[1] Among the splice variants, *NOTCH2-Va* resulting from a complete deletion of exon 12 (111 bp) was of particular interest because it was detected at a very high frequency (73%) in AML and its high expression was associated with poor outcome in patients with an intermediate-risk cytogenetics.

Incidentally, we found that this splice variant can be observed in normal hematopoietic cells undergoing apoptosis. To further investigate this phenomenon, we obtained 8 bottles of blood specimens each from 3 healthy individuals and stored samples in a dark chamber at room temperature. RNA was extracted immediately after venipuncture and also sequentially after scheduled storage times: 1, 3, 6, and 12 hours and 1, 3, and 7 days. Fluorescence polymerase chain reaction (PCR) and capillary electrophoresis using primers to detect *NOTCH2-Va* and full-length wild-type (WT) transcript (*NOTCH2-FL*)[1] showed a gradual increase of *NOTCH2-Va* and a decrease of *NOTCH2-FL* during specimen aging (Figure 1A-B). Gel extraction and sequencing analysis for the abnormal PCR band confirmed skipping of the entire exon 12 of *NOTCH2* (Figure 1C).

In line with our observations, another study also identified aberrant splicing of *NOTCH2* in incubated normal blood cells by RNA sequencing,[2] suggesting that the presence of *NOTCH-Va* may reflect the illegitimate splicing typically observed in specimen aging.[3] The aberrant splicing is suggested to be widespread across the genome and associated with apoptosis pathways.[4,5] However, Adamia et al[1] have also demonstrated some functional consequences of the *NOTCH2* splicing variants to induce downregulation of its target genes, such as *HES1* and *DTX1*. Considering that deregulation of apoptosis is a key mechanism in the pathogenesis of AML,[6] there might be some commonality between aging-related and leukemia-related missplicing events. And of special note, *NOTCH2* is proven to have important roles on the regulation of apoptosis.[7,8] The original investigators have suggested *NOTCH2* variants as attractive targets for novel therapeutics, and we may say that the targeting strategy should be considered in the context of cell apoptosis. For clinical diagnostics, it is strongly recommended that these variants be tested using fresh specimens and to interpret the results with an understanding of the dynamics of splicing during cellular aging.

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