to the editor:

Cryptic XPO1-MLLT10 translocation is associated with HOXA locus deregulation in T-ALL

Biological subclasses of T-cell acute lymphoblastic leukemia (T-ALL) can be defined by recurrent gene expression patterns, which typically segregate with specific chromosomal anomalies. The HOX A\(^+\) subgroup is characterized by deregulated homeobox A (HOXA) gene expression and is associated with translocations involving the mixed lineage leukemia (MLL) and/or MLLT10 loci, SET-NUP214, or TCRB-HOXA.\(^1\)\(^2\) Nevertheless, the genetic basis for many HOXA\(^+\) cases remains unexplained.

Diagnostic assessment of a 33-year-old man with T-ALL revealed high leukemic blast expression of HOXA9 at levels comparable to those in known HOXA\(^+\) cases (Figure 1A). For PICALM-MLLT10, SET-NUP214, MLL-AF6, and TCRB-HOXA were negative. Leukemic cells exhibited a complex karyotype with translocations involving the mixed lineage leukemia (MLL) and/or MLLT10 loci, SET-NUP214, or TCRB-HOXA.\(^1\)\(^2\) Nevertheless, the genetic basis for many HOXA\(^+\) cases remains unexplained.

Diagnostic assessment of a 33-year-old man with T-ALL revealed high leukemic blast expression of HOXA9 at levels comparable to those in known HOXA\(^+\) cases (Figure 1A). We hypothesized that the common involvement of MLLT10 would result in similar deregulation of HOXA locus expression in XPO1-MLLT10\(^+\) and PICALM-MLLT10\(^+\) T-ALL. We tested the expression of a range of HOX genes by quantitative RT-PCR. As predicted, the pattern of HOX gene transcription in the XPO1-MLLT10\(^+\) case was very similar to that in the PICALM-MLLT10\(^+\) cases (Figure 1C). A targeted RT-PCR screen of 84 HOXA\(^+\) T-ALL samples that lacked known explicable genetic anomalies identified no further XPO1-MLLT10\(^+\) cases (Figure 1D), suggesting rarity and/or breakpoint heterogeneity.

Each of the genes involved in this fusion has been previously implicated in leukemia. Notably, MLLT10 (which encodes the AF10 protein) is involved in the recurrent PICALM-MLLT10\(^+\) and MLL-MLLT10\(^+\) translocations in both T-ALL and acute myeloblastic leukemia. Recently reported results of RNA-sequencing have identified HRNRPH1 and DDX3X as MLLT10 fusion partners in HOXA\(^+\) T-ALL.\(^3\)\(^\text{7}\) Our data provide further evidence of shared expansion of this fusion partner–independent mechanisms of AF10-mediated transcriptional dysregulation, and this case adds to the repertoire of MLL and/or AF10-rearranged T-ALL that might be candidates for targeted DOT1L-directed therapy.\(^6\) MLLT10 breakpoints are heterogeneous, and increasing truncation of the transcript was reported to correlate with an earlier maturation block in T-ALL, although this was not confirmed in a later series.\(^3\)\(^\text{7}\) In this case, detailed characterization of T-cell receptor (TR) gene configuration revealed monoallelic TRG and TRD and incomplete TRB diversity-joining rearrangements (data not shown), consistent with an immature pre-β-selection immunogenotype.\(^8\)

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Figure 1. XPO1-MLLT10 fusion detected by RNA-sequencing is associated with deregulation of HOXA locus expression. (A) Expression of HOXA9 in genetic subgroups of T-ALL. Levels were determined by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and calculated relative to an ABL housekeeping gene control. Boxes encompass the 25th through 75th percentiles, with the horizontal bar denoting the median expression level. Whiskers indicate the 10th and 90th percentiles. The numbers of cases tested in each group were as follows: XPO1-MLLT10, n = 1; PICALM-MLLT10, n = 36; SET-NUP214, n = 14; MLL-AF6, n = 9; TLX1, n = 18; TLX3, n = 67; SIL-TAL, n = 40. (B) Upper panel: Genomic mapping of the XPO1-MLLT10 fusion by poly(A)-enriched strand-specific RNA-sequencing using the SOLiD HG550olx system (Life Technologies). Mapping, coverage, and fusion discovery were determined by using LifeScope (Life Technologies), with reference to version hg19 of the human genome. A schematic representation of paired-end and fusion-spanning reads that revealed fusion between exon 24 of XPO1 (chr2:61708320-61708416) and exon 6 of MLLT10 (chr10:21901277-21901380) is shown. Solid lines indicate split reads spanning 2 exons, and dotted lines indicate 2 reads of the same fragment. The numbers of unique reads for the wild-type XPO1 (exons 24 and 25) and MLLT10 (exons 5 and 6) transcripts are also depicted. Lower panel: Confirmation of expression of an in-frame XPO1-MLLT10 fusion transcript by direct (Sanger) sequencing. The positions of the nucleotide (NT) and amino acid (AA) at the breakpoint of each gene are annotated. (C) Expression of HOXA genes in XPO1-MLLT10 (n = 1; denoted by triangles) and PICALM-MLLT10 (n = 4; mean levels denoted by circles with error bars indicating standard error of the mean) blasts. Transcript quantification was determined by qPCR using a TaqMan Low-Density Array, and the results of 2 experimental replicates were combined. Expression was calculated relative to a GAPDH housekeeping gene control. (D) RT-PCR for XPO1-MLLT10; 84 cases of HOXA1-T-ALL lacking known explicable genetic anomalies were screened by using primers specific for the XPO1-MLLT10 fusion transcript (product size, 618 bp). A representative PCR result is shown. T-ALL 1 is the index XPO1-MLLT10 case. NTC, no template control; PE, paired-end; SE, single-end.

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To the editor:

Two types of amyloid in a single heart

Amyloidosis is a heterogeneous group of diseases, in which amyloidogenic precursor proteins misfold and adopt a β-pleated sheet conformation. Several proteins can form amyloid fibrils in vivo including transthyretin, apolipoprotein A-I and A-II, lysozyme, fibrinogen, serum amyloid A protein and immunoglobulin light chains, but “cross-fibril” seeding appears to be rare such that 2 types of amyloid are rarely identified in the same individual. Congo red (CR) staining with apple green birefringence under polarized light is used to confirm the presence of amyloid, whereas immunohistochemistry, staining the biopsy tissue with a panel of monospecific antibodies against known amyloidogenic proteins, is the technique most widely used to characterize the amyloid fibril protein, but it is flawed. Proteomic analysis involves proteolytic cleavage of proteins within microdissected amyloidotic tissue and identification by mass spectrometry. This additional tool is being increasingly used in conjunction with immunohistochemistry to identify the amyloid fibril protein.

We describe a case in which 2 amyloid fibril proteins were isolated in an individual patient both by immunohistochemistry and by proteomic analysis. An 81-year-old man was referred to our center with a 6- to 7-month history of exertional dyspnea and New York Heart Association class II symptoms. Baseline investigations included an echocardiogram showing characteristic features of cardiac amyloidosis with a thickened interventricular wall of 18 mm, moderate diastolic dysfunction, and preserved left ventricular ejection fraction with a 6- to 7-month history of exertional dyspnea and New York Heart Association class II symptoms. Baseline investigations included an echocardiogram showing characteristic features of cardiac amyloidosis with a thickened interventricular wall of 18 mm, moderate diastolic dysfunction, and preserved left ventricular ejection fraction, thus confirming the immunohistochemistry results of 2 types of amyloid in the same heart. Our patient is due to receive chemotherapy for AL amyloidosis shortly.

In summary, the exceptionally rare occurrence of 2 different amyloid fibril proteins was suggested by immunohistochemistry in this patient. The coexistence of 2 amyloid types in the same heart was confirmed by laser capture microdissection and proteomic analysis of 2 distinct areas.

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