Identification of ANLN as ETV6 partner gene in recurrent t(7;12)(p15;p13): a possible role of deregulated ANLN expression in leukemogenesis

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Abstract

The ETV6 gene encodes an ETS family transcription factor that is involved in a myriad of chromosomal rearrangements found in hematological malignancies and other neoplasms. A recurrent ETV6 translocation, previously described in patients with acute myeloid leukemia (AML) (Genes Chromosomes Cancer 51:328–337, 2012, Leuk Res 35:e212-214, 2011), whose partner has not been identified is t(7;12)(p15;p13). We herein report that the t(7;12)(p15;p13) fuses ETV6 to ANLN, a gene not previously implicated in the pathogenesis of hematological malignancies, and we demonstrate that this translocation leads to high expression of the fusion transcript in the myeloid and lymphoid lineages.

Keywords: Leukemia, Myeloid, Acute, Gene fusion, Translocation, Genetic

Findings

Background

Balanced translocations leading to chimeric fusion genes play a major role in the pathogenesis of cancer. ETV6 is a frequently rearranged gene involved in least 30 fusion genes in myeloid and lymphoid neoplasms [1]. A recurrent ETV6 translocation, previously described in patients with acute myeloid leukemia (AML), whose partner has not been identified is t(7;12)(p15;p13) [2, 3]. In the present work we describe the identification of ANLN as a novel ETV6 fusion partner as a result of the t(7;12)(p15;p13).

Results

After Institutional Review Board approval (IRB-25000.179520/2011-36) and signing of consent form, we studied a 40 year-old female patient (P005) with diagnosis of JAK2 V671F-positive primary myelofibrosis (PMF) and karyotype compatible with 46,XX,del(5)(q12q33) since 2010. She had been treated with supportive care and ruxolitinib. In 2013, the patient presented worsening of blood counts and increase in spleen size, suggesting disease progression. A bone marrow (BM) biopsy showed an increase in BM fibrosis (grade 3 out of 3) and 9 % blasts in the BM aspirate. Chromosomal analysis of BM showed 46,XX,del(5)(q12q33),t(7;12)(p15;p13) [20] (Fig. 1a). The patient was treated with an allogeneic BM transplantation from her HLA-haploidentical sister. Seven months after transplantation, she progressed to refractory acute megakaryoblastic leukemia that presented with two BM blast populations, a CD34-positive (4.9 %) and a CD34-negative (49.6 %).

At the time of disease progression before transplantation, a sample of her BM aspirate was analyzed by a hybrid-capture-based comprehensive genomic profiling assay (FoundationOne Heme), employing both DNAseq
and RNAseq in a CLIA certified laboratory (Foundation Medicine, Cambridge, MA, USA). This method evaluates the entire coding sequence of 405 cancer-related genes, 31 selected introns frequently involved in rearrangements and RNA sequencing of 265 genes commonly fused in cancer. The result revealed three genomic alterations: JAK2 V617F, NRAS G13D and an ETV6-ANLN fusion. The fusion breakpoints occurred in intron 1 of both genes, leading to a putative transcript carrying ETV6 first exon fused to ANLN exons 2 to 25. We confirmed the expression of the fused gene by means of cDNA polymerase chain reaction (PCR) and Sanger sequencing (Fig. 1b).

ANLN encodes an actin-binding protein essential to cytokinesis that is expressed at low levels in most normal tissues [4]. The protein encoded by ANLN consists of one,125 amino acids and contains an actin-binding region (amino acids 231 to 676), an Anilin domain (amino acids 799–953), a C-terminal pleckstrin homology domain (amino acids 983–1107) and a nuclear localization region (amino acids 1–230) [4, 5]. The putative protein encoded by the ETV6-ANLN fusion described here substitutes the first 6 ANLN amino acids (MDPFTE) by the first 11 ETV6 amino acids (MSETPAQSSIK), resulting in a protein almost identical to ANLN, without disrupting its main functional domains. It is unknown at this time if this change disrupts ANLN function. On the other hand, this protein lacks all ETV6 functional domains. We therefore hypothesized that the leukemogenic mechanism operating in this case could be related to the overexpression of the fusion protein, which can have very similar functional characteristics to wild type ANLN.

We thus designed two Taqman qPCR assays (Life Technologies) to study the expression level of the fusion gene and also the expression of wild type ANLN in healthy volunteer donors and patients with myeloid malignancies. In order to evaluate only the expression of wild type ANLN, we designed primers complementary to ANLN exon 1 and exon 2. Since the fusion gene lacks ANLN exon 1, only wild type ANLN was amplified. For the fusion assay, primers were complementary to ETV6 exon 1 and ANLN exon 2. We evaluated the expression of both transcripts in the following magnetic bead selected cell populations: granulocytes (CD66b+) from 20 patients with PMF and 8 healthy volunteers, in CD34+ cells from 10 AML patients and on myeloblasts (CD34+), bone marrow mononuclear cells enriched for megakaryoblasts (CD34-), T cells (CD3+) and granulocytes (CD66b+), from P005. All cell populations had > 95 % purity.

Expression of wild type ANLN was absent in granulocytes from healthy subjects, PMF patients and P005. On the other hand, ANLN expression was present in CD34+ cells from a subset of AML patients and in both CD34+ and CD34- mononuclear populations from P005 (Fig. 2a). These data suggest that wild type ANLN is
not expressed in mature granulocytes, but only in CD34+ cells from a subset of AML patients. We did not study the expression of ANLN in CD34+ cells from healthy donors, therefore we cannot rule out ANLN expression in normal CD34+ cells.

On the other hand, the fusion transcript was present in all P005 cell subpopulations (Granulocytes, T lymphocytes, CD34+ blasts and CD34- mononuclear cells), suggesting that the translocation may have occurred in a pluripotent hematopoietic stem cell or an early precursor (Fig. 2b). As expected, no fusion transcript was detected in other individuals.

While more common in lymphoid malignancies, such as acute lymphoblastic leukemia, ETV6 translocations are uncommon in myeloid neoplasms. In a study of 9,550 patients with myeloid neoplasms, ETV6 translocations were found in 0.5 % of patients, occurring in only 0.3 % of myeloproliferative neoplasms [1].

Since the fusion gene we identified retains only the first exon of ETV6, while maintaining the integrity of the ANLN gene, we speculate that the oncogenic mechanism can be related to deregulated ANLN expression. Indeed, previous studies have reported that ANLN is overexpressed in a variety of human cancers such as lung [6], breast [7] and endometrial cancer [8]. In addition, increased ANLN expression has been linked to tumor progression [8], and inhibition of ANLN in lung cancer cells decreases cell viability and increases cell size and ploidy, probably secondary to defective cytokinesis [6]. Thus, ANLN seems to play an important role in cell division, and increased expression of ANLN has been shown to induce DNA synthesis in lung cancer cells [6]. Similar results have been shown in breast cancer, where inhibition of ANLN expression abrogates cell proliferation and colony forming ability of breast cancer cell lines. We are not aware of published data about ANLN expression in hematological malignancies, and the precise role of the ETV6-ANLN fusion transcript in the pathogenesis of myeloid malignancies with the t(7;12)(p15;p13) translocation still needs to be defined, but we believe that deregulated ANLN expression leading to increased cellular proliferation might play a role.

Conclusions
We have demonstrated that the recurrent AML associated translocation t(7;12)(p15;p13) leads to the formation of the novel fusion gene ETV6-ANLN that is expressed in the myeloid and lymphoid lineages. To the best of our knowledge, this is the first report implicating the actin-binding protein ANLN in the pathogenesis of AML and other myeloid neoplasms.

Competing interests
Paulo Vidal Campregher, Jeffrey S. Ross, Siraj Ali and Vincent Miller are employees of Foundation Medicine.

Authors’ contributions
PVC - designed the study and wrote the paper (paulo.campregher@einstein.br). BL - performed experiments (lisboa.bianca@gmail.com). RP - performed bioinformatics analysis and revised the paper (renato.puga@einstein.br). RH - participated in patient selection and revised the paper (helman@einstein.br). MM - performed experiments (marimiyagi@hotmail.com). EHAM - performed experiments (elvira.velloso@einstein.br). EDRPV - performed cytogenetics analysis (evelyn.asc@hotmail.com). NSB - performed flow cytometry experiments (nsbacal@einstein.br). JRR - performed the comprehensive genomic profile and revised the paper (jross@foundationmedicine.com). SA - performed the comprehensive genomic profile and revised the paper (sali@foundationmedicine.com). VM - performed the comprehensive
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