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Original Article

A novel t(11;14)(q13;q32) translocation involving Pellino3 links innate immune and inflammatory signaling to a myeloproliferative neoplasm

Hua Guo[1], Nayyara Mahmood[1], Judith Brody[1], Dipti Gheewala[1], Craig Devoe[2], Steven L Allen[2], Silvat Sheikh-Fayyaz[1], Chandrika Sreekantaiah[1], Gokul Kandala[1*], Xinmin Zhang[1*]

[1]Department of Pathology and Laboratory Medicine, Northwell Health, Hofstra Northwell School of Medicine, Lake Success, NY, USA; [2]Division of Hematology, Department of Medicine, Northwell Health, Hofstra Northwell School of Medicine, Lake Success, NY, USA. [*]Equal contributors.

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Abstract: Toll-like receptor (TLR)/interleukin-1 receptor (IL-1R) plays a central role in innate immune response and inflammation. Pellino3 is an E3 ubiquitin ligase which has important regulatory functions in TLR signaling. We identified a novel t(11;14)(q13;q32) translocation in a patient with *JAK2* positive myeloproliferative neoplasm who later developed acute myeloid leukemia. FISH analysis revealed no *CCND1-IGH* fusion. By sequential FISH using a series of bacterial artificial clone (BAC) probes, we demonstrated that the translocation involved *Pellino3,* resulting in its translocation to der(14) and overexpression of Pellino3 protein. This is the first report linking the Pellino3 signaling to myeloproliferative neoplasm.

Keywords: Pellino3, myeloproliferative neoplasm, ubiquitin ligase, TLR/IL-R, JAK2

Introduction

Toll-like receptor (TLR)/interleukin-1 receptor (IL-1R) plays an important role in innate immune response and inflammation. The engagement of TLR/IL-1R recruits the adaptor protein MYD88 or TRIF, which initiates a cascade of downstream events, ultimately resulting in NF-KB activation and the induction of proinflammatory cytokines. The ubiquitin proteasome system plays a central role in the regulation of the TLR/IL-1R pathway by controlling levels and activities of signaling molecules. Pellino is a ring class of E3 ubiquitin ligase and has three human family members: Pellino1, Pellino2 and Pellino3 [1].

Pellino3 has diverse functions in the TLR/IL-1R pathway: (1) It catalyzes K63 polyubiquitination of TLR/IL-1R signaling molecules such as IL-1R-associated kinase 1 (IRAK1) and TNF receptor-associated factor 6 (TRAF6) [1]. (2) It is a negative regulator for TLR3 and TLR4 induced interferon β (IFNβ) expression and tumor necrosis factor (TNF) induced apoptosis [1]. (3) It is a mediator of the nucleotide-binding oligomerization domain-containing protein 2 (NOD2) signaling pathway that is important in the homeostatic control of intestinal inflammation [2]. Overexpression of Pellino3 triggers activation of ERK, JNK and P38 MAPK pathways [3]. Pellino3 is required for P38 mediated activation of cAMP-responsive element-binding protein (CREB) [4]. Overexpression of CREB in myeloid cells in transgenic mice induced a myeloproliferative process mimicking human myeloproliferative neoplasm (MPN) [5]. Accumulating evidence suggests that Pellino3 may play a role in the pathogenesis of MPN.

The chromosomal 11q13 region is frequently rearranged in hematological malignancies [6]. Of the chromosomal translocations involving this region, t(11;14)(q13;q32) is the most well-studied and is characteristic for mantle cell lymphoma. However, it is extremely rare in myeloid neoplasms. We identified a novel t(11;14)(q13;q32) in a patient with JAK2 positive MPN, which involved *Pellino3* and resulted in its over-

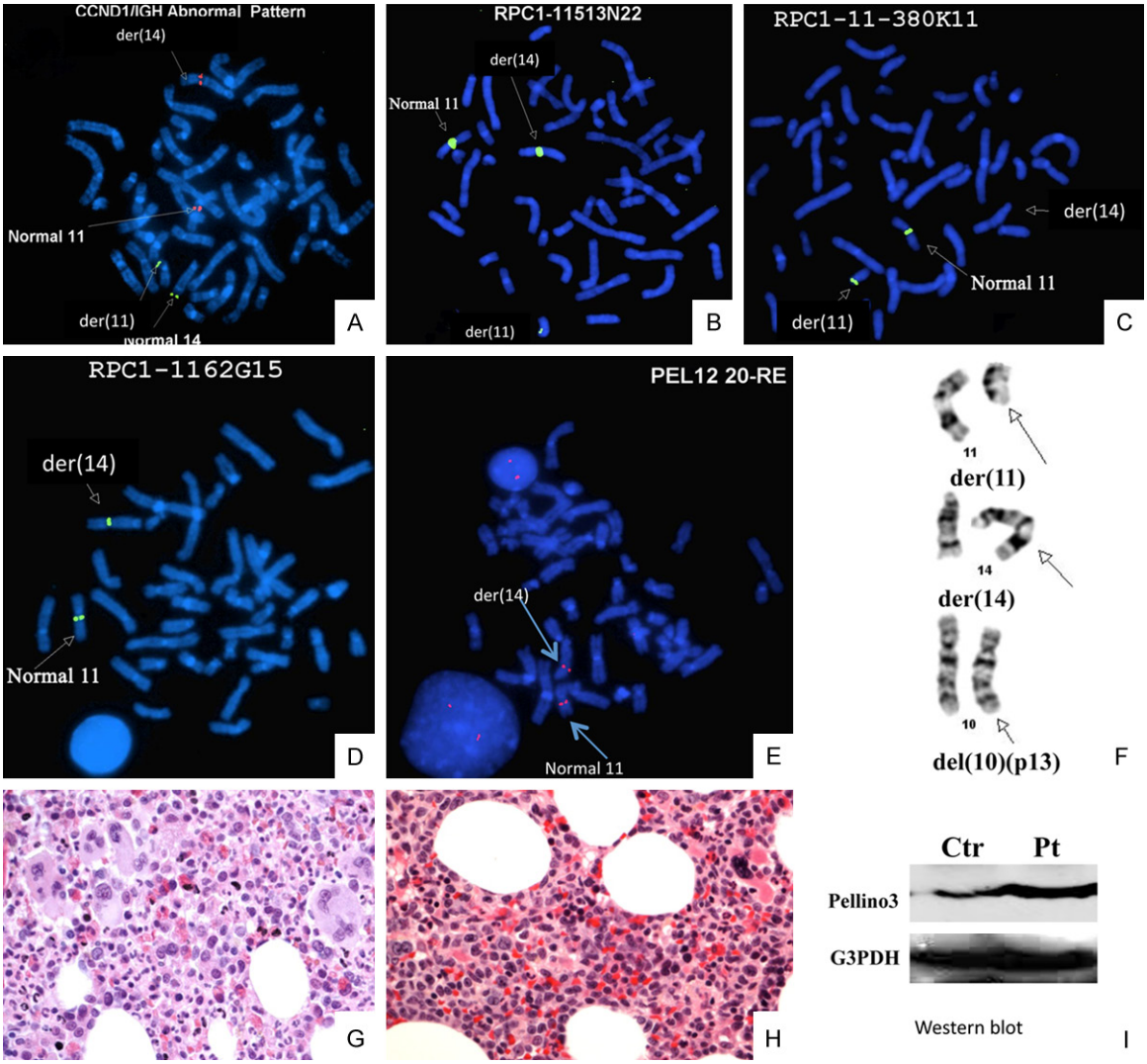

Figure 1. A summary of findings for morphology, cytogenetics, FISH and Western blot. FISH studies are performed on metaphase nuclei of the AML bone marrow using *CCND1 IGH* break-apart probe (A) and probes prepared from BAC clones including RPC1-11-513N22 (B), RPC1-11-380K11 (C), RPC1-11-62G15 (D), and Pellino3 specific probe (E). The cytogenetic abnormalities are shown in (F). (G and H) represent the histologic findings of bone marrow biopsy (Hemotoxin and Eosin stain) for MPN stage and AML stage, respectively. Western blot studies for Pellino3 and G3PDH are shown in (I).

expression. Our findings suggest a link between Pellino3 mediated signaling and MPN.

Patient and methods

Case report

A 71 year-old man with a history of pulmonary embolism and recurrent deep venous thrombosis was found to carry *JAK2V617F* mutation. Complete blood count (CBC) showed white blood cell count (WBC) 7.7 × 10^9 with 11.7% eosinophils, hemoglobin (Hb) 13.3 g/dL, and platelets (Plt) 409 × 10^9/L. Bone marrow biopsy revealed a JAK2 positive MPN. He was treated with low dose of hydroxyurea to prevent recurrent thrombosis. Four years after diagnosis, CBC showed Hb 13.7 g/dL, Plt 35 × 10^9/L, and WBC 15.4 × 10^9/L with 24% blasts. The serum lactate dehydrogenase (LDH) was elevated with a value of 620 U/L. Computed tomography (CT) Scan revealed splenomegaly with a large infarct. Bone marrow findings were consistent with acute myelomonocytic leukemia (FAB M4). While he was prepared for induction therapy in the hospital, his condition rapidly deteriorated and he died.

Table 1. BAC clone probes for FISH studies and their chromosomal localization

Chromosomal band	Probe	Localization	Covered genes	Ch11	Ch14
11q13.2	RP11-149G19	67,774,672-67,942,131	C11orf24, LRP5	-	+
11q13.2	RP11-783K16	63,751,866-63,918,223	PLCB3, RPS6KA4, DKFZP566E164, VEGFB, ESRRA, FKBP2, DNAJC4, PRDX5, HSPC152, GPR137, CCDC88B, BAD, KCNK4, PPP1R14B, NUDT22	+	-
11q13.2	RP11-937I17	66,842,920-67,012,980	PPP1CA, TMEM134, RPS6KB2, RAD9A, PTPRCAP, CLCF1, CORO1B, KIAA1394, POLD4, CABP4, TBC1D10C, GPR152, AIP	-	+
11q13.2	RPC1-11-856B14	65,043,422-65,254,836	FAM89B, EHBP1L1, MAP3K11, KCNK7, HTATIP, SSSCA1, SIPA1, SCYL1, LTBP3, RELA, RNASEH2C, PCNXL3	+	-
11q13.2	RPC1-11-506O3	65,641,026-65,815,317	YIF1A, KLC2, RAB1B, CNIH2, PACS1	+	-
11q13.2	RPC1-11-513N22	65,941,482-66,124,845	NPAS4, MRPL11, PELI3, ACTN3, CTSF, CCS, CCDC87, BBS1, DPP3, ZDHHC24	+	+
11q13.2	RPC1-11-246B18	66,521,062-66,659,893	RHOD, SYT12, FBXL11	-	+
11q13.2	RPC1-11-380K11	65,802,873-65,980,447	BRMS1, SLC29A2, RIN1, B3GNT1, CD248, YIF1A, TMEM151A, CNIH2, NPAS4, MRPL11	+	-
11q13.2	RPC1-11-62G15	65,980,453-66,140,055	PELI3, ACTN3, CTSF, CCS, CCDC87, BBS1, DPP3, ZDHHC24	-	+

Abbreviation: ZDHHC24, Zinc Finger DHHC-Type Containing 24.

Cytogenetics and FISH

Conventional cytogenetics and FISH studies were performed following standard protocols.

Western blot

Cells collected from bone marrow aspirate smear were lysed in RIPA buffer and incubated on ice for 30 minutes followed by centrifugation for 30 minutes at 12,000 rpm at 4°C. The supernatants were transferred to a new Eppendorf tube and protein concentration measured. For each sample, 20 ug of protein was loaded on SDS-PAGE gel for electrophoresis and transferred onto nylon membrane using standard transfer system. The membranes were blocked in 1% bovine serum albumin (BSA), and then sequentially incubated with primary antibodies (1:500 for Pellino3 and 1:1000 for G3PDH; Santa Cruz Biotechnology, Dallas, TX) and HRP conjugated polyclonal rabbit anti-mouse antibody (1:1000). The bands were visualized using Enhanced chemiluminescence (ECL) reagents (Amersham Pharmacia Biotech, Piscataway, NJ).

Results

Myeloproliferative stage

Bone marrow biopsy revealed hypercellularity (85 to 90%) with myeloid hyperplasia with complete maturation, decreased erythropoiesis, moderate megakaryocytosis including occasional clusters, and moderate eosinophilia (**Figure 1G**). Stainable iron stores were present; no ring sideroblasts were seen. There was mild fibrosis. Flow cytometry of bone marrow aspirate showed normal myeloid maturation patterns with no increased immaturity. Conventional cytogenetics revealed 46,XY,t(11;14)(q13;q32)[19]/46,XY [1]. No BCR-ABL fusion, PDGFRα or PDGFRβ rearrangement was detected by FISH.

AML stage

Bone marrow aspirate smear showed 69% blasts including myeloblasts and immature monocytic cells. Bone marrow biopsy revealed hypercellularity (90%) with extensive infiltration of immature cells. Mature myeloid and erythroid elements were focally present. Megakaryocytes were normal in number with occasional clusters (**Figure 1H**). Cytochemistry revealed >3% myeloblasts and >20% monocytic cells. Flow cytometry analysis showed 27% myeloblasts (positive for CD34, CD13, CD33 (partial), CD7, and CD4 (partial); negative CD15, CD117) and 20% immature monocytic cells (positive for HLA-DR, CD34, CD13, CD33, CD64, CD4, CD11b, and CD56 (partial); negative CD14). Conventional cytogenetics showed 46,XY,t(11;14)(q13;q32),del(10)(p13)[20] (**Figure 1F**).

Mapping the breakpoint by FISH

We initially performed FISH on metaphase nuclei of the AML bone marrow using a *CCND1/IGH* break-apart probe (Abbott Molecular, Des Plaines, IL). Surprisingly, we did not observe the typical signal pattern for *CCND1-IGH* fusion. Instead, two signals for each color were observed, with *CCND1* on der(14) and *IGH* on der (11), suggesting that the breakpoint is centromeric to *CCND1*. After performing sequential metaphase FISH using a series of BAC probes (Empire Genomics, Buffalo, NY) (**Table 1**), we identified one probe that displayed a split signal between der(11) and der(14). Based on the FISH information and human genome map, we focused on a small region containing three genes: *NSP3*, *MTLP11* and *Pellino3*. Two additional BAC probes were identified, which contains either *MTLP11* or *Pellino3*, but do not overlap. FISH showed that *MTLP11* and *Pellino3* probes hybridized to der(11) and der(14), respectively, with no split signals observed. The FISH findings suggest that the breakpoint is located between *Pellino3* and *MTLP11* and *Pellino3* is translocated to der(14), which was confirmed by a *Pellino3* specific probe (**Figure 1A-E**).

To determine if the translocation affects the expression of *Pellino3* and *MTLP11*, we performed Western blot using bone marrow samples from the patient and control. **Figure 1I** shows that there is much higher level of Pellino3 protein in patient sample than in control. However, we did not observe any difference for MTLP11 (data not shown).

Discussion

To the best of our knowledge, t(11;14)(q13;q32) has only been previously reported once in myeloid neoplasms [7]. In that case, FISH analysis revealed no *IGH-CCND1* fusion and the

breakpoint was located on the telomeric side of *CCND1* locus. Unlike that case, the breakpoint in our case is located between *MRPL11* and *Pellino3*, which is on the centromeric side of *CCND1* locus. We further showed that Pellino3 was upregulated as a result of the translocation while MRPL11 was not affected. Since there were no fusion proteins detected by Western blot, it is most likely that *Pellino3* was placed under the control of a promoter or enhancer which drives its constitutive expression.

Dysregulation of TLR/IL1-R signaling has been linked to autoimmunity, hematologic malignancies, and some solid tumors. IRAK1 was recently found to be activated or ovexpressed in myeloid neoplasms [8]. Studies showed that IRAK1 was a target of MIR146a and knockdown of MIR146a resulted in overexpression of IRAK1 and subsequent NF-KB activation [9, 10]. MIR146a deficient mice were normal at birth but started to develop myeloproliferation in spleen and bone marrow at age 8 months, which was secondary to expansion of hematopoietic stem cells and differentiation into myeloid lineage. At a later stage, the mice developed bone marrow failure characterized by hypocellularity and myelofibrosis and transformed into AML [10]. Pellino3 mediates K63-polyubiquitylation of IRAK1 and promotes its stabilization. Modified IRAK1 directly phosphorylates STAT3 at serine 727 and induces IL-10 gene expression [11]. In chronic myeloid leukemia, it was found that BCR-ABL fusion protein greatly induced STAT-S727 phosphorylation and to a lesser extent STAT3-T705 phosphorylation [12]. A recent study showed that STAT-S727 phosphorylation was indispensible for K-RASG12D induced MPN in mice [13]. In addition, the binding of Pellino3 to IRAK1 mediates activation of CREB in a P38-dependent manner and transgenic CREB mice developed MPN. These findings suggest that Pellino3 may play a role in the pathogenesis of MPN through activation of STAT3 and P38 MARK pathway.

It is recently suggested that crosstalk between inflammatory signaling and JAK2 signaling exists. STAT3-S727 phosphorylation was found to enhance the transcriptional activation of STAT3-T705. IL10 is induced by activation of STAT3 and P38 MAPK, which in turn activate JAK2-STAT3 pathway in a feed-forward manner. Therefore, it is reasonable to assume that constitutive activation of Pellino3 and JAK2 synergistically drives development of MPN in our case. However, these signals alone appear to be insufficient to induce AML transformation as del(10)(p13) was identified in the AML clone. Interestingly, RAS suppressor protein 1 (*RSU-1*) gene is located at 10p13, which encodes a protein that inhibits V-RAS mediated transformation and JNK activity [14]. RSU-1 may be responsible for the AML transformation in our case.

In summary, we identify a novel t(11;14)(q13;q32) translocation that links the innate immune response and inflammatory pathway to MPN. Our findings uncover a previously undescribed role of Pellino3 in MPN.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xinmin Zhang, Department of Pathology and Laboratory Medicine, Northwell Health, Hofstra Northwell School of Medicine, 6 Ohio Drive, Suite 202, Lake Success, NY 11042, USA. Tel: 516-304-7242; Fax: 516-224-8586; E-mail: xzhang2@nshs.edu

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