**MYEOV gene overexpression in primary plasma cell leukemia with t(11;14)(q13;q32)**

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**Abstract.** Primary plasma cell leukemia (pPCL) is an uncommon form of plasma cell dyscrasia, and the most aggressive of the human monoclonal gammapathies. The t(11;14)(q13;q32) rearrangement is the most common alteration in pPCL, promoting *cyclin D1 (CCND1)* gene overexpression caused by its juxtaposition with the *immunoglobulin heavy locus* chromosome region. The *myeloma overexpressed (MYEOV)* gene maps very close to the *CCND1* gene on chromosome 11, but its overexpression is rarely observed in multiple myeloma. The present study describes a case of pPCL with t(11;14) characterized by a breakpoint on der(11), unlike the one usually observed. Droplet digital polymerase chain reaction analysis revealed overexpression of *CCND1* and *MYEOV*. To the best of our knowledge, *MYEOV* gene overexpression has never been previously described in pPCL.

**Introduction**

Primary plasma cell leukemia (pPCL) is an uncommon form of plasma cell (PC) dyscrasia, and the most aggressive human monoclonal gammapathies (1,2). pPCL is characterized by the presence of $>20\%$ circulating PCs in peripheral blood and/or an absolute circulating PC count exceeding 2x$10^9$ cells/l (1). Peripheral blood flow cytometry is an important tool to demonstrate the presence of PCs and to confirm their clonality, as well as to exclude other lymphoproliferative disorders such as low-grade B-cell and lymphoplasmacytic lymphoma (2). In regard to cytogenetic findings, the t(11;14)(q13;q32) rearrangement is the most common alteration in pPCL (3), promoting *cyclin D1 (CCND1)* gene overexpression due to its juxtaposition with the *immunoglobulin heavy locus* (IGH) chromosome region. The subsequent deregulation of *CCND1* is considered to perturb the G1-S phase transition of the cell cycle and, therefore, to contribute to tumor development (4). However, the *IGH/CCND1* rearrangement alone may be insufficient to cause hematologic malignancies, and may require other additional genetic aberrations to boost its oncogenic activity (4). In the present study, a case of pPCL with t(11;14) characterized by the overexpression of *CCND1* and the *myeloma overexpressed (MYEOV)* gene, which maps very close *CCND1* on chromosome 11, is described.

**Materials and methods**

**Clinical history.** In July 2014, a previously healthy 65-year-old male was admitted to the Department of Emergency and Organ Transplantation, Hematology Section, University of Bari (Bari, Italy) for anemia, thrombocytopenia and mild leukocytosis [hemoglobin levels, 11.0 g/dl (normal range, 13.0-16.0 g/dl); platelets, 49x$10^9$ cells/l (normal range, 150-450x$10^9$ cells/l); and leukocytes, 13x$10^9$ cells/l (normal range, 4-10x$10^9$ cells/l)]. Peripheral blood smear analysis demonstrated the presence of $\sim40\%$ apparently undifferentiated cells, a number of which had a large eccentric nucleus and scattered chromatin, while others had a scanty and intensely basophilic cytoplasm with protrusions (Fig. 1A-C). Immunophenotypic analysis of bone marrow (BM) demonstrated the specimen to be cluster of differentia tion (CD)38+, CD138+, CD20-, CD23-, CD56+, CD9-, CD117, human leukocyte antigen-antigen D related- and cytoplasmic immunoglobulin (CyIg)x+. Primary antibodies used were as follows: CD38+ (catalog no. 340926), CD138+ (catalog no. 341097), CD20- (catalog no. 340954), CD23- (catalog no. 341008), CD56+ (catalog no. 340724), CD9- (catalog no. 341639), CD117 (catalog no. 340867), HLA-DR- (catalog no. 335813), Cylgx+ (catalog no. 643774) (BD Biosciences, Franklin Lakes, NJ, USA). BM aspirate and biopsy revealed the presence of $\sim80\%$ immature plasma cells and plasmablasts, the majority with considerable atypia (Fig. 1D). Serum protein electrophoresis identified a monoclonal protein in the gamma region, with a concentration of 0.55 g/dl. This was classified on immunofixation electrophoresis as an intact monoclonal immunoglobulin AK. Total body computed tomography did...
not reveal the presence of swollen nodes or lytic bone lesions. Viral serological tests specific for human immunodeficiency virus, hepatitis B and C viruses, and human herpes viruses 6 and 8 resulted negative. Conventional cytogenetic analysis identified the following karyotype: 56, XY, +Y, +del(1p), +2, +3, +7, +8, +9, t(11;14)(q13;q32), +der(14) (t(11;14)(q13;q32), +18, +22) [20]. Molecular analysis revealed the presence of the B-Raf V600E gene mutation. According to these data, a diagnosis of pPCL was made. The patient refused to start chemotherapy treatment and succumbed to sepsis 3 months later.

The study was approved by the Ethics Committee of the Azienda Ospedaliero-Universitaria Consorziali Policlinico di Bari (Bari, Italy) and written informed consent was obtained from the patient.

Cytogenetic analysis. Karyotyping was performed at diagnosis on BM cells according to standard methods (5,6). BM cells were cultured for 24-48 h, and chromosomes were G-banded with trypsin-Giemsa staining, according to the recommendations of the International System for Human Cytogenetic Nomenclature (5). At least 20 metaphases were analyzed.

Fluorescence in situ hybridization (FISH) analysis. FISH analyses were performed on BM samples using bacterial artificial chromosomes (BACs) and fosmid clones (Children's Hospital Oakland Research Institute, Oakland, CA, USA), according to the University of California (Santa Cruz, CA, USA) database (http://genome.ucsc.edu/; February 2009 release). Chromosome preparations were hybridized in situ with probes labeled by nick translation (7).

Molecular analyses. Total RNA was extracted from BM cells using the RNeasy Mini kit (Qiagen, Inc., Valencia, CA, USA). The RNA concentration was assessed using a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). A total of 1 µg RNA was reverse transcribed into complementary (c)DNA using the Quantitect Reverse Transcription kit (Qiagen, Inc.). Gene expression analysis was conducted by droplet digital polymerase chain reaction (ddPCR) using the QX200 droplet generator (Bio-Rad Laboratories, Hercules, CA, USA). The principle of ddPCR technology is to combine water-oil emulsion droplet technology with microfluidics and to quantify the absolute target number present in a sample, thus implementing PCR data with Poisson statistics to quantify the absolute target number present in a sample, thus revealing the presence of swollen nodes or lytic bone lesions.

The target concentration in each sample was achieved by the QX200 droplet generator (Bio-Rad Laboratories, Hercules, CA, USA). Gene expression analysis was conducted by ddPCR using the QX200 droplet generator (Bio-Rad Laboratories, Inc.). The generated droplets were transferred to a 96-well PCR plate (Eppendorf, Hamburg, Germany). The plate was sealed with a pierceable foil heat seal (Bio-Rad Laboratories, Inc.), and the samples were amplified on the T100 thermal cycler (Bio-Rad Laboratories, Inc.). The thermal cycling conditions were 95°C for 5 min (1 cycle), 95°C for 30 sec (ramp rate, 2°C/sec; 40 cycles), 60°C for 1 min (ramp rate, 2°C/sec; 40 cycles), 4°C for 5 min (1 cycle), 90°C for 10 min (1 cycle) and 4°C (hold). After amplification, the 96-well PCR plate was loaded onto the QX200 droplet reader (Bio-Rad Laboratories, Inc.), which counts the fluorescence-positive and -negative droplets to define the target concentration using QuantaSoft analysis software version 1.7.4 (Bio-Rad Laboratories, Inc.). The target concentration in each sample was expressed as number of copies/ng. Patient's gene expression was compared with that of a healthy BM control (Human Bone Marrow Total RNA; Clontech Laboratories, Inc., Mountainview, CA, USA) and with the myeloma cell lines RPMI-8266 and U266 [American Type Culture Collection, Manassas, VA, USA; cultured at 1x10⁶ cells/ml in RPMI-1640 medium supplemented with 10% fetal bovine serum (Euroclone Spa, Pero, Italy), 100 U/ml penicillin, and 100 µg/ml streptomycin at 37°C and 5% CO₂]. Molecular analysis of B-Raf V600E was performed as previously reported (8).

Results

To confirm the presence of the t(11;14) rearrangement observed in the cytogenetic analysis, FISH experiments were performed using fosmids and BACs. For chromosome 14q32, the BAC clone RP11-1145H5 and the fosmid G248P83912C7, specific for the IGH variable (IGHV) region, and the BACs RP11-815O20 and RP11-676G2, specific for the IGH constant (IGHC) region, were employed. To study the breakpoint on chromosome 11, the RP11-156B3 and RP11-378E8 clones were selected; the former was specific for CCND1, and the latter mapped upstream, in the region where the breakpoint is usually localized. The FISH pattern revealed the breakpoint on normal chromosome 11 and on the derivative chromosome 14, respectively. Unexpectedly, the RP11-378E8 signal was not split, but produced signals on the two derivative chromosomes in addition to the normal chromosome 11 (Fig. 2A). In order to perform a precise characterization of the breakpoint mapping on chromosome 11, further FISH experiments using fosmids G248P8216G1, G248P87301E9 and G248P87014D2, specific for the MYEOV gene and which map upstream to RP11-378E8, demonstrated that they were retained on der(11) (Fig. 2B). By using these results, the breakpoint on der(11) was assumed to be within the RP11-378E8 and G248P87301E9 regions. The CCND1 and MYEOV genes were then translocated on der(14) and retained on der(11), respectively. The breakpoint on the 14q32 locus was identified in the IGHV region, since all the probes specific for the 14q32 locus induced a signal on the normal and on the derivative chromosome 14, respectively. Based on the evidence of a difference from the recurrent breakpoint at the basis of the t(11;14)(q13;q32) rearrangement in the present patient, CCND1 and MYEOV gene expression was investigated by ddPCR. The analysis revealed that, as expected, the CCND1 gene was overexpressed in the patient sample (670 copies/ng) and in the two myeloma cell lines tested (U266, 294 copies/ng; RPMI-8266, 431 copies/ng).
Figure 1. Wright-Giemsa staining of peripheral blood and BM smears. (A-C) Peripheral blood smear revealed numerous (A) small and (B and C) medium-sized bizarre-looking cells, with pleomorphic nuclear shapes, basophilic cytoplasm and a high nuclear-cytoplasmic ratio, which were identified as plasma cells by immunophenotyping. (D) BM analysis revealed numerous plasmablasts characterized by a high nuclear-cytoplasmic ratio, finely dispersed chromatin and a prominent nucleolus. Magnification, x100. BM, bone marrow.

Figure 2. FISH experiments. FISH co-hybridization experiments with (A) the bacterial artificial chromosomes clones RP11-378E8 (red), RP11-156B3 (green) and RP11-1145H5 (yellow), and (B) with the fosmid clones G248P87014D2 (green), G248P87014D2 (green), mapping upstream to RP11-378K8, demonstrated that the cyclin D1 and MYEOV genes were translocated on der(14) and retained on der(11), respectively. Arrows indicate chromosomes involved in the rearrangement. (C) Expression analysis by droplet digital polymerase chain reaction revealed that, in the present primary plasma cell leukemia patient, the MYEOV gene was expressed ~70-fold more (54.0 copies/ng) than in normal bone marrow samples (0.8 copies/ng) and myeloma cell lines (0.0 copies/ng). CCND1, cyclin D1; MYEOV, myeloma overexpressed; IGHV, immunoglobulin heavy locus variable; pPCL, primary plasma cell leukemia; NBM, normal bone marrow; FISH, fluorescence in situ hybridization.
compared with normal BM (47 copies/ng). In addition, the MYEOV gene was expressed ~70-fold more (54.0 copies/ng) in the patient than in the normal BM control (0.8 copies/ng) and in the myeloma cell lines evaluated, where no MYEOV gene expression was detected (Fig. 2C).

Discussion

The present study reports a pPCL case characterized by a t(11;14) chromosomal rearrangement associated with overexpression of MYEOV, a gene mapped in close proximity to the CCND1 locus (9). In fact, MYEOV is located 390 kb centromeric of CCND1, and its activation [which is concurrent to that of CCND1 through juxtaposition of MYEOV to either the 5′ intronic Em gene located in the intron between the IGH joining and switch sequences, or the 3′ regulatory region (RR) IGH enhancers located downstream of the constant region genes] was first described in a subset of multiple myeloma (MM) cell lines with t(11;14) (9). Since then, MYEOV overexpression has rarely been reported in MM patients with t(11;14) (10). Since the enhancers are joined to both CCND1 and MYEOV with a breakpoint in switch sites, and MYEOV expression is lost in the majority of these cases, the authors concluded that MM does not favor MYEOV expression (10). Furthermore, gene expression profile experiments revealed that the MYEOV gene was expressed in malignant PCs in 79% of newly diagnosed patients with MM, and that MYEOV is a prognostic factor, partly through a role of MYEOV in the control of neoplastic cell proliferation (11). The MYEOV gene was reported to be co-amplified and co-overexpressed with CCND1 in a subset of esophageal squamous cell carcinomas, breast cancers, gastric cancers, neuroblastomas and colorectal cancers (12,13). The oncogenic role of MYEOV has also been investigated in functional studies, where in vitro small interfering RNA-mediated knockdown of MYEOV resulted in an inhibition of the proliferation, invasion and migration of colorectal cancer cell lines (13). To the best of our knowledge, MYEOV gene overexpression has never been described in pPCL. In MM, IGH translocations were shown to be definite, non-random chromosomal fusions of IGHC with the loci of the fibroblast growth factor receptor 3 (4p16.3), CCND1 (11q13.3), CCND3 (6p21.1), v-Maf avian musculosarcoma fibrosarcoma oncogene homolog (MAF) (16q23.2) and MAFB (20q12) genes, and of IGH with the locus of the multiple myeloma SET domain (4p16.3) gene (14). On the contrary, in the present pPCL case, the breakpoint on the 14q32 region was in the IGHV region, resulting in the juxtaposition of the MYEOV and CCND1 genes near to Em, which could promote their transcription. Therefore, in the present pPCL case, it was the MYEOV proximity to Em that could favor its expression rather than the RR enhancer. On this basis, as this rearrangement occurred with a high frequency, cytogenetic molecular characterization of the t(11;14) rearrangement in pPCL cases could highlight the role and the mechanism regulating MYEOV overexpression. The current pPCL case was also characterized by the presence of the B-Raf V600E gene mutation. B-Raf mutations were observed in 4% of MM cases (15) and were found to be significantly associated with relapsed myeloma, extensive extramedullary disease and a decreased overall survival (16). Molecular analyses of 15 pPCL cases identified the presence of B-Raf V600E gene mutation in 1 case (6.7%), together with t(14;16) (3). Notably, an MM patient was described to exhibit the B-Raf V600E gene mutation together with a plasmablastic differentiation during the relapse (17). The current case presented a marked plasmablastic differentiation, a phenotype difficult to link with the molecular characteristics reported above. There are few reports in the literature describing the morphological features of circulating PCs in pPCL (2,18). However, certain cases were described with an irregular nuclear contour or containing multiple immature cells with a high nuclear-cytoplasmic ratio, finely dispersed chromatin, prominent nucleolus and limited or absent Golgi zone, which corresponded to blasts or plasmablasts (18). In patients with sepsis, viral infections, autoimmune conditions and less common peripheral T-cell lymphomas such as angioimmunoblastic T-cell lymphoma, polyclonal plasmacytosis in the peripheral blood may mimic pPCL, particularly when PCs display atypical features (19). Furthermore, in MM, the plasmablastic morphology is highly associated with adverse clinical risk features and a high proliferation rate, but not with prognostically adverse IGH rearrangements (20).

In conclusion, the present study reports the first pPCL case with t(11;14) in which the breakpoint located in the IGHV region was associated with a concomitant MYEOV and CCND1 gene overexpression. The pathogenic role of the MYEOV gene in pPCL remains to be elucidated.

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References


