

Case Report

Detection of t(12;14)(p13;q32) in a patient with IGH-CCND1 negative mantle cell lymphoma resembling ultra-high risk chronic lymphocytic leukemia

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Abstract: T(12;14)(p13;q32) is a rare recurrent chromosomal translocation, which has only been identified in a small subgroup of mantle cell lymphoma (MCL) without typical t(11;14)(q13;q32). This rearrangement causes aberrant over-expression of cyclin D2 (CCND2), which disrupts the normal cell cycle. Here we report a subtle case of MCL with t(12;14)(p13;q32) that was initially misdiagnosed as ultra-high risk chronic lymphocytic leukemia (CLL). A 60-year-old male patient presented with obvious leukocytosis and progressive weakness. Morphology of peripheral blood and immunophenotyping by flow cytometry pointed to a diagnosis of chronic lymphocytic leukemia. Fluorescence *in situ* hybridization (FISH) using IGH-CCND1 probe was negative for CCND1 abnormality, but demonstrated IGH breakapart signals. The initial diagnosis of CLL was established and the patient was treated with six courses of immunochemotherapy with fludarabine, cyclophosphamide and rituximab (FCR). Complete remission (CR) was achieved at the end of treatment, but disease relapsed quickly. The patient was transferred to our hospital, flow cytometry using additional markers showed that the clonal cells were CD200+(dim), CD148+(strong), and chromosome analysis revealed a complex karyotype, 47, XY, t(12;14)(p13;q32), +12, del(9p21), which indicated over-expression of CCND2, and immunostaining showed strong positivity of SOX11 further confirming the characteristics of CCND1-negative MCL. The final diagnosis was revised to rare subtype of MCL with CCND2 translocation and intensive regimens were employed. This confusable MCL case illustrates the importance of cytogenetic analysis and clinicopathologic diagnosis of this rare category of MCL.

Keywords: t(12;14), mantle cell lymphoma, cyclin D2, CD148, SOX11

Introduction

Mantle cell lymphoma (MCL) is an aggressive lymphoma characterized by the presence of t(11;14)(q13;q32) translocation resulting in IGH-CCND1 fusion gene. More than 90% of MCL cases carry the characteristic IGH-CCND1 fusion gene, which results in over-expression of cyclin D1 (CCND1) [1, 2]. In recent years, increasing evidences point to the existence of a particular subgroup of MCL without typical t(11;14) and nuclear CCND1 expression [2-7]. In those CCND1-negative MCL, cyclin D2 (CCND2) over-expression is the most frequent genetic event, and more than half of CCND1-negative MCL harbor CCND2 translocation, which predominantly fuses with immunoglobulin light chain genes [3, 6]. Here we report a

case of atypical MCL with a complex karyotype including t(12;14)(p13;q32), trisomy 12, as well as 9p21 deletion that was initially diagnosed as ultra-high risk chronic lymphocytic leukemia (CLL).

Case presentation

A 60-year-old male was admitted to the local hospital because of palpation and progressive weakness. Laboratory parameters included leukocytes $378 \times 10^9/L$, hemoglobin 56 g/L, platelet $107 \times 10^9/L$, and reticulocytes 0.5%. The bone marrow aspirate showed extensive marrow replacement by small mature lymphocytes with 8% prolymphocytes (**Figure 1A, 1B**). The immunophenotyping by flow cytometry revealed that the tumor cells were CD19+,

Mantle cell lymphoma with t(12;14)

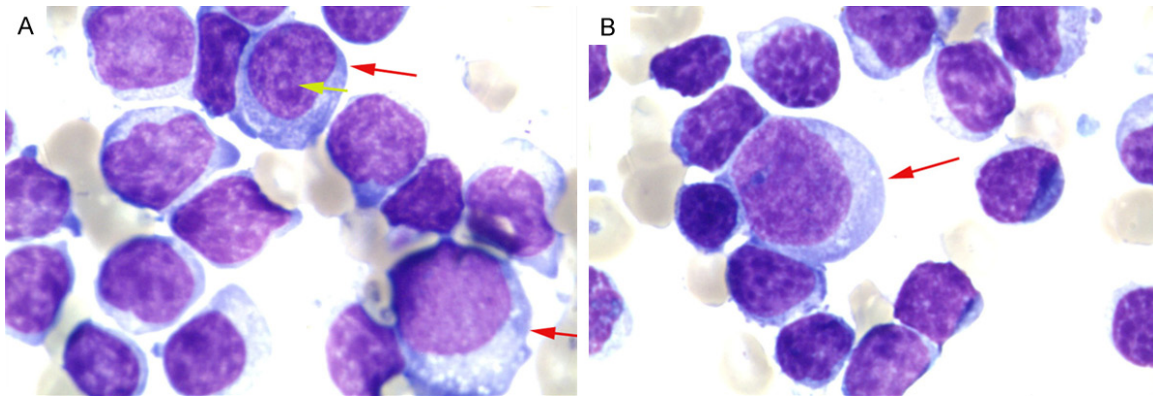


Figure 1. Bone marrow smear at presentation (A, B). The polymphocytes (red arrows) were large, about twice the size of surrounding small lymphocytes, and a vesicular nucleus (yellow arrow) could be seen (A, B: Giemsa, $\times 1000$).

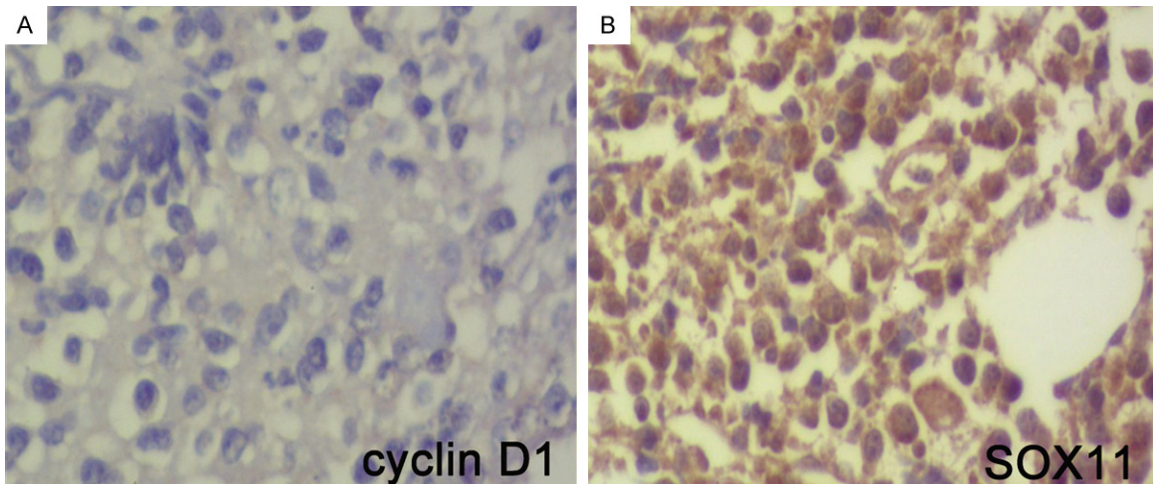


Figure 2. Bone marrow biopsy (A) the specimen was negative for CCND1 staining (B) immunohistochemistry using monoclonal SOX11 antibody showed bright nuclear staining.

CD23+ (dim), CD5+, FMC7-, CD22+ (dim), CD20+ (strong), sIg λ + (moderate). The analysis of karyotype failed as there were no metaphases detected. CCND1 immunohistochemistry (**Figure 2A**) and fluorescence in situ hybridization (FISH) using dual fusion probes for IGH-CCND1 were performed, and both the results were negative. However, IGH gene break apart signals were detected. No IGHV somatic mutations were detected using polymerase chain reaction. Based on the above laboratory findings, an initial diagnosis of CLL was made.

Complete remission (CR) was reached after six cycles of fludarabine, cyclophosphamide and rituximab (FCR) regimen. However, his disease soon relapsed six months later. Because of

rapid progression of disease treating with combined immunochemotherapy, ultra-high risk CLL was diagnosed. Therefore, the patient was referred to our hospital and re-evaluation was initiated. Results of flow cytometry were consistent with the previous one, and immunophenotyping using additional markers revealed that the tumor cells were CD200+ (dim), CD148+ (strong), which was not typical of CLL (unpublished observation). FISH analysis utilizing IGH-CCND1 probes were identical to the previous one, and split signal IGH FISH confirmed the existence of IGH gene translocation (**Figure 3A, 3B**). And no deletions of p53, ATM, 13q14 or 6q23 were detected by the corresponding FISH probes. Moreover, no p53 mutation was detected by Sanger sequencing. Further analysis of

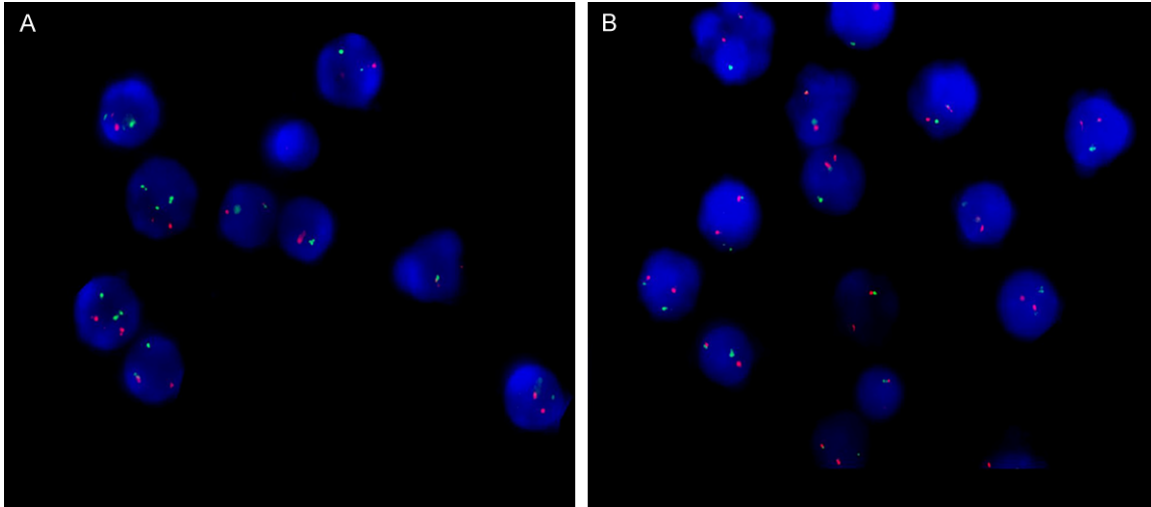


Figure 3. FISH analysis detecting translocation using IGH-CCND1 (A) and IGH (B) probes in our hospital. (A) two red signals indicated two wild-type CCND1 and three green signals indicated a translocated IGH (B) one red/green fusion signal pattern indicated a wild-type IGH, while separation of one red/green signal pair confirmed the break apart rearrangement of IGH gene.

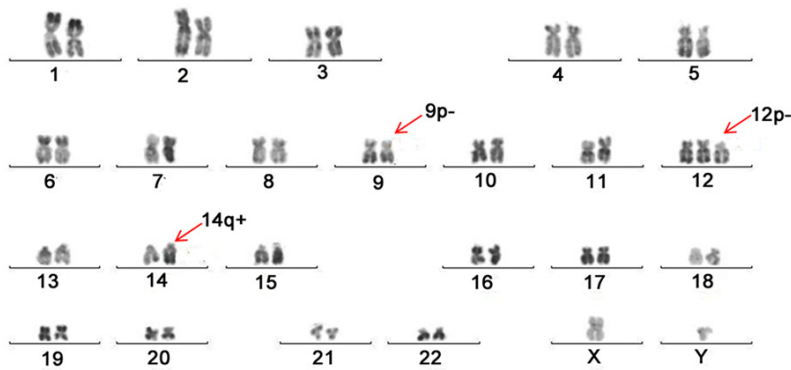


Figure 4. karyotype analysis of bone marrow in our hospital: complex karyotype including t(12;14)(p13;q32), +12 and del(9p21), red arrows indicated the abnormal chromosomes.

chromosome using CpG oligonucleotide revealed a complex karyotype: 47, XY, t(12;14)(p13;q32), +12, del(p21), which suggested over-expression of CCND2 (Figure 4). And this cytogenetic aberration was detected in 3 of 6 metaphases analyzed. Immunostaining utilizing monoclonal SOX11 antibody (clone number: CL0142) showed strong SOX11 expression (Figure 2B). The expression of ki67 was investigated immunohistochemically in the neoplastic cells, which revealed a Ki67 labeling of 20%. Characteristic t(12;14)(p13;q32) and strong expression of SOX11, as well as aggressive clinical course were highly suggestive of a diagnosis of MCL. A diagnosis of CCND1-negative MCL was established. Then the patient received

four courses of modified hyper-fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (hyper-CVAD) regimen and subsequent two courses of rituximab. Despite active therapy, the disease progressed rapidly, and the patient died of severe sepsis two months later.

Discussion

Distinguishing MCL from other indolent B cell lymphomas including CLL is essential due to different therapeutic strategies and clinical outcomes. Clinically, FISH analysis detecting IGH-CCND1 rearrangement and/or immunostaining for nuclear CCND1 are routinely used to differentiate MCL from other B cell lymphomas and confirm the diagnosis of MCL [8]. Recently, emerging cases of lymphomas similar to conventional MCL both morphologically and phenotypically but without CCND1 expression and t(11;14) have been identified. Over-expression of CCND2 or cyclin D3 was reported in this subset of lymphomas, and gene expression profiling study showed that these cases had a gene expression signature typical of MCL [4]. CCND2 rearrangements are more frequently detected in CCND1-negative MCL, in

which CCND2 break with IGH@, IGK@ or IGL@, resulting in aberrant CCND2 expression [3].

To identify these CCND1-negative MCL, SOX11, a neural transcription factor that is involved in central nervous system development has recently been proposed as a novel differential diagnostic marker [9]. SOX11 is expressed in virtually all aggressive MCL and to a lesser extent in some cases of Burkitt lymphoma and acute lymphoblastic leukemia (ALL) but not in other mature B cell neoplasms including CLL [10, 11]. Immunostaining for SOX11 nuclear expression using monoclonal antibody targeting its C-terminal peptide can identify MCL in absence of t(11;14)(q13;q32) and CCND1 expression [12].

T(12;14)(p13;q32) with IGH-CCND2 fusion was firstly described in a case of MCL [2]. MCL with IGH-CCND2 juxtaposition is rare. Until now, only 3 cases were reported [2, 3]. In this unique case of MCL, in addition to t(12;14)(p13;q13), other cytogenetic abnormalities including trisomy 12 and 9p21 deletion were also confirmed. Both +12 and del(9p21) were previously reported in MCL, and +12 is a cytogenetic abnormality that is frequently detected in CLL [13, 14]. However, according to a recent study, del(9p21) is not observed in CLL cases suggesting that del(9p21) might be helpful in differential diagnosis between MCL and CLL [15]. Deletion of 9p21 frequently occurred in both t(11,14) positive and negative MCL, and this region contains two tumor suppressor genes CDKN2A/B, which play a pivotal role in the regulation of cell cycle [14, 16]. Although the mechanism triggering 9p21 deletion has been investigated in ALL, it remains to be determined in MCL, so further studies are needed to elucidate the underlying mechanism [3, 17]. In this case, the immunophenotype was atypical for MCL, but dim CD200 expression and relative strong CD148 expression were suggestive of a diagnosis of MCL (unpublished observation). Further, characteristic t(12;14)(p13;q13), 9p21 deletion, strong SOX11 nuclear expression as well as aggressive clinical course led us to make the final diagnosis of MCL.

Like most CCND1-positive MCL, MCL with CCND2 rearrangement usually has an aggressive clinical course, so intensive therapy is warranted [3]. This case highlights the existence of CCND2 positive MCL. Much more attention

should be paid to this special group for both better understanding of molecular pathogenesis of MCL and appropriate therapy [10]. Moreover, for cases with atypical CLL immunophenotype or increasing number of prolymphocytes but negative for t(11;14)(q13;q32) and CCND1 expression, the diagnosis of CLL should be made with precaution and any possibility of MCL should be excluded, and in these situations comprehensive cytogenetic analysis and immunostaining for SOX11 nuclear expression are strongly recommended.

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Disclosure of conflict of interest

None.

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References

- [1] Rosenberg CL, Wong E, Petty EM, Bale AE, Tsujimoto Y, Harris NL, Arnold A. PRAD1, a candidate BCL1 oncogene: mapping and expression in centrocytic lymphoma. *Proc Natl Acad Sci U S A* 1991; 88: 9638-9642.
- [2] Herens C, Lambert F, Quintanilla-Martinez L, Bisig B, Deusings C, de Leval L. CyclinD1-negative mantle cell lymphoma with cryptic

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- t(12;14)(p13;q32) and cyclin D2 overexpression. *Blood* 2008; 111: 1745-1746.
- [3] Salaverria I, Royo C, Carvajal-Cuenca A, Clot G, Navarro A, Valera A, Song JY, Woroniecka R, Rymkiewicz G, Klapper W, Hartmann EM, Sujobert P, Wlodarska I, Ferry JA, Gaulard P, Ott G, Rosenwald A, Lopez-Guillermo A, Quintanilla-Martinez L, Harris NL, Jaffe ES, Siebert R, Campo E, Beà S. CCND2 rearrangements are the most frequent genetic events in cyclin D1 (-) mantle cell lymphoma. *Blood* 2013; 121: 1394-1402.
- [4] Wlodarska I, Dierickx D, Vanhentenrijk V, Van Roosbroeck K, Pospisilova H, Minnei F, Verhoef G, Thomas J, Vandenberghe P, De Wolf-Peeters C. Translocations targeting CCND2, CCND3, and MYCN do occur in t(11;14)-negative mantle cell lymphomas. *Blood* 2008; 111: 5683-5690.
- [5] Fu K, Weisenburger DD, Greiner TC, Dave S, Wright G, Rosenwald A, Chiorazzi M, Iqbal J, Gesk S, Siebert R, De Jong D, Jaffe ES, Wilson WH, Delabie J, Ott G, Dave BJ, Sanger WG, Smith LM, Rimsza L, Braziel RM, Müller-Hermelink HK, Campo E, Gascoyne RD, Staudt LM, Chan WC; Lymphoma/Leukemia Molecular Profiling Project: Cyclin D1-negative mantle cell lymphoma. a clinicopathologic study based on gene expression profiling. *Blood* 2005; 106: 4315-4321.
- [6] Shiller SM, Zieske A, Holmes H 3rd, Feldman AL, Law ME, Saad R. CD5-positive, cyclin D1-negative mantle cell lymphoma with a translocation involving the CCND2 gene and the IGL locus. *Cancer Genet* 2011; 204: 162-164.
- [7] Gesk S, Klapper W, Martin-Subero JI, Nagel I, Harder L, Fu K, Bernd HW, Weisenburger DD, Parwaresch R, Siebert R. A chromosomal translocation in cyclin D1-negative/cyclinD2-positive mantle cell lymphoma fuses the CCND2 gene to the IGK locus. *Blood* 2006; 108: 1109-1110.
- [8] Li JY, Gaillard F, Moreau A, Harousseau JL, Labosse C, Milpied N, Bataille R, Avet-Loiseau H. Detection of translocation t(11;14)(q13;q32) in mantle cell lymphoma by fluorescence in situ hybridization. *Am J Pathol* 1999; 154: 1449-1452.
- [9] Seto M. Cyclin D1-negative mantle cell lymphoma. *Blood* 2013; 121: 1249-1250.
- [10] Xu W, Li JY. SOX11 expression in mantle cell lymphoma. *Leuk Lymphoma* 2010; 51: 1962-1967.
- [11] Lu TX, Li JY, Xu W. The role of SOX11 in mantle cell lymphoma. *Leuk Res* 2013; 37: 1412-1419.
- [12] Mozos A, Royo C, Hartmann E, De Jong D, Baró C, Valera A, Fu K, Weisenburger DD, Delabie J, Chuang SS, Jaffe ES, Ruiz-Marcellan C, Dave S, Rimsza L, Braziel R, Gascoyne RD, Solé F, López-Guillermo A, Colomer D, Staudt LM, Rosenwald A, Ott G, Jares P, Campo E. SOX11 expression is highly specific for mantle cell lymphoma and identifies the cyclin D1-negative subtype. *Haematologica* 2009; 94: 1555-1562.
- [13] Matutes E, Carrara P, Coignet L, Brito-Babapulle V, Villamor N, Wotherspoon A, Catovsky D. FISH analysis for BCL-1 rearrangements and trisomy 12 helps the diagnosis of atypical B cell leukaemias. *Leukemia* 1999; 13: 1721-1726.
- [14] Sander S, Bullinger L, Leupolt E, Benner A, Kienle D, Katzenberger T, Kalla J, Ott G, Müller-Hermelink HK, Barth TF, Möller P, Lichter P, Döhner H, Stilgenbauer S. Genomic aberrations in mantle cell lymphoma detected by interphase fluorescence in situ hybridization. Incidence and clinicopathological correlations. *Haematologica* 2008; 93: 680-687.
- [15] Edelman J, Holzmann K, Miller F, Winkler D, Bühler A, Zenz T, Bullinger L, Kühn MW, Gerhardinger A, Bloehdorn J, Radtke I, Su X, Ma J, Pounds S, Hallek M, Lichter P, Korbel J, Busch R, Mertens D, Downing JR, Stilgenbauer S, Döhner H. High-resolution genomic profiling of chronic lymphocytic leukemia reveals new recurrent genomic alterations. *Blood* 2012; 120: 4783-4794.
- [16] Iacobucci I, Ferrari A, Lonetti A, Papayannidis C, Paoloni F, Trino S, Storlazzi CT, Ottaviani E, Cattina F, Impera L, Abbenante MC, Vignetti M, Vitale A, Potenza L, Paolini S, Soverini S, Pane F, Luppi M, Foà R, Baccarani M, Martinelli G. CDKN2A/B alterations impair prognosis in adult BCR-ABL1-positive acute lymphoblastic leukemia patients. *Clin Cancer Res* 2011; 17: 7413-7423.
- [17] Novara F, Beri S, Bernardo ME, Bellazzi R, Malovini A, Ciccone R, Cometa AM, Locatelli F, Giorda R, Zuffardi O. Different molecular mechanisms causing 9p21 deletions in acute lymphoblastic leukemia of childhood. *Hum Genet* 2009; 126: 511-520.