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Short communication

Inversions of chromosomes 2 and 6 in mantle cell lymphoma. Cytogenetic, FISH, and molecular studies

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Abstract Inversions are infrequent events in hematological malignancies. We here report the cytogenetic, fluorescence in situ hybridization (FISH), and molecular studies of 2 patients diagnosed with mantle cell lymphoma (MCL) that showed inversions of chromosomes 2 and 6 as part of complex karyotypes. Both patients showed a cytogenetically identical inv(6)(p23q11) detected as a secondary aberration. In addition, both patients had a derivative chromosome 2 which originated by partial deletion of the short arm and a pericentric inversion with different breakpoints on the long arm: der(2)del(2)(p21)inv(2)(p21q11) and der(2)del(2)(p21)inv(2)(p21q13), respectively. The presence of t(11;14)(q13;q32) was confirmed by interphase FISH and by molecular study. Residual normal cells were found in both cases. The patients showed a different clinical evolution with a poor outcome for one case and a favorable course of the disease for the other one. The review of the literature in MCL showed a total of 9 inversions affecting different chromosomes. Considering that inversions are very infrequent events in MCL, our findings could be important for detecting genes potentially involved in development and/or progression of this aggressive non-Hodgkin lymphoma subtype.

1. Introduction

Mantle cell lymphoma (MCL) is a malignant non-Hodgkin lymphoma (NHL) that accounts for approximately 6% of all lymphomas. It is characterized by a male predominance, frequent advanced clinical stage at diagnosis, and poor prognosis with a median survival range of 3–4 years [1]. MCL is associated with the t(11;14)(q13;q32), which is considered the primary genetic event. This translocation juxtaposes the immunoglobulin heavy chain gene (*IGH*)(14q32) with the *BCL1* locus on 11q13, leading to the overexpression of *cyclin-D1* gene. Experimental studies have shown that *cyclin-D*1 activation by itself may not be sufficient to induce the malignant transformation of affected cells and other genomic changes are required to reach the neoplastic phenotype.

Inversions are infrequent events in hematological malignancies, accounting for 0.5% of the chromosome aberrations listed in the Mitelman's catalog [2]. Among them, only 4 inversions are characteristic of a specific malignant disorder: inv(3)(q21q26) in acute myeloid leukemia with abnormal megakaryopoiesis and thrombocytosis [3], inv(16)(p13q22) in acute myelomonocytic leukemia with eosinophilia ($M4_{Eo}$) [4], inv(14)(q11q32) in T-cell lymphoprolipherative disorders [5], and inv(2)(p23q35) in ALK-positive anaplastic large cell lymphomas [6]. We here report the cytogenetic, fluorescence in situ hybridization (FISH), and molecular

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Table 1
Clinical features at diagnosis in two mantle cell lymphoma patients

Case	Age/Sex	Hb (g/dL)	WBC (10^9/L)	Plateles (10^9/L)	LDH (IU/L)	Immunophenotype
1	35/M	13	12600	136000	435	CD19+, CD20+, CD22+, FMC7+, $CD79_b$+, CD5+, CD23-, CD10-, kappa light chain restriction
2	72/F	10.7	29100	154000	303	CD19+, CD20+, FMC7+, $CD79_b$, CD5+, CD23-, kappa light chain restriction

Abbreviations: Hb, hemoglobin; WBC, white blood count; LDH, lactic dehydrogenase.

studies of 2 patients diagnosed with MCL that showed inversions of chromosomes 2 and 6 as part of complex karyotypes. A review of the literature for the presence of inversions in MCL was performed.

2. Case reports

The diagnosis of MCL was made according standard international criteria. Clinical characteristics and immunophenotype at diagnosis are shown in Table 1. In both patients, the cyclin D1 immunostain showed nuclear positivity.

2.1. Case 1

A 35-year old man with stage IVB MCL showed axillary and inguinal adenopathies, hepato-splenomegaly, and bone marrow (BM) involvement. The patient started with CHOP

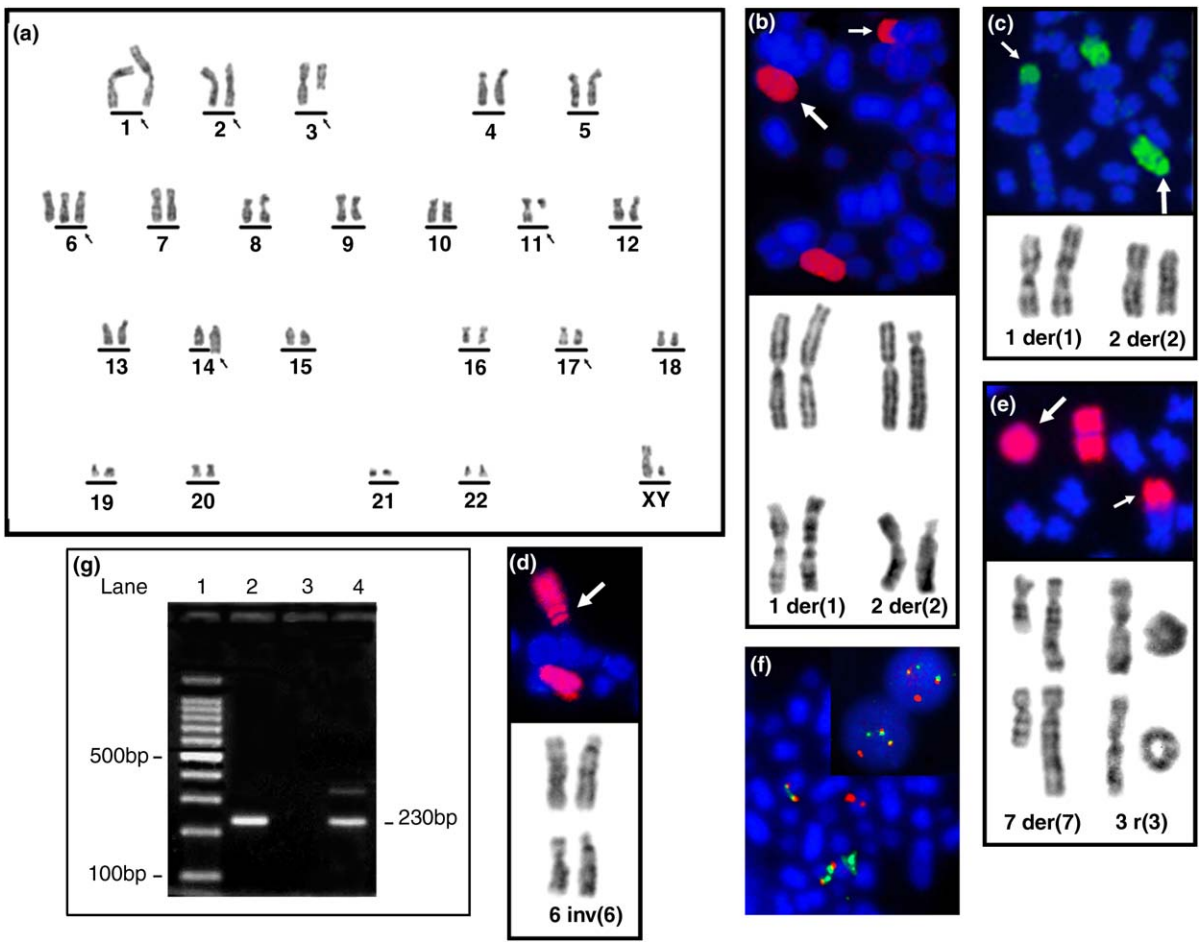

Fig. 1. *(a)* Karyotype of case 1 showing der(1)t(1;2)(p36;p21), der(2)del(2)(p21)inv(2)(p21q11), del(3)(q11), inv(6)(p23q11), t(11;14)(q13;q32), and del(17)(p11)(arrows). *(b,c)* Partial metaphases hybridized with chromosome 2 painting probes from case 1 (green) and case 2 (red), showing the normal chromosomes 2, der(1)t(1;2)(small arrows), and der(2)(large arrows), and partial karyotypes showing normal chromosomes 1 and 2, der(1), and der(2). *(d)* Partial metaphase of case 2 hybridized with chromosome 6 painting probe showing normal chromosome 6 and inv(6)(arrow), and partial karyotypes showing the same chromosomes. *(e)* Partial metaphase of case 2 hybridized with chromosome 3 painting probe showing normal chromosome 3, der(7)t(3;7)(p11;q36)(small arrow), and r(3)(large arrow), and partial karyotypes showing normal chromosomes 3 and 7, der(7), and r(3). *(f)* Partial metaphase and interphase nuclei of case 1 hybridized with LSI IgH Spectrum Green/Cyclin D1 Spectrum Orange probes showing 2 fusion signals (yellow), 1 red and 1 green normal signals. *(g)* Agarose gel electrophoresis of PCR products showing CCND1/IGH rearrangement in case 2. Lane 1: molecular weight marker; lane 2: positive control; lane 3: negative control; lane 4: patient.

Table 2
Karyotypes of patients with mantle cell lymphoma and chromosome inversions

Reference	Age/Sex	Karyotypes
Siebert et al. [11]	71/M	91~95,XXYY,**inv(1)(p13p32)**x2,del(2)(q12q24)x2,add(3)(q21)x2,dup(3)(p14p24)x2,t(11;14)(q13;q32)x2, +13,+13,del(13)(q12~13q14)x4,i(17)(q10)x2,+mar
Wlodarska et al. [10]	?/M	45,XY,der(1;6)t(1;6)(p21;q12)**inv(1)(p21q42)**,-8,der(11)del(11)(q13q14)t(11;14)(q13;q32),der(14)t(11;14), der(19)t(8;19)(q13;p13)x2
Wlodarska et al. [10]	?/M	47,XY,t(1;5)(q25;p15),**inv(2)(p22q21)**,+3,t(11;14)(q13;q32)
Huret et al. [8]	75/M	47,XY,ins(1;?)(q31;?),del(3)(p22),del(5)(q33q34),**inv(6)(p22q22)**,ins(17;?)(q22;?),+18,-19,+mar
Cuneo et al. [9]	?/M	46,XY,t(11;14)(q13;q32)/45,idem,del(1)(p22p32),**inv(6)(p21q2?3)**,-15
Nodit et al. [12]	41/F	46,XX,+del(3)(p12),**inv(8)(p21q22)**,t(11;14)(q13;q32)
Wlodarska et al. [10]	?/M	47,XY,del(7)(q22q32),**inv(11)(p11q23)**,+mar
Espinet et al. [13]	65/M	44~46,XY,del(1)(q32),dup(1)(q25q44),t(4;9)(p16;q12),add(7)(p22),-10,t(11;14)(q13;q32),**inv(12)**,add(17)(p13), -21/45~46,idem,+dup(1),del(14)(q24)
Wong & Chang [14]	81/F	39~41,XX,der(1)?t(1;21)(p11;q11)del(21)(q22),-2,t(2;14;11)(p11;q32;q13),-6,der(9;15)(q10;q10),+11,-14, -15,-17,add(18)(p11),**inv(19)(p13q12)**,?der(20)t(14;20)(p13;q11),-21,+2mar
Present study		
Case 1	35/M	44~46,XY,der(1)t(1;2)(p36;p21),**der(2)del(2)(p21)inv(2)(p21q11)**,del(3)(q11),**+inv(6)(p23q11)**,+7,-8, t(11;14)(q13;q32),-12,del(17)(p11),-18,-20,-22
Case 2	72/F	44~46,X,del(X)(q13),der(1)t(1;2)(p36;p21),**der(2)del(2)(p21)inv(2)(p21q13)**,r(3),der(7)t(3;7)(p11;q36), **inv(6)(p23q11)**,i(17)(q10),-21

Bold indicates the chromosome inversions described in mantle cell lymphomas.

(cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy (6 cycles). He had a short partial remission (5 months) and a progression of disease with leukemic outcome was observed. The patient started HiperCVAD regimen. He died in disease progression 19 months after presentation.

2.2. Case 2

A 72-year old woman with stage IVA showed splenomegaly and BM involvement. A complete remission was achieved with CHOP-Mabthera (6 cycles). The patient had a favorable course of the disease. She is still alive and in molecular remission 21 months after diagnosis.

3. Cytogenetic, FISH, and molecular studies

Cytogenetic study was performed on lymph node biopsy in Case 1 and on unstimulated BM in Case 2. Cells were cultured by short time (24–48 h) in RPMI 1640 medium supplemented with 15% fetal calf serum and L-glutamine. Slides were prepared using the G-banding technique. Karyotype abnormalities were described according to the International System for Human Cytogenetic Nomenclature [7]. FISH analysis was performed according to manufacturer's protocols. Locus specific LSI CCND1/IGH (11q13/14q32) dual fusion and LSI *ALK* (2p23) probes (Vysis, Downers Grove, IL), and whole chromosomes 1, 2, 3, 6, 7, and 17 painting probes (CAMBIO) were used. Furthermore, genomic DNA was extracted and a semi-nested PCR for CCND1/IGH rearrangement was done.

Both cases showed complex karyotypes. All the structural aberrations were confirmed by FISH (Fig. 1). The combined G-banded and FISH karyotypes of both cases are shown in Table 2. Both patients showed a cytogenetically identical inv(6)(p23q11) that could be a new recurrent aberration in MCL. They also showed another marker originated by deletion and inversion of chromosome 2: der(2)(:2q11→2p21::2q11→2qter) for case 1 and der(2)(:2q13→2p21::2q13→2qter) for case 2. Moreover, a der(1)t(1;2)(p36;p21) was also observed in both patients. FISH using LSI ALK probe on metaphases showed one fusion signal on the normal chromosome 2 and the other on the derivative chromosome 1, confirming the involvement of the short arm of chromosome 2 in this rearrangement. There were no signals of chromosome 1 on both der(2). It is not clear if der(1) and der(2) have originated as a part of one complex rearrangement or if they correspond to different events. All these abnormalities were not previously described in MCL. In both patients, alterations of chromosomes 3, 7, and 17 were also found. The presence of t(11;14)(q13;q32) was confirmed by interphase FISH with the CCND1/IGH probe and also by molecular study in Case 2. Residual normal cells were found in both cases.

4. Discussion

Inversions are uncommon events in MCL, accounting for 2.2% of all MCL listed in the Mitelman's catalog [2]. Thus, our findings provide interesting and uncommonly reported abnormalities in this pathology. To our knowledge, in MCL only 2 cases with inversions of chromosome 6 [8,9] and a patient with an inv(2) [10] (Table 2) have been reported. As shown in Table 2, the other chromosomes involved in this type of alteration were: chromosomes 1 (2 cases) [10,11] and 8, 11, 12 and, 19 (1 case each) [10,12–14], all of them as part of complex karyotypes.

Our cases showed a different clinical evolution with a poor outcome for Case 1 and a favorable course of the

disease for Case 2. Nodit et al. [12] described a patient with an inv(19) as part of a complex karyotype with an indolent MCL, while the patient referred by Espinet et al. [13] with an inv(12) had a survival of 87 months. There is no information about the clinical evolution of the other cases listed in Table 2.

Inv(6)(p23q11) was observed as a secondary aberration identical in both patients on the cytogenetic level. In the present study, no attempts were made to map the breakpoints of this cytogenetic anomaly. However, it is interesting to point out that *CD83* gene maps at 6p23 [2] and its soluble form (sCD83) has an immunosuppressive role in vitro and suppresses in vivo anti-tumor responses [15]. Recently, Hock et al. [16] have reported elevated levels of sCD83 in patients with chronic lymphocytic leukemia and MCL, suggesting that it may have functional and/or prognostic significance in these pathologies. In addition, breakpoint 2p21, site in which cyclin T2 *(CCNT2)* gene maps [2], was also recurrent in our patients.

In conclusion, new inversions as secondary genetic changes in MCL are reported. If we consider that this type of alteration is a very infrequent event in hematological malignancies, our finding of an identical inversion at the cytogenetic level in both cases could be important to detect genes potentially involved in development and/or progression of this aggressive NHL subtype.

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