

CASE REPORT

Insertion of the CCND1 gene into the IgH locus in a case of leukaemic small cell mantle lymphoma with normal chromosomes 11 and 14

A Aventín, J Nomdedéu, J Briones, I Espinosa, R Bordes, J Sierra

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The t(11;14)(q13;q32) translocation is considered to be the cytogenetic hallmark of mantle cell lymphoma. This report describes a case of leukaemic mantle cell lymphoma in which conventional cytogenetics on stimulated peripheral blood cells showed a 46,XY, t(1;12)(p21;q23)/46,XY karyotype. Fluorescence in situ hybridisation analysis using a dual colour immunoglobulin heavy chain (IgH)/CCND1 probe showed a fusion hybridisation signal on one normal chromosome 14, indicating that an insertion of the CCND1 gene into the 14q32/IgH locus had taken place. Overexpression of the cyclin D1 protein was demonstrated on bone marrow trephine by immunohistochemical staining.

Leukaemic mantle cell lymphoma is considered to be the peripheral expression of mantle cell lymphoma, which is characterised by the t(11;14)(q13;q32) translocation. As a consequence of this translocation, the CCND1 gene locus at 11q13 is juxtaposed with the immunoglobulin heavy chain (IgH) gene locus at 14q32, resulting in overexpression of the cyclin D1 protein.

We identified a unique case of mantle cell lymphoma with leukaemia harbouring a submicroscopic insertion of the CCND1 gene in the 14q32/IgH locus, producing a hybridisation fusion signal on an apparently normal chromosome 14 and overexpressing cyclin D1 on bone marrow cells.

CASE REPORT

A 47 year old man presented at the hospital in October 2000 with lymphocytosis. Physical examination showed neither lymphadenopathy nor hepatosplenomegaly. The white blood cell count was 15×10^9 /litre, with 63% atypical lymphocytes; haemoglobin, platelet count, liver, and renal biochemistry were normal. A computed tomography scan of the body was normal. Cytomorphological examination of a May Grünwald's stained peripheral blood smear revealed a predominance of small sized mature lymphocytes; the nuclei of these cells were round to slightly irregular without a prominent nucleolus. Bone marrow aspirate and trephine showed an interstitial infiltration by mature lymphocytes with similar morphology to those of peripheral blood. Surface markers of peripheral lymphocytes revealed an immunophenotype consistent with mantle cell lymphoma; that is: CD5⁺, CD10⁻, CD19⁺, CD22⁺, CD23⁻, CD79b⁺, FMC7⁺, and surface membrane Ig⁺⁺. A cytogenetic study of TPA (phorbol 12-myristate 13-acetate) stimulated peripheral blood cells revealed a 47,XY,t(1;12)(p21;q23)/10/46,XY/10/ karyotype (fig 1A). Interphase fluorescence in situ hybridisation (FISH) analysis using a commercial dual colour probe for IgH (green)/CCND1 (red) (Vysis, Downers Grove, Illinois, USA) displayed two red signals, one green signal, and a small single yellow fusion signal (fig 1C). An additional metaphase FISH

experiment showed a single yellow fusion signal at band q32 of an apparently normal chromosome 14 (fig 1D). DNA polymerase chain reaction (PCR) analysis did not detect a bcl1/J_H clonal band, indicating that the rearrangement at the genomic level was localised outside the major translocation cluster.

Immunohistochemistry on paraffin wax embedded material from a bone marrow trephine showed overexpression of the cyclin D1 protein in atypical lymphocytes (fig 1F). The patient remained asymptomatic with no treatment at last follow up in May 2003.

DISCUSSION

Differentiating between leukaemic presentation of B cell non-Hodgkin lymphomas and chronic B cell leukaemias based on morphological and immunophenotyping studies alone can be very difficult. The World Health Organisation recognises that genetic abnormalities are one of the most reliable criteria for the classification of malignant lymphomas.¹ Although t(11;14)(q13;q32) or its molecular counterpart, CCND1 rearrangement, can be detected using various methods such as conventional cytogenetics, Southern blot, and PCR analysis, the FISH technique has proved to be a highly sensitive tool for the detection of the t(11;14) translocation, irrespective of the localisation of the breakpoints in the CCND1 gene or the presence of cycling cells.²⁻⁴

In our present case, chromosomes 11 and 14 were normal by standard cytogenetics. However, metaphase and interphase FISH analysis showed the occurrence of a microinsertion of the CCND1 gene into the IgH locus, leading to overexpression of cyclin D1 in lymphoid bone marrow cells. The hybridisation pattern in interphase cells in our patient was identical to that described in cases with t(11;14)(q13;q32) associated with a deletion involving the variable region of IgH.² Therefore, additional conventional cytogenetic studies are advisable in cases with this interphase FISH pattern to rule out this microinsertion not previously described in mantle cell lymphoma.

"The fluorescence in situ hybridisation technique has proved to be a highly sensitive tool for the detection of the t(11;14) translocation, irrespective of the localisation of the breakpoints in the CCND1 gene"

Similar submicroscopic insertions have been reported in other cytogenetic-clinical entities, such as chronic and acute myeloid leukaemias in which fusion genes are generated without morphologically altering the chromosomes.^{5,6} Moreover, insertional events arising during aberrant immunoglobulin switch recombination have been described in a

Abbreviations: FISH, fluorescence in situ hybridisation; IgH, immunoglobulin heavy chain; PCR, polymerase chain reaction

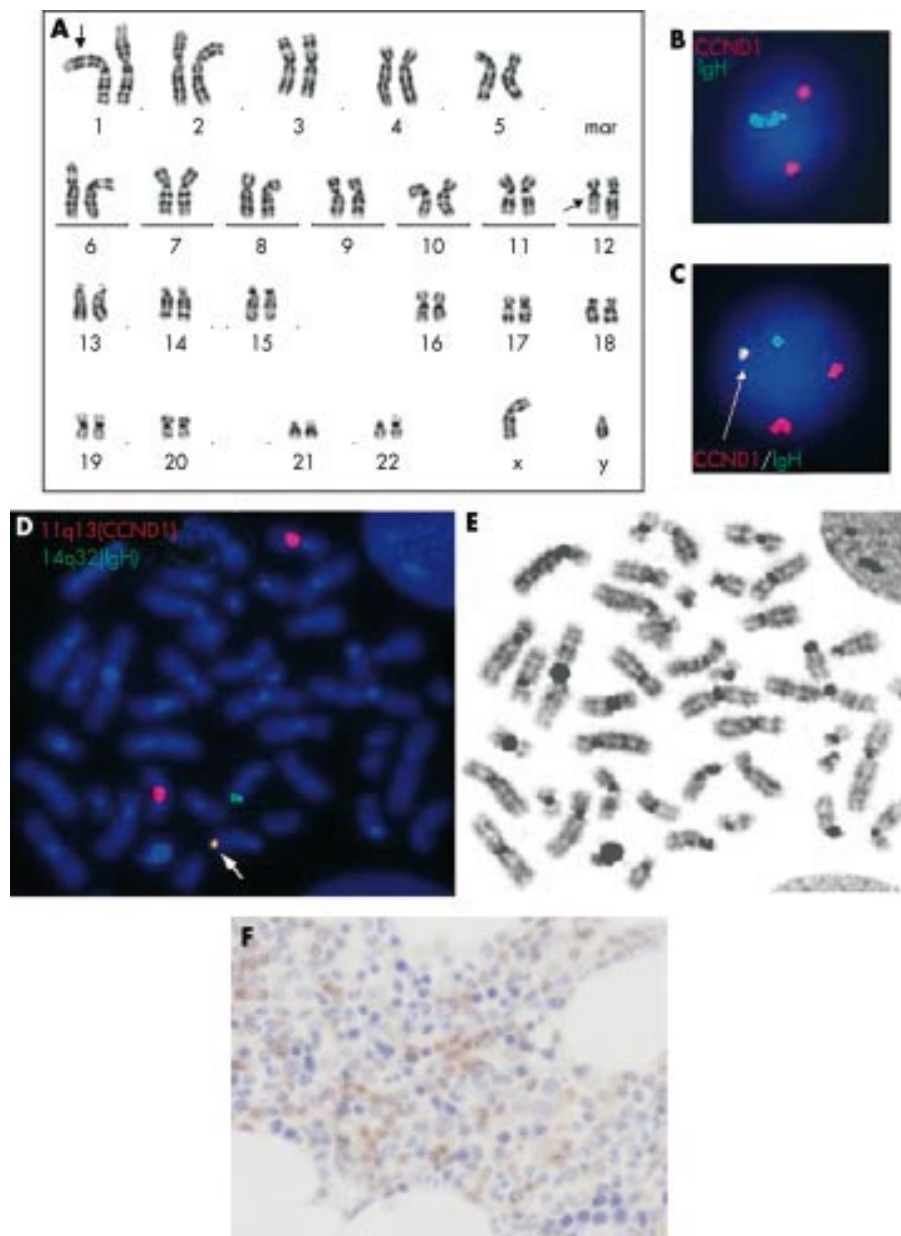


Figure 1 (A) Complete Giemsa (G) banding karyotype showing the $t(1;12)(p21;q13)$ translocation (arrows). (B) Interphase nuclei displaying two red signals and two green signals (normal hybridisation pattern). (C) Interphase nuclei displaying a single fusion hybridisation signal, two red signals and one green signal (abnormal hybridisation pattern). (D) Fluorescent *in situ* hybridisation on metaphase spreads using the LSI dual colour immunoglobulin heavy chain (IgH; green) and CCND1 (red) probes, showing the single fusion hybridisation signal on an apparently normal chromosome 14 (arrow). (E) The same metaphase G banded; the abnormal chromosomes 1 and 12, namely $t(1;12)(p21;q23)$, and the insertion of the CCND1 gene at the 14q32 position are part of the same clone. (F) Cyclin D1 immunohistochemistry showing positive reactivity in bone marrow lymphoma cells.

myeloma tumour cell line in which an IgH sequence containing the 3' IgH enhancer was inserted into chromosome 11, resulting in overexpression of cyclin D1.⁷ In addition, an insertion of 132 bp of chromosome 22q12 sequence into the 5' region flanking $\Sigma\mu$ on chromosome 14q32 has been reported in a patient with multiple myeloma.⁸ With regard to secondary

chromosomal abnormalities, the most frequent rearrangements described in mantle lymphoma are genomic imbalances, which appear to indicate poor prognosis.^{9,10} In our patient, no chromosomal imbalance was detected by interphase cytogenetics for $del17p53$, $del13q14$, and trisomy 12 or by comparative genomic hybridisation analysis (data not shown). Given the indolent clinical course in our patient, presenting features such as morphological small cell type, non-nodal disease, and no chromosomal imbalances may be of clinical interest in the management of patients with mantle cell lymphoma.

Take home messages

- Leukaemic mantle cell lymphoma is characterised by the $t(11;14)(q13;q32)$ translocation, in which the CCND1 gene locus is juxtaposed with the immunoglobulin heavy chain (IgH) gene locus, resulting in overexpression of the cyclin D1 protein
- We describe a unique case of leukaemic mantle cell lymphoma with a submicroscopic insertion of the CCND1 gene at the IgH locus, producing a hybridisation fusion signal on an apparently normal chromosome 14 and overexpressing cyclin D1 on bone marrow cells

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