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Translocation t(2;7)(p12;q21-22) with dysregulation of the CDK6 gene mapping to 7q21-22 in a non-Hodgkin's lymphoma with leukemia

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Background and Objectives. A female patient presented with splenomegaly and lymphocytosis with atypical lymphoid cell morphology. We identified t(2;7)(p12;q21) prompting studies of the translocation breakpoint and its consequences on protein expression to confirm or otherwise the recently reported involvement of CDK6 and IG κ genes in the t(2;7) leading to over-expression of CDK6 protein.

Design and Methods. A variety of clinical and laboratory techniques including cell marker, cytogenetic and histologic studies were applied in order to establish the diagnosis. Fluorescence *in situ* hybridization (FISH) and Southern blotting were used for mapping the translocation breakpoint and Western blotting for assessing protein expression.

Results. Immunophenotyping showed the presence of a B-cell population with strong expression of FMC7, CD22, CD79b, CD5 and κ restricted surface immunoglobulins. Based on morphology and immunophenotypic markers the diagnosis of B-cell non-Hodgkin's lymphoma was made. Karyotyping revealed a clone with t(2;7)(p12;q21-22). Evidence for clonal evolution with additional abnormalities including a deletion of the TP53 was present. We established by FISH and Southern blotting that the breakpoint on 7q21-22 fell in a region 66kb telomeric to the previously reported breakpoint for the t(2;7) and was the same as that observed in a t(7;21). CDK6 protein was over-expressed. The patient received alkylating agents and splenectomy and is alive but the lymphocytosis persists with evidence of disease progression.

Interpretations and Conclusions. We have demonstrated that CDK6 expression is dysregulated even when the breakpoint on 7q21-22 is located 66kb upstream from the coding region. Interestingly, the precise assignment of the lymphoma type in our case was not possible even when the splenic histology was analyzed.
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Key words: t(2;7)(p12;q21-22), CDK6; p53 deletion, atypical SMZL.

Cyclins, cyclin-dependent kinases (CDK) and cyclin-dependent kinase inhibitors (CKI) regulate the cell cycle and control cell division. In a variety of cancers, genes encoding these proteins are mutated or deleted resulting in loss of cell cycle control. Drugs that modulate CDK activities are being developed.[1] A specific chromosome translocation resulting in the over-expression of cyclin D1 gene on 11q13 is a common feature of mantle cell lymphoma (MCL).[2] Splenic lymphoma with villous lymphocytes (SLVL) is an indolent low grade lymphoma with a gradual accumulation of neoplastic cells arrested in the G0/G1 phase of the cell cycle and is the leukemic counterpart of splenic marginal zone lymphoma (SMZL).[3] It is characterized by splenomegaly and the presence of circulating villous lymphocytes in the peripheral blood.[4] Two translocations have recently been described: a t(2;7) (p12;q21-22) in three patients with SLVL and a t(7;21)(q21-22;q22) in a patient with SMZL, both involving the CDK6 gene on

7q21-22. The breakpoints on the t(2;7) clustered to a 3.6kb region upstream of the CDK6 gene while the t(7;21) contained a breakpoint 66kb further upstream from the t(2;7) cluster on 7q21.[5] We describe a patient with a diagnosis of B-cell lymphoma carrying a t(2;7)(p12;q21-22) involving the κ immunoglobulin light chain gene locus on 2p12 and the CDK6 gene on 7q21-22 with over-expression of the latter gene.

Case report and laboratory features

A 75-year old female presented in April 1999 with back pain and was found to have lymphocytosis. Physical examination showed splenomegaly 3cm below the costal margin and no lymphadenopathy. Computer tomography scanning confirmed spleen enlargement with intra-abdominal lymphadenopathy. Peripheral blood counts were as follows: hemoglobin 11.7 g/dL, platelets 287× 10^9/L and white blood cells (WBC) 46×10^9/L with 40×10^9/L atypical lymphocytes. The majority of cells in the peripheral blood were of medium size and a few were large. The chromatin was condensed and some cells had an indented or irregular nucleus. A proportion of cells had mildly basophilic cytoplasm without cytoplasmic villi. Immunophenotyping by flow cytometry showed the presence of a clonal B-cell population with strong expression of surface immunoglobulin (SmIg) with κ light chain restriction (88%). The majority of lymphocytes were FMC7$^+$ (74%), strongly positive CD22 (84%), and CD79b (78%$^+$) and co-expressed CD5 (88%) while they were negative for CD2, CD23 and CD38. According to the chronic lymphocytic leukemia (CLL) scoring system[6] this case was scored as 1. The presence of the CD5 molecule on the cell surface, in the absence of a phenotype typical of chronic lymphocytic leukemia prompted the analysis of cyclin D1 protein expression, but this was not detected. Examinations of the peripheral blood morphology and immunonologic markers were consistent with a diagnosis of B-cell lymphoma.

Because of disease progression with rising WBC and anemia, the patient was treated with alkylating agents, first chlorambucil (2 courses) and thereafter with low dose cyclophosphamide. There was no response to therapy and further deterioration of the blood counts and increase in the spleen size. The patient underwent splenectomy leading to improvement of the blood counts but the lymphocytosis persisted. Current blood counts are hemoglobin 10.5 g/dL; platelets 149×10^9/L and WBC 96×10^9/L. Renal and liver function tests are normal except for a mildly raised alkaline phosphatase. At present the patient is alive, 24 months after diagnosis, showing evidence of disease progression with high white blood counts and decreases in hemoglobin and platelet counts.

Spleen histology showed a nodular and interstital lymphoid infiltrate effacing the white pulp and involving the red pulp. The nodules had a biphasic appearance with the inner zone composed of small lymphoid cells with round nuclei; while the outer zone contained cells with slightly more abundant cytoplasm and scattered large cells with prominent nucleoli. The cytology of the cells in this area was not typical of marginal zone type since the nuclei were more irregular than would be expected in SMZL. Phenotypically the cells expressed CD20, CD79b, CD5 antigens with κ light chain restriction. They were negative for CD10, CD23, IgD, CD3, and cyclin D1. Proliferation was low to moderate. A similar population infiltrated the lymph node. Although the organization of the infiltrate was reminiscent of splenic marginal zone B-cell lymphoma the detailed morphology and immunophenotype (CD5$^+$/IgD) was not typical of this lymphoma. For these reasons histologically the diagnosis was that of an *unclassified low grade primary splenic B-cell lymphoma* or an atypical form of SMZL without villous lymphocytes.[3]

Design and Methods

Karyotype and fluorescent *in situ* hybridization (FISH) studies were carried out on peripheral blood cells cultured with TPA (tetradecanoyl phorbol 12 myristate 13 acetate). G-banding was performed according to standard procedures. Probes used for FISH included whole chromosome paints (WCP) for chromosomes 2 labeled with FITC and chromosomes 7, 10 and 13 labeled with biotin (Cambio), BAC H_2NH0029A11 mapping upstream to and containing the first 2 exons of the CDK-6 gene (labeled with biotin by nick translation) and PAC for κ immunoglobulin light chain gene (labeled with digoxigenin by nick translation). Commercial probes LSI *p53* and *RB-1* Spectrum Orange (Vysis) were also applied. FISH was performed according to standard methods and probes were prepared following the manufacturer's protocols.[21] Comparative genomic hybridization (CGH) was performed as described elsewhere.[7]

Patient's and control (CEM cell line, normal individual and patient's granulocytes) DNA for Southern blotting was extracted according to standard procedures and digested with the following enzymes: BamHI, XbaI, SacI, EcoRI, and PvuII (all from New England Biolabs, USA). DNA blots were

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probed with genomic probes p1305.0 and p1303.0 derived from cosmid 130a6 which spans the cluster of breakpoints 3.6kb from the CDK6 gene reported in 3 SLVL with t(2;7)(p12;q21) and p67tel derived from cosmid BMTH9 detecting rearrangements 66-67kb from the cluster and reported in a SMZL with t(7;21)(q21;q12).[5]

Cells for evaluating protein expression by Western blotting were lysed at 1×10^7 cells/mL in HB buffer (50 mM Hepes pH 7.4, 150 mM NACI, 20 mM EDTA, 10 mM B-glycerophosphate, 0.5% NP40, 1 mM PMSF, 2.5 μg/mL leupeptin and 1% aprotinin). Samples were fractionated on 12% SDS-polyacrylamide gels, blotted on to PVDF membranes and probed with anti-tubulin antibody (Neomarkers) to confirm equal loading of the lanes. They were subsequently reprobed with anti-CDK6 antibody (Santa Cruz). Antibody-antigen complexes were detected by incubation of the membranes with HRP-conjugated secondary antibody and enhanced chemiluminiscence (Amersham).

Results

Karyotype/molecular cytogenetics

G-banded metaphases revealed a clone with a balanced translocation t(2;7)(p12;q21-22) in all the cells (Figure 1A). Clonal evolution was evident in the presence of two subclones. One subclone

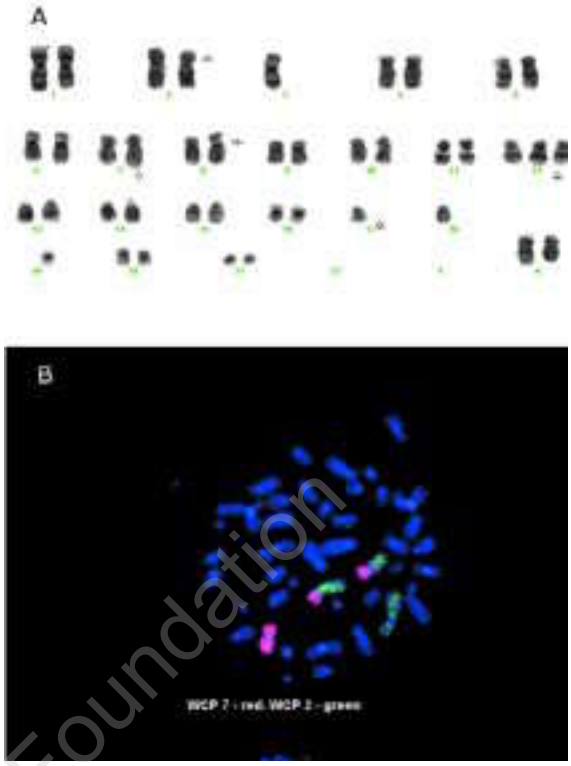

Figure 1. A. Karyotype of a metaphase from subclone 1 with arrows pointing to the clonal abnormalities: t(2;7)(p12;q21-22), -17, der(8)t(8;17)(p12;p11) and +12.
B. WCP for chromosome 2 (green) and 7 (red) depicting the t(2;7)(p12;q21-22).

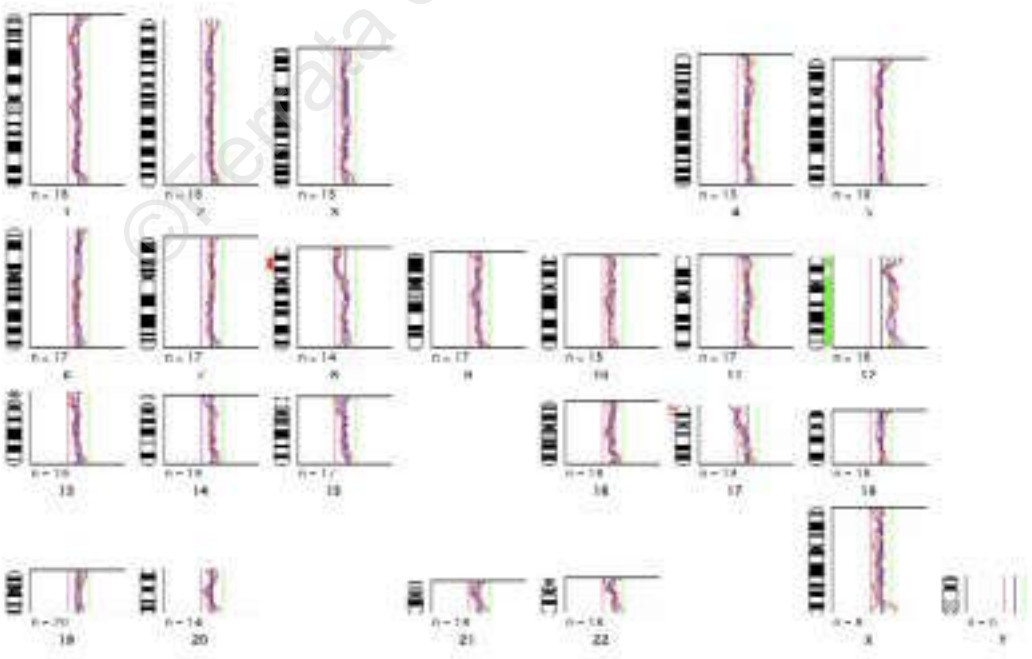

Figure 1C. CGH profile showing dim(8p12-pter); dim(17p11-pter) and enh(12). The profile arose from analysis of 10 metaphases. The gain/loss ratios used were 1.20/0.80. The red lines represent the 95% confidence interval of the average ratio (the blue line).

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contained an unbalanced translocation t(8;17) and trisomy 12 (Figure 1A). The other displayed complex rearrangements involving the q arm of the other chromosome 7 homolog (7q34-35) with 13q and the q arm of chromosome 10 with the p arm of the 13 resulting in 45, XX, -10, t(2;7)(p12;q21-22), der(7)t(7;13)(q34-35;q21), der(13)t(13;10)(p11;q?). Translocation t(8;17) and trisomy 12 were absent from this latter clone. Chromosome 7 abnormalities were confirmed by experiments using WCP 2, 7, 10 and 13 (Figure 1B).

CGH detected a gain of chromosomal material from the whole of chromosome 12 as well as a loss of material from the short arms of chromosomes 8 and 17 (Figure 1C). The gain of 12 profile was stronger than the loss of 8p and 17p.

Interphase FISH revealed monoallelic TP53 gene deletion mapping to 17p13 in 52% of the cells. RB-1 mapping to 13q14 was diploid.

Mapping of the 7q21-22 breakpoint

The breakpoint on t(2;7)(p12;q21-22) was defined more precisely by FISH and showed co-localization of signals with a PAC for κ light chain gene and BAC H_2NH0029A11 (Figure 2A). This BAC spanned the breakpoints on 7q21.2 of all 4 patients reported by Corcoran *et al.*[5] DNA rearrangement was only observed by Southern blotting in the PvuII digests probed with p67tel (Figure 2B) which identifies a breakpoint ~66-67kb upstream from the CDK6 gene on 7q21.

Protein expression

Marked overexpression of CDK6 by Western blotting was observed in the patient with t(2;7), while the 2 control cases of SLVL without chromosome 7 abnormalities showed weak to negative and low levels of CDK6 expression (Figure 2C).

Discussion

Deregulation of genes contributing to tumorigenesis by juxtaposition to Ig gene enhancers is common in B-lymphoid disorders. These result from errors in VDJ recombination of the IGH genes.[8] Less frequently, the rearrangements occur during the processes of class switch recombination or somatic hypermutation.[8] Some translocations, such as t(11;14)(q13q32) resulting in the over-expression of cyclin D1, occur in almost all cases of MCL but may occasionally occur in other lymphoproliferative disorders.[9,10] The present investigation is a case study of a patient with a diagnosis of B-cell lymphoma that was difficult to classify by standard diagnostic methods. The diagnosis of CLL was considered but excluded as although the cells were CD5 positive and a clone with +12 was identified, neither of these features is specific for CLL and tumor cells from up to 20% of patients with SLVL express surface CD5.[11] Furthermore, the phenotype and spleen histology of the reported case were different from those normally encountered in CLL. The patient's circulating peripheral blood lymphocytes were not villous and spleen histology although not inconsistent was *not* typical of SLVL/SMZL. However, the presence of chromosome 7 abnormalities and more precisely t(2;7)(p12;q21.2), a translocation described previously only in cases with SLVL/SMZL[5,12] made the diagnosis of atypical SMZL most likely.

Chromosome 7 abnormalities were confirmed by experiments with WCP but remained undetected by CGH. The explanation for this discrepancy is the fact that the t(2;7)(p12;q21-22) is a balanced translocation and does not involve any loss or gain of DNA. Furthermore, the subclone with rearrangements involving chromosomes 7, 13 and 10 constituted a small percentage of cells, below the threshold of sensitivity for CGH (<50%) (Figures 1A, B). The presence of a subclone containing t(8;17) with loss of 8p and 17p as well as +12 was confirmed by CGH (Figure 1C). The stronger intensity of the CGH profile for +12 as compared with the loss of 8p and 17p may reflect that +12 arose during clonal evolution before the t(8;17). However, karyotypes with +12 but without t(8;17) were not detected on cytogenetic analysis suggesting that these cells failed to divide in culture.

CDK4 and CDK 6 in concert with their D type cyclin partners are responsible for progression through G1 of the cell cycle while CDK2 with cyclin E is responsible for progression from G1 to S phase. These CDKs are serine/threonine kinases which phosphorylate retinoblastoma protein (pRB) leading to the release of E2F transcription factor that controls the S phase genes.[13] CDK6 is ubiquitously expressed in precursors of normal B- and T-cells and strong expression has been described in acute lymphoblastic leukemia.[14-16] Although the mechanism of dysregulation does not involve DNA rearrangements of the CDK6 gene, inactivation of p16, an inhibitor of CDK6, has been observed in acute T-cell lymphoblastic leukemia.[16] Overexpression of CDK6 is considered to contribute to leukemia advantage through dysregulation of cell multiplication and prolonging the life span of the blasts by favoring progression through the G1 phase in these aggressive leukemias.[17]

In two of the 3 reported SLVL patients with t(2;7)[5] CDK 6 was dysregulated by juxtaposition to the κ light chain gene, while in the third, and in a

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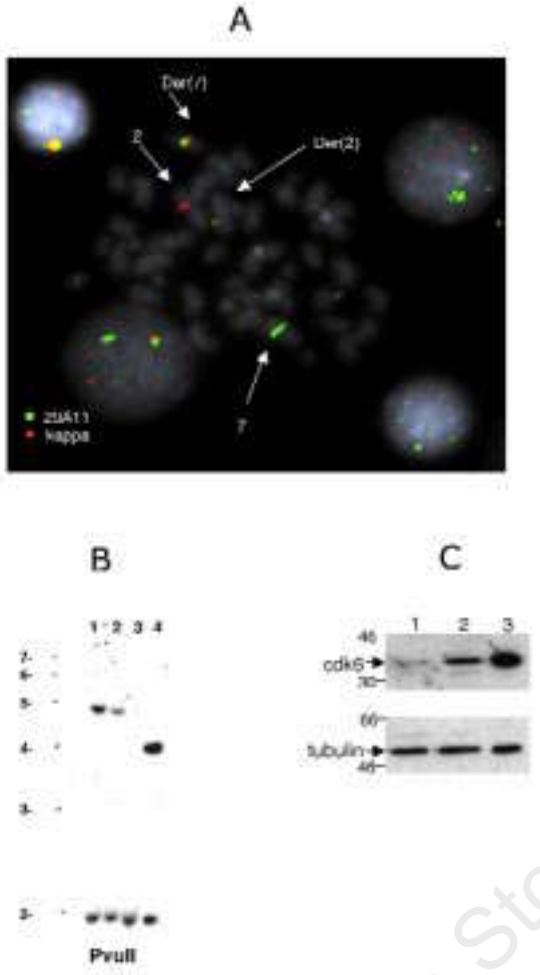

Figure 2. A. Metaphase showing co-localization by FISH of BAC H_2NH0029A11 (green) and PAC for κ gene (red) on the two derivative chromosomes resulting from t(2;7)(p12;q21-22) and normal signals on unaffected homologs of chromosomes 2 and 7. B. Southern blot showing the PvuII digest of DNA from a normal individual (lane 1), patient's granulocytes (lane 2), cell line K1718 (lane 3) and the patient's lymphocytes (lane 4) probed with p67tel which identifies a breakpoint ~66kb upstream from the CDK6 gene. The presence of a band lower than the germline (normal individual and the patient's granulocytes) was identified in the patient's lymphocytes. C. Western blotting image showing overexpression of CDK6 in the presented case. Lanes 1 and 2 were loaded with protein from 2 SLVL patients without chromosome 7 abnormalities and lane 3 with a sample from the patient with t(2;7)(p12;q21-22) described here.

case with a t(7;21) with a breakpoint 66kb further upstream from the breakpoint cluster, CDK6 expression was not tested due to lack of material.[5] We observed a marked overexpression of CDK6 despite the fact that the breakpoint of t(2;7) in this case was 66kb upstream from the reported cluster and similar to that found in t(7;21).[5] Low levels of CDK6 have been described in a variety of non-Hodgkin's lymphoma (NHL)[14] and this was seen in the two cases of SLVL tested which did not have chromosome 7 abnormalities (Figure 2C). In Corcoran's study, the disease course was not progressive in the 2 patients with t(2:7) as a sole abnormality, suggesting that this translocation on its own in SLVL is not sufficient to cause aggressive disease.[5] In the present case clonal evolution with additional abnormalities including the der(7) t(7;13)(q34-35;q24) was observed. The chromosomal band 7q34-35 harbors the CDK5 and the apoptosis regulatory gene.[18] Other cytogenetic abnormalities in the patient reported here, namely trisomy 12, loss of 8p and 17p both of which latter loci are known to harbor tumor suppressor genes,[19–21] together with clinical features such as persistent lymphocytosis, anemia and a decrease in the platelet count maybe be prognostic of future disease progression. In particular p53 abnormalities, though not common in SLVL are associated with an aggressive clinical course and poor prognosis[21] and the patient described here had contained a mutation in exon 8 (*data not shown*).

Thus the present study confirms CDK6 deregulation by juxtaposition of the CDK6 gene to the κ immunoglobulin light chain gene and also demonstrates over-expression of CDK6 even when the breakpoint is 66kb away from the reported cluster. The atypical nature of this B-NHL suggests that dysregulation of CDK6 by translocation could be a recurrent abnormality common to primary splenic B-cell lymphomas including SLVL/SMZL.

Contributions and Acknowledgments

VBB was responsible for the conception and design of the project, conventional karyotyping and drafting of the manuscript. AMGW was involved in the laboratory part of this project, analyzing data and finalizing the manuscript. GP, CLA, and MOE were involved in the laboratory part and data analysis. EM, ACW, SGWS and DC were involved in analysis of data, discussion and preparation of the manuscript.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

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PEER REVIEW OUTCOMES

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Sakari Knuutila, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Professor Knuutila and the Editors. Manuscript received November 21, 2001; accepted February 12, 2002.

What is already known on this topic

Cyclins regulate the cell cycle and control cell division. There are reports that show specific translocations involving cyclin dependent kinase 6 gene.

What this study adds

Here the authors describe another translocation activating CDK6. In t(2;7)(p12;q21-22) the gene fusion of κ immunoglobulin light chain and CDK6 results in overexpression of CDK6.

Potential implications for clinical practice

This translocation is highly likely to be an abnormality that can be diagnostically and prognostically associated with primary splenic B-cell lymphoma.

Sakari Knuutila, Associate Editor