Human Pathology (2017) **64**, 207–212

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Case study

Mantle cell lymphoma with a novel t(11;12)(q13;p11.2): a proposed alternative mechanism of *CCND1* up-regulation ☆

Joshua R. Menke MD [a], George Vasmatzis PhD [b], Stephen Murphy PhD [b], Lin Yang PhD [b], David M. Menke MD [c], Han W. Tun MD [d], Rebecca L. King MD [e], Stephanie A. Smoley BA [e], Rhett P. Ketterling MD [e], William R. Sukov MD [e,*]

[a]*Department of Pathology, University of California, San Francisco, CA 94143*
[b]*Center for Individualized Medicine, Mayo Clinic, Rochester MN 55905*
[c]*Department of Pathology, Mayo Clinic, Jacksonville, FL 32224*
[d]*Department of Hematology and Oncology, Mayo Clinic, Jacksonville, FL 32224*
[e]*Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester MN 55905*

Received 25 July 2016; revised 3 January 2017; accepted 5 January 2017

Keywords:
Mantle cell lymphoma;
CCND1;
Mate-pair sequencing;
Cytogenetics;
Translocation;
3′-UTR;
microRNA

Summary Mantle cell lymphoma (MCL) is typically characterized by t(11;14), which places the *IGH@* enhancer elements upstream of *CCND1*. This fusion results in up-regulation of *CCND1* and consequently its protein product cyclin D1. Recent studies have shown that in MCL, mutations or translocations occurring within the 3′ untranslated region (UTR) of the *CCND1* gene can result in a truncated mRNA transcript that is more stable and associated with more aggressive disease. We identified a case of MCL showing cyclin D1 overexpression by immunohistochemistry and a t(11;12)(q13;p11.2) by conventional cytogenetic studies. Next-generation genomic sequencing indicated a chromosomal break through the *CCND1* 3′-UTR and fusion with a non-coding region of chromosome 12. We suggest that, in the absence of the typical *CCND1/IGH@* fusion, this rearrangement promotes MCL pathogenesis by eliminating miRNA interaction elements within the 3′-UTR of the *CCND1* mRNA transcript consequently resulting in *CCND1* overexpression.

1. Introduction

Mantle cell lymphoma (MCL) is traditionally characterized by a chromosomal translocation between the *CCND1* gene region on chromosome 11 and the *IGH@* gene region on chromosome 14. In the proper clinicopathologic context, detection of t(11;14)(q13;q32) by conventional cytogenetics, *CCND1/IGH@* fusion by fluorescence in situ hybridization (FISH), or cyclin D1 overexpression by immunohistochemistry (IHC) is considered diagnostic of MCL [1]. Alternatively, *CCND1* fusion with other immunoglobulin gene partners, such as *IGK@* or *IGL@*, has been described in MCL, albeit rarely [2-4]. Regardless of the gene partner, these rearrangements result in the position of immunoglobulin regulatory elements upstream to *CCND1*, ultimately resulting in up-regulation of *CCND1* [2,5,6].

☆ Disclosures: None.

* Corresponding author at: Department of Laboratory Medicine and Pathology, Divisions of Laboratory Genetics and Anatomic Pathology, Mayo Clinic, 200 1st Street SW, Rochester, MN 55905.
E-mail address: Sukov.william@mayo.edu (W. R. Sukov).

http://dx.doi.org/10.1016/j.humpath.2017.01.001
0046-8177/

We present a case of MCL lacking *CCND1* fusion with an immunoglobulin region, but instead demonstrating t(11;12)(q13;p11.2) resulting from a breakpoint through the *CCND1* 3′ untranslated region (UTR) and fusion with a non-coding region of chromosome 12, as demonstrated by next-generation sequencing. We postulate this rearrangement, at least in part, promotes MCL pathogenesis by eliminating miRNA interaction elements within the 3′-UTR of the *CCND1* mRNA transcript in the absence of the typical t(11;14) translocation.

2. Case report

A 68-year-old man presented to an outside hospital where he underwent routine examination including a peripheral blood evaluation, which showed an absolute lymphocytosis. At the time, he was asymptomatic. A subsequent bone marrow biopsy showed 40% involvement by a proliferation of small monomorphic lymphocytes. Immunohistochemical studies performed on the bone marrow biopsy material showed tumor cells to express CD20, cyclin D1, focal CD5, and to lack expression for CD23. Flow cytometry of the bone marrow aspirate demonstrated a population of κ light chain restricted B-lymphocytes with expression of CD19, CD20, CD22, and CD5 without coexpression of CD23 or CD10.

Conventional chromosome analysis of G-banded, trypsin- and Leishman-stained metaphases from unstimulated bone marrow cultures showed a normal 46, XY karyotype. However, bone marrow cultures stimulated with CpG-oligonucleotide mitogen (CPG-ODN2006 (ODN-B) + IL-15 + IL-2 for 60–72 hours) showed an abnormal karyotype of 46, XY, t(11;12)(q13;p11.2)i(17)q10 in 3 of 10 metaphases. Interphase FISH studies performed on biopsy material using a commercially available *CCND1/IGH@* fusion probe (Abbott Molecular, Des Plaines, IL) did not show a fusion but instead three *CCND1* signals consistent with one intact *CCND1* allele and one *CCND1* allele split into two parts. Sequential FISH studies performed on the abnormal metaphase cells identified one *CCND1* signal on the normal chromosome 11, one *CCND1* signal on the abnormal chromosome 12 and one *CCND1* signal on the abnormal chromosome 11, consistent with a *CCND1* rearrangement. Subsequent interphase FISH studies using a *CCND1* break-apart probe confirmed *CCND1* rearrangement. In addition, FISH for *TP53* (Abbott Molecular) showed loss of one copy of *TP53* consistent with the isochromosome 17q identified by conventional chromosome studies. Based on the histologic, immunophenotypic, and cytogenetic findings, a diagnosis of mantle cell lymphoma with variant translocation t(11;12)(q13;p11.2) was made.

DNA from CpG-stimulated bone marrow culture was additionally isolated for Illumina mate-pair (MP) sequencing. Briefly, a MP library was assembled according to previously published protocols using the Illumina Nextera MP kit and was sequenced on 1/2 lane of the HiSeq2000 [7,8]. Paired sequencing reads were aligned to the CRCh38 reference human genome using a32-bit binary indexing mapping algorithm (BIMA) as previously described. Discordant MP reads mapping >15 kb apart or in different chromosomes were selected for further analysis. A mask was used to eliminate common variants and discordant fragments from experimental or algorithmic errors. MP sequencing confirmed the t(11;12)(q13;p11.2) and predicted breakpoints within the *CCND1* 3′-UTR (between genomic coordinates chr11:69 651 870 and 69 651 884) and a non-coding region of chromosome 12p11.2 [Fig. 2]. Polymerase chain reaction (PCR) validation of the patient sample and human germline control verified fusion events 1 (chr11–12) and 2 (chr12–11). Sanger sequencing of the yielded PCR products characterized the sequence at the event junctions. No translocations between chromosome 11 and IGH@, IGK@ or IGL@ or associated regulatory elements were identified.

The patient was subsequently treated with the modified Nordic lymphoma group regimen and completed 6 cycles of R-CHOP (rituximab, cyclophosphamide, hydroxycaunorubicin, oncovin, prednisone) alternating with R-HiDAC (rituximab plus high-dose cytarabine). Autologous stem cell transplant was unsuccessful. Twenty-five months after initial diagnosis, positron-emission tomography/computed tomography (PET/CT) scan demonstrated enlarged, PET-avid left axillary lymph nodes. Excisional biopsy of one of the lymph nodes demonstrated involvement by the patient's MCL. At this time, however, IHC and flow cytometry showed the lymphoma to express CD19, CD20 and cyclin D1, lack expression of SOX11, CD3, CD10 and CD23 and maintain κ light chain restriction. In addition, the lymphoma no longer showed expression of CD5 (Fig. 1). The patient underwent local radiation treatment.

At 30 months, the patient had a bone marrow recurrence, at which time he received additional therapy and underwent an autologous stem cell transplant. He continues to be in remission 12 months later.

3. Discussion

The classic t(11;14)(q13;32) resulting in *CCND1/IGH@* fusion is identified in the vast majority of cases of MCL, in the proper context, is regarded as pathognomonic for MCL [9]. Rare variant translocations have been identified, but these exploit the same mechanism for *CCND1* overexpression with *IGK@* or *IGL@* substituting for those from *IGH@* [3]. In the current case, the t(11;12) is the only structural alteration involving *CCND1* identified. Furthermore, there were no structural alterations involving chromosome 11 and the immunoglobulin genes *IGH@*, *IGHK@* or *IGL@*. Therefore, in the context of overexpression of cyclin D1, we assume this t(11;12)(q13;p11.2) to be the defining abnormality resulting in up-regulation of *CCND1*. Despite this atypical chromosomal rearrangement, the clinical, histologic, flow cytometric, and IHC findings are consistent with the diagnosis of MCL. MP sequencing predicts that the break at 11q13 occurs through the 3′-UTR of *CCND1* rather than 5′ to the gene, as would