Case Report

A Rare Case of Chronic Lymphocytic Leukemia Transformed to B-Cell Lymphoma, Unclassifiable, with Features Intermediate Between Diffuse Large B-Cell Lymphoma and Classic Hodgkin Lymphoma

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Richter's transformation refers to chronic lymphocytic leukemia (CLL) transforming into diffuse large B-cell lymphoma (DLBCL) or classic Hodgkin lymphoma (CHL). The transformation is associated with TP53 and NOTCH1 mutation, CDKN2A/B deletion, and/or MYC translocation. Here we report a rare case of CLL evolving to B cell lymphoma, unclassifiable, with features intermediate between diffuse large B cell lymphoma and classic Hodgkin lymphoma (BCL-U). The patient was a 61-year-old male with long history of CLL who developed right inguinal lymphadenopathy. The lymph node was biopsied and submitted to flow cytometry, immunohistochemistry, cytogenetics, FISH, and NGS molecular profiling. His peripheral blood and bone marrow were also submitted for similar tests. Our results showed that the peripheral blood and bone marrow were involved by CLL (CD5+, CD23+) with trisomy 12. The lymph node was infiltrated by pleomorphic large lymphoid cells with a subset showing Reed-Sternberg-like lymphoma cells. The lymphoid cells were positive for CD5, CD20, CD45, and CD30. Trisomy 12 was detected, while gene rearrangement for BCL2, BCL6, C-MYC was negative. NGS study on peripheral blood and the lymph node revealed similar mutation profiles (10 mutated genes in a 250 gene panel), including NOTCH1 and CDKN2C mutation. This is the first reported case of CLL transforming to BCL-U. Cytogenetics and molecular profiling confirmed the CLL and BCL-U were clonally related. Among the reported "transformation driving force" mutations, we only detected NOTCH1 mutation. However, we discovered a potential new mutation candidate that might belong to "transformation driving force": CDKN2C, a molecule closely related to the reported CDKN2A/B.
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INTRODUCTION

About 2-10% CLL cases transform into an aggressive lymphoma with poorer prognosis. This process is called Richter's transformation, in which 85-95% cases transformed to DLBCL and 5-15% cased transformed to classical Hodgkin lymphoma.[1] In addition, CLL has also been rarely reported to transform into plasmablastic lymphoma or B-lymphoblastic leukemia/lymphoma.[2-5]

B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B cell lymphoma and classic Hodgkin lymphoma (BCL-U) is a high-grade B-cell lymphoma demonstrating dual features of DLBCL and CHL, both morphologically and immunophenotypically. Majority of the cases are associated with mediastinal diseases, with a portion of which having been reported in peripheral lymph nodes. Histologically this entity is pleomorphic, with sheets of tumor cells resembling DLBCL in one area and CHL-like in another area. Immunophenotypically, the tumor cells are usually positive for CD45, CD20, CD30, variable for Bcl6, but negative for CD10, ALK, or EBER. Gene rearrangement for BCL2, BCL6, and C-MYC is usually negative.[6]

Here we report a rare case of CLL transforming into a BCL-U. By using genetic and molecular profiling, the molecular mechanism behind this phenomenon was also explored.

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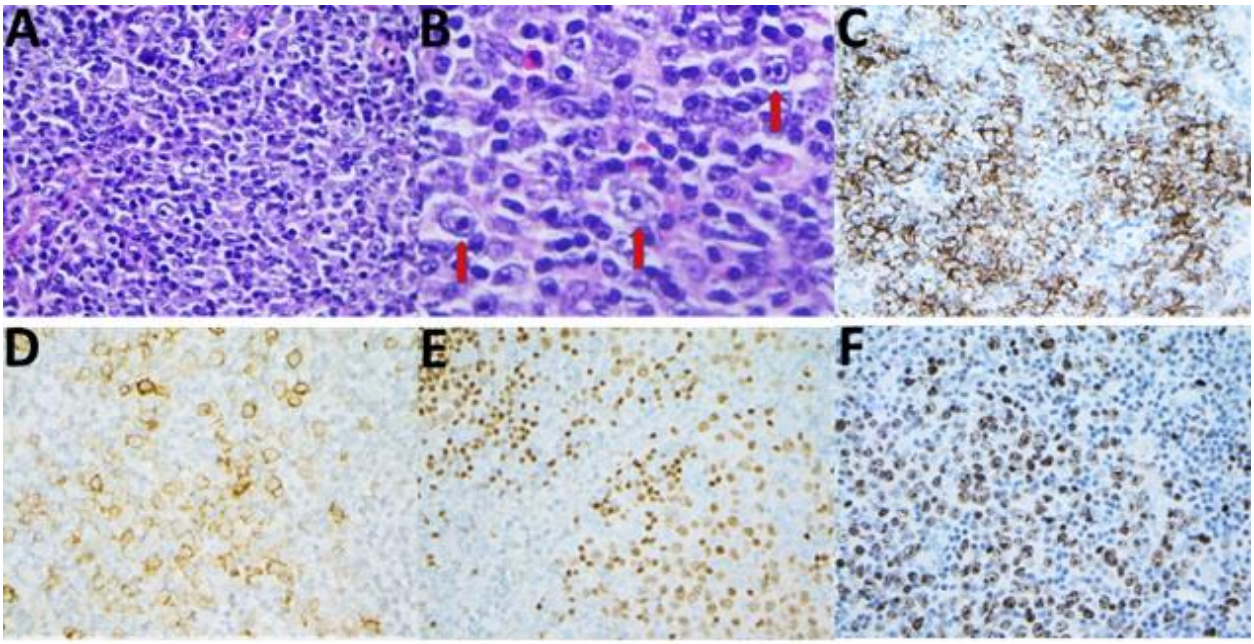

Figure 1. Morphology and immunohistochemistry of the B-cell lymphoma-U. **A.** H&E section (630X), sheets of large lymphoma cells resembling DLBCL. B. H&E section (1,000X), Examples of pleomorphic lymphoma cells resembling CHL (pointed by red arrows). In the background, there are eosinophils and small lymphocytes. **C.** Stain for CD20. The lymphoma cells are strong positive for CD20, both for DLBCL and CHL. **D.** Stain for CD30. Majority of the lymphoma cells are positive for CD30, both for DLBCL and CHL. **E.** Stain for Pax5. The lymphoma cells are positive for Pax5. **F.** Stain for Ki-67. The Ki-67 proliferative index for the lymphoma cells is more than 90%.

CASE REPORT

A 61-year-old male with long history of CLL (diagnosed 8 years ago, under monitoring without treatment) presented with right inguinal lymphadenopathy. An excisional lymph node biopsy was performed. On H&E sections, the lymph node was effaced, containing sheets, clusters, and singly scattered pleomorphic large atypical cells. In some areas the large cells are relatively uniform, with vesicular chromatin, moderate amount of cytoplasm, and single prominent nucleoli. In other areas the large cells are Reed-Sternberg cell-like, with double nucleation and eosinophilic nucleoli. Focally there were sporadic eosinophils, plasma cells, and small lymphocytes. Immunohistochemistry study revealed these large atypical cells were positive for CD45, CD20, CD30, CD5 (dim), Pax5 (dim), Bcl2, Bcl6, and MUM-1, but negative for CD10, Cyclin D1, and EBER (**Figure 1**). FISH analysis detected trisomy 12, but no gene rearrangement for BCL2, BCL6, or C-MYC (**Figure 2**). Based on all the findings, a diagnosis of B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classic Hodgkin lymphoma (BCL-U) was rendered based on WHO classification of tumors of hematopoietic and lymphoid tissues.[6]

To investigate whether this lymphoma was transformed from patient's existing CLL, tests were performed on patient's peripheral blood and bone marrow including flow cytometry, cytogenetics, and FISH for CLL panel. The results revealed that the CLL cells were positive for CD5 and CD23, and were carrying trisomy 12 (Integrated Oncology, Manhattan, NY). The bone marrow contained CLL only, with no evidence of involvement by BCL-U. Next generation sequencing performed both on peripheral blood and lymph node revealed similar mutation profiles (10 mutated genes in a 250 gene panel, Foundation Medicine, RTP, NC), including NOTCH1 and CDKN2C mutation (**Table 1**). Therefore, it's evident that the BCL-U and the CLL are clonally related, consistent with Richter's transformation.

Table 1. Gene mutation in peripheral blood/CLL and lymph node/B-cell lymphoma-U.[#]

specimen	Mutated genes										
blood	**NOTCH1 T2466fs*11**	**CDKN2C T69A**	C11ORF30 (EMSY) I668V	CD36 Y325*	CREBBP Q1002H	KMT2C (MLL3) A779T	MAP2K2 (MEK2) V215L	MLL2 P2349L	MSH2 R444L	RNF43 Q324K	**DNMT3A - R635Q - subclonal**
Lymph node	**NOTCH1 T2466fs*11**	**CDKN2C T69A**	C11ORF30 (EMSY) I668V	CD36 Y325*	CREBBP Q1002H	KMT2C (MLL3) A779T	MAP2K2 (MEK2) V215L	MLL2 P2349L	MSH2 R444L	RNF43 Q324K	

#: FoundationOne®Heme 250 gene panel was used to analyze gene mutations in CLL (blood) and B-cell lymphoma-U. As shown in the table, 10 identical gene mutations were detected in both entities. A subclonal DNMT3A mutation was detected in CLL only.

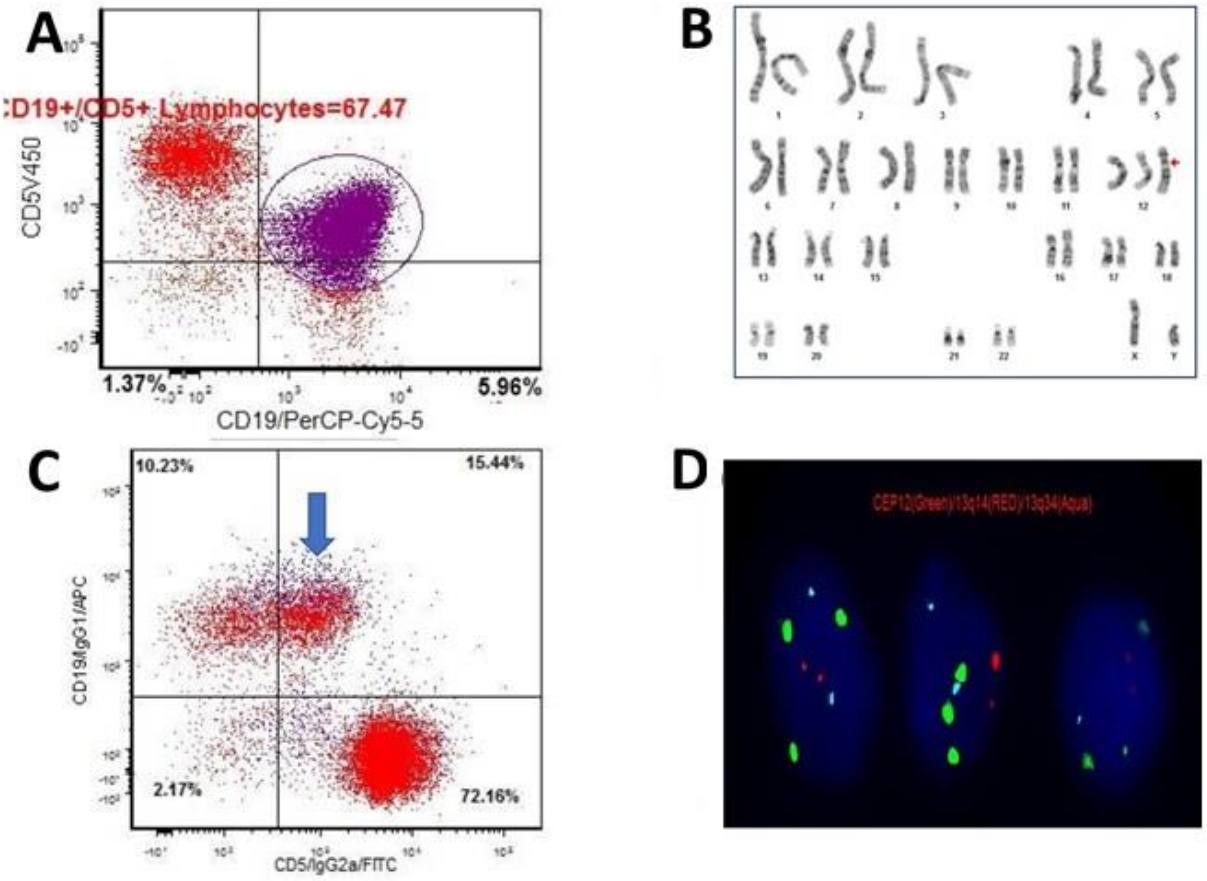

Figure 2. Flow cytometry analysis and cytogenetic study in CLL (A and B) and BCL-U (C and D). **A.** Peripheral blood flow cytometry analysis revealed CD19+/CD5+ CLL population (circled). **B.** Chromosome analysis on the CLL cells revealed trisomy 12 (red arrow). **C.** Flow cytometry analysis on the lymph node revealed the lymphoma cells are CD19+/CD5+ (blue arrow). Benign B-cells (CD19+/CD5-) are shown in the left upper quadrant. **D.** FISH analysis on the paraffin slide of BCL-U revealed trisomy 12 (CEP12/green; 13q14(red)/13q34(Aqua)).

DISCUSSION

Here we report for the first time a case of CLL transforming to BCL-U, and propose CDKN2C mutation as a novel "transformation driving force" in this process.

Understanding the molecular pathways of how CLL transforms into a more aggressive lymphoma potentially provides therapeutic targets. Most of the studies have been focused on transformation to DLBCL, since it is the most common CLL transformation. Two patterns of clonal evolution in CLL transformation have been proposed: 1) the linear clonal evolution model, in which the lymphoma is clonally related to CLL, accounts for 80-90% of all cases; and 2) the rarer branched clonal evolution model, in which the lymphoma is independent of the pre-existing CLL clone.[7-8] In the linear clonal evolution model, the DLBCL arises from a dominant CLL clone after having gained additional somatic mutations. Two main genetic pathways are proposed leading to the transformation of CLL to DLBCL.[9] One is the deregulation of cell cycle, characterized by TP53 inactivation, CDKN2A/B loss, and MYC activation. The second pathway is characterized by the presence of trisomy 12 and NOTCH 1 mutation. The two pathways are known to be independent and mutually exclusive.[9]

Both CDKN2A/B form complex with cyclin-dependent kinase 4 (CDK4) and cyclin-dependent kinase 6 (CDK6) to prevent their activation and therefore arrest the cell cycle at S1 phase. CDKN2A/B are frequently mutated/deleted in a wide variety of tumors. They are thought to be one of "transformation driving force" in CLL transforming to DLBCL.[9]

Similarly, NOTCH1 has emerged as one of the most commonly mutated genes in CLL, and the mutation frequency rises with disease progression.[10,11] The mutated form of NOTCH1 continuously keeps its biological activity by preventing ubiquitination/degradation, which, constantly

activating downstream target genes that are contributing tumor cell survival and resistance to apoptosis.[11] In addition, Notch 1 mutation associates with trisomy 12 in CLL and induces a particular transcriptional profiling, which triggers a high proliferative/survival advantage that leads to the clinically aggressive behavior, including Richter transformation.[10]

In this patient, Both the CLL cells and the BCL-U cells carry trisomy 12 and NOTCH1 mutation (**Figure 2** and **Table 1**), suggesting the second pathway is involved for the transformation. No CDKN2A/B or TP53 mutation was identified on the NGS profiling. However, a novel mutation, CDKN2C mutation, was identified. Similar to CDKN2A/B, CDKN2C functions as a cyclin-dependent kinase inhibitor. It interacts with CDK4 and CDK6 to prevent their activation and thus inhibits the cell cycle progression from G1 to S phase.[12] Therefore, it would be reasonable to ascribe this molecule to the first pathway, just as CDKN2A/B.

The above findings in this patient raised some interesting questions, such as 1) are the two pathways for Richter's transformation indeed mutually exclusive as reported in literature? [9] and 2) if both pathways are involved, will the transformed lymphoma be more aggressive (like in this case)?

CONCLUSION

Taken together, here we report a rare case of CLL transforming into BCL-U. We identified a novel molecule, CDKN2C, which might function similarly to the known transformation driving force molecules CDKN2A/B. We propose the involvement of both pathways, that is, deregulation of cell cycle by CDKN2C mutation and enhancing proliferation/survival by trisomy 12 and NOTCH1 mutation, contributes to transforming CLL into a more aggressive entity, such as BCL-U. Further study is needed to understand this complicated molecular process.

CONFLICT OF INTEREST

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