Mutational profile of immunoglobulin heavy chain genes of chronic lymphocytic leukemia and diffuse large B-cell lymphoma in Richter's syndrome

Ph.D. theses

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I. SUMMARY

Patients with chronic lymphocytic leukemia (CLL) may develop diffuse large B-cell lymphoma (DLBCL), also known as Richter’s syndrome. Mutational status of immunoglobulin heavy chain variable region (IgV_H) genes have prognostic impact in CLL. Patients with mutated IgV_H genes have stable disease, whereas patients with unmutated IgV_H gene have more aggressive disease. The mutational status of CLLs that transform to DLBCL is unknown. To reveal whether Richter’s syndrome occurs in CLLs with mutated or unmutated IgV_H genes, we have performed mutational analysis on serial specimens from eight patients. CLL and DLBCL tumorclones were identical in five cases and they were different in three cases. Six CLL expressed unmutated and two cases expressed mutated IgV_H genes. In five of the six unmutated CLLs, the DLBCL clones evolved from CLL tumorclones and the IgV_H genes expressed by DLBCLs were also unmutated. In one unmutated and two mutated CLLs, the DLBCL expressed mutated IgV_H genes, but in these three cases the DLBCL tumorclones developed as independent secondary neoplasm. These results suggest that Richter’s syndrome may develop in both mutated or unmutated CLLs, but clonal transformation of CLL to DLBCL occur only in the unmutated subgroup of CLL.
II. INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most common form of leukemia and mainly affects elderly individuals. The disease is characterized by the monoclonal expansion of B lymphocytes in the peripheral blood, bone marrow and lymphoid organs. The disease follows a variable course. Recently, there has been considerable progress in the identification of molecular and cellular markers that may predict the tendency for disease progression in patients with CLL. Particularly, the mutational profile of immunoglobulin (Ig) genes have been demonstrated to display strong prognostic value. Cases expressing unmutated Ig variable region (IgVH) genes have a worse prognosis than cases expressing mutated IgVH genes.

In approximately 5-10 percent of CLL patients, a diffuse large B-cell lymphoma (DLBCL) may develop over time. This process is commonly referred to as Richter's syndrome (RS). The molecular mechanisms associated with RS is a critical issue to understand the pathogenesis of these lymphomas. It has been demonstrated that DLBCL may occur in two distinct pathways in CLL: DLBCL may evolve from preexisting CLL tumorclone or develop as a secondary, unrelated neoplasm. Therefore, it has been proposed to use the term 'lymphoma transformation' denoting cases where DLBCL evolved from CLL, in contrast to the term 'composite lymphoma', indicating different clonal origin of CLL and DLBCL tumorclones.

Before modern genetic methods were available, immunophenotyping of membrane and/or the cytoplasmic Ig light chains (IgL) was used to examine the clonal relationship between CLL and RS. When neoplastic B cells of two different types carried the same IgL, RS was
suggested to progress from the original CLL. However, when neoplastic B cells expressed different IgL, RS was a de novo DLBCL.

Molecular techniques can provide convincing results for clonal relationships. Southern blot hybridization with the immunoglobulin heavy chain (IgH) gene and IgL gene was expected to be the most useful key. But with modern techniques like polymerase chain reaction (PCR) we can detect clonality using primers directed against the third complementary determining region (CDR3) of the Ig variable region (IgVH). The CDR3 region is unique in each B-cell. Clonality can be determined by sequencing the IgVH genes. The IgVH gene sequences of CLL and DLBCL samples in Richter’s syndrome have been analyzed only in a few cases before.

Our main goals were the following:

1. To search and analyze additional Richter’s syndrome cases than reported before, where both the CLL and the consecutive DLBCL sample is available.
2. To reveal whether CLL and DLBCL samples are clonally related within each case.
3. To analyze somatic hypermutation of the IgVH gene in both the CLL and the DLBCL sample.
4. Is there any relationship between the lymphoma transformation and the mutational profile of the IgVH gene in CLL?
III. MATERIALS AND METHODS

Materials

Peripheral blood, bone marrow or lymph node biopsy samples from eight patients with CLL that progressed to DLBCL were elected for this study based on the availability of frozen tissue for the molecular analyses. Diagnoses were based on histopathologic, immunophenotypic and immunogenotypic analyses according to the World Health Organization Classification of lymphoid tissues. In all DLBCL samples, the percentage of tumor cells were more than 90%. In all the eight cases, tumor cells of CLLs and corresponding DLBCLs coexpressed CD5, CD19, CD20 and CD23 antigens.

DNA isolation

Genomic DNAs were prepared from frozen tissue samples using the salting out technique. DNA concentration was measured in spectrophotometer at 260nm. DNAs were stored at 4°C.

PCR amplification of the CDR3 region

DNAs were amplified by PCR using specific sense and antisense primers to amplify the CDR3 region. PCR products were visualized using agarose gel electrophoresis.
PCR amplification, cloning and sequencing of the IgV<sub>H</sub> genes

DNAs were amplified by PCR using sense IgV<sub>H</sub> gene family specific primers in conjunction with an antisense consensus J<sub>H</sub> primer in independent reactions. PCR products were cloned in the pCR2.1-TOPO Vector using the TOPO Cloning system. After the transformation of competent cells, colonies found to contain an insert of appropriate size were sequenced by an automated DNA sequencer. The sequences obtained were compared to the corresponding germline sequences using the Immunogenetics database to estimate the respective homology of each gene with its closest germline counterpart. Where there was more than 2% deviation from the closest germline sequence, the a binomial formula was used to determine whether the replacement mutation had undergone antigenic selection.

IV. RESULTS

The clonal relationship of the CLL and DLBCL samples has been identified by PCR and nucleic acid sequence analysis of the IgH gene. All samples included in this study displayed monoclonal IgH gene rearrangement. In five cases, the first and the corresponding second biopsy samples showed identical bands of the PCR products and identical gene sequences, indicating the common clonal origin of the tumor samples. In three cases, the first and second corresponding biopsy samples displayed different patterns of the PCR products and different IgV<sub>H</sub>-D-J<sub>H</sub> sequences, indicating different clonal origin of the two tumors.
The rearranged IgVH-D-JH sequences identified for each sample seemed to represent functional rearrangements because no stop codons or crippling mutations were identified. Comparing the IgVH genes to reported germline sequences revealed that four of the eight CLL cases used VH1 family gene segments, three used VH3 family gene segments and one case used a gene segment from the VH4 family. In five cases, the DLBCLs and corresponding CLLs used the same IgVH genes. In three cases, DLBCL samples used VH genes different from the corresponding CLL samples. Two of these three DLBCLs used VH3 family gene segments and one used a VH5 family gene.

Based on the number of nucleotide differences from germline genes, the eight CLL cases could be divided into two groups. Samples in which fewer than 2% of base pairs differed from those of the consensus sequence for IgVH gene were considered unmutated, whereas cases with more than 2% of base pairs different from consensus VH gene sequence fell into the mutated group. In five cases of Richter’s syndrome where CLL and DLBCL samples were identical, IgVH genes were unmutated. In the three cases where CLL and corresponding DLBCL samples were different, the IgVH sequences of DLBCL samples showed 91.8–96.9% homology to the closest germline gene sequences. None of the tumor samples showed evidence of intraclonal IgVH gene diversity.
V. DISCUSSION AND CONCLUSIONS

The characterization of IgV<sub>H</sub> mutational status of CLL cells may provide prognostic information for a risk-adapted management of CLL patients. To determine whether Richter’s syndrome develops in CLLs that express mutated or unmutated IgV<sub>H</sub> genes, we have analyzed IgV<sub>H</sub> gene mutational status of sequential CLL and DLBCL tumor samples in eight patients where Richter’s syndrome developed during the course of the disease. However, the number of Richter’s syndrome cases analyzed in this study are small; our results demonstrate that DLBCL may develop in CLL patients with both mutated or unmutated VH genes, but DLBCLs with unmutated VH genes evolved from CLL tumorclone in contrast to DLBCLs with mutated IgV<sub>H</sub> genes that developed as clonally unrelated, secondary neoplasm. The development of DLBCLs in Richter’s syndrome comprises two alternative pathways in a biological aspect. DLBCL may evolve from pre-existing CLL tumorclone or develop as a secondary, unrelated neoplasm. Therefore, it has been proposed to use the term ‘lymphoma transformation’ denoting cases where DLBCL evolved from CLL, in contrast to the term ‘composite lymphoma’, indicating different clonal origin of CLL and DLBCL tumorclones.

The tumor samples of patients with Richter’s transformation analyzed in this study demonstrate that during the histological transformation of CLL, the IgV<sub>H</sub> gene mutational status remained stable and the mutational mechanism was not activated in Richter’s transformation.

In three of the eight cases with Richter’s syndrome, the DLBCL developed as secondary independent lymphoma, also designed as ‘composite lymphoma’. These DLBCLs developed in both CLL cases whose tumor cells displayed mutated or unmutated VH genes, but all the secondary DLBCLs expressed mutated IgV<sub>H</sub> genes with no evidence of intraclonal
heterogeneity. The mutated IgV<sub>H</sub> gene status in these lymphomas is consistent with the findings that secondary DLBCLs develop from germinal center experienced B cells; however, the CD5 expression in these cases suggests a unique way of pathogenesis. CD5-positive DLBCL may also develop 'de novo' without a prior history of CLL. The similar patterns of IgV<sub>H</sub> gene mutations found in de novo CD5-positive DLBCLs and DLBCLs developed in Richter's syndrome as secondary unrelated neoplasm suggests that both lymphomas are derived from a CD5-positive B cell that express stable mutated IgV<sub>H</sub> genes.

In summary, our findings suggest that DLBCLs developing as clonal transformation of CLLs and DLBCLs arising as secondary unrelated neoplasm in patients with CLLs represent different subgroups of DLBCLs that differ in cellular origin. These findings also suggest that different pathways of Richter's syndrome can be associated with neoplastic transformation of different B-cell subpopulation.

Original findings:

1. Lymphoma progression in Richter's syndrome occurs in both mutated and unmutated CLLs,
   - A) but only CLLs expressing unmutated IgV<sub>H</sub> genes develop clonally related DLBCL, (Lymphoma transformation)
   - B) otherwise a secondary, unrelated neoplasm (DLBCL) will develop. (Composite lymphoma)

2. Mutational profile of secondary, unrelated DLBCL developed in Richter's syndrome is similar to activated B-cell like DLBCL. Mutational profile in clonally transformed DLBCL suggests a new unique way of pathogenesis.
VI. PUBLICATION RECORD

Publications related to the thesis:


Abstracts:


