Mutational patterns of IGHV and BCL6 genes in B-cell Non-Hodgkin lymphomas

PhD thesis

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1. INTRODUCTION

B-cell non-Hodgkin lymphomas are a heterogeneous group of malignant disorders characterized by variable morphologic, immunophenotypic, genetic, and clinical features. The malignant lymphocytes in each of the entities are considered to be clonal descendants of a transformed progenitor cell arrested at various stages of lymphocyte differentiation. A highly characteristic and specific feature of physiological B-lymphocyte maturation is the rearrangement and affinity maturation of the immunoglobulin (Ig) genes ultimately producing a functionally active, antigen specific and broad B-cell receptor (BCR) repertoire. Molecular analysis of the Ig gene rearrangement is able to deliver valuable insight into the character of the different lymphoproliferative entities. At the most basic level, it provides genetic support for the monoclonal nature of the diseases by demonstrating uniform Ig gene rearrangement in all tumor cells. The in depth analysis of the somatic mutation pattern of the Ig heavy-chain gene variable regions (IGHV) provides valuable information about exposure to antigen selection in a suitable microenvironment such as the germinal center (GC).

The evaluation of the mutational status of the IGHV gene also enables us to establish the cellular origin of the malignant clone with respect to the germinal center (pre-follicular, follicular or post-follicular) supplying additional clues for lymphoma classification. The prefollicular stage is characterized by germline configuration of the IgH gene found in precursor B-cell entities, or rearranged, but unmutated IGHV sequences, for entities originating from naïve, antigen inexperienced B-cells. All mature B-cell non-Hodgkin

lymphomas harbor rearranged IgH sequences. A mutated IgH gene indicates an antigen experienced B-cell that has transited through the GC seen in the majority of mature B-cell lymphomas. The entities originating directly from the germinal center stage of development, regarded as of follicular origin, display significant intraclonal heterogeneity (same IGHV rearrangement, but variable V-region somatic mutations) indicating presence active mutational machinery leading to continuously evolving (ongoing) V-region mutations. The postgerminal, mature B-cell lymphomas contain rearranged, mutated IGHV sequences without evolving intraclonal diversity since the mutational machinery is forced to shut-down when the B-cell exits the germinal center.

The information gained from mutation analysis of IGHV genes can also be used to determine the clonal composition/relatedness of the malignant cell population allowing us to monitor the clonal evolution and disease progression. In a similar fashion, the study of aberrant somatic hypermutation, targeting key regulatory genes of lymphocyte maturation outside of the IGHV loci, can also be related to high-grade transformation and progression.

The initiation of the GC reaction is dependent on the induction of several transcriptional modulators, including BCL6. Due to its essential role in GC reactions, the BCL6 expression is tightly regulated on several levels and its expression is restricted to the GC B-cells. Its regulatory mechanisms include signaling through the B-cell receptor, stimulation of the CD40 receptor by CD40 ligands expressed on T-cells, ATM-promoted BCL6 phosphorylation followed by degradation in response to massive DNA damage, acetylation

and through an autoregulatory circuit by binding to its own promoter. The 5' non-coding region of the BCL6 gene contains a promoter region that plays a vital role this autoregularory circuit. The disruption of this region by aberrant somatic hypermutation (ASHM) has been implicated in the pathogenesis in several B-NHL.

2. OBJECTIVES

1. Determine the cellular origin of primary mediastinal B-cell lymphoma based on the mutational profile of the IGHV and BCL6 genes.

The histogenetic derivation and cellular origin of PMBL has been a matter of debate for many years in the past. Early immunophenotypic analysis suggested that these lymphomas originate from post germinal center B-cells. In later years, a germinal center derivation was proposed based on the BCL6 and CD10 expression, despite the fact that only a proportion of the cases express these antigens. Therefore, we aimed to determine the cellular origin of primary mediastinal B-cell lymphoma based on the mutational patterns of IGHV and BCL6 genes and compare it to reactive tymic B-cells.

2. Compare the mutational landscape of the IGHV gene in lymph node and bone marrow involvement of follicular lymphoma.

It has been well established that the neoplastic B-cells of follicular lymphoma retain their intraclonal heterogenity upon progression to adjacent lymphoid organs. With natural progression the lymphoma may also involve extranodal sites. We were curious to find out whether the hypermutation mechanism characterizing this entity remained active beyond borders of the germinal center.

3. Correlate the mutation pattern of IGVH gene to the various grades and progression of follicular lymphoma.

The histological classification or grading system of FL has been shown to correlate with the clinical prognosis suggesting that FL consist of lymphomas with different biological behavior. The ongoing nature of somatic hypermutations of IGHV genes in low grade follicular lymphomas is well established in the literature. On the other hand, several of our previous studies have demonstrated the lack of IGHV intraclonal heterogenity in the transformed DLBCL cases. It is not clear however, that at what timepoint during the clonal evolution of follicular lymphoma does this transition occur. In an attempt to correlate the IGHV molecular signature with the cytological grades of FL, we have analyzed the IGHV genes different cytological grades of FL.

4. Elucidate the role of the BCL6 gene alterations in the histological transformation and clonal progression of FL.

Clonal evolution and histological transformation of FL is frequently associated with accumulation of secondary genetic alterations. The common targets include the BCL6 gene, that can be altered by chromosomal translocations and mutations clustering in its 5' noncoding region. We have analyzed the BCL6 gene in sequential biopsy specimen of 12 FL cases that either showed no sign of histological progression or that underwent morphological transformation to DLBCL.

3. METHODS

To achieve our objectives, we have employed the following methods. The procedures are described in detail in the methods sections of the respective publications.

1. Immunohistochemistry

2. FACS sorting of thymic B-cells

3. PCR amplification of IGHV and BCL-6 genes with appropriate primer sets

4. Single-stand conformation polymorphism (SSCP) assay to screen for mutations

5. TA subcloning of PCR products

6. Manual and automated sequencing

7. Mutation analysis of IGHV region by Chang and Casali binomial distribution formula

4. RESULTS

1. Cellular origin of primary mediastinal B-cell lymphoma based on the mutational profile of the IGHV and BCL6 genes.

The IGHV sequences of the thymic B- cells showed 90.08% to 97.95% homology to their closest germline in 9 out of the 10 clones, compatible with mutated IGHV status and previous exposure to the somatic mutational machinery.

The PCR analysis was able to amplify V_H-D-J_H rearrangements in only five out of the six PMBL cases. One case showed an unmutated IGHV profile, while the other 4 cases showed displayed 86.1%-95.8% sequence homology with the germline, compatible with mutated IGHV status. The binomial distribution model revealed molecular evidence for clonal selection in four sequences of the thymic B-cells and in two PMBL cases.

The reactive thymic B-cells harbored a total of 22, while the six PMBL cases contained 18 BCL6 51 non-coding region mutations. An overall mutation frequency of 3×10^{-3}/bp was calculated for the PMBL cases.

2. Comparison of the IGHV mutational profile of lymph node and bone marrow involvement of follicular lymphoma.

Evaluation and comparison of the somatic mutations between the lymph node and bone marrow samples of the same

patient revealed the presence of shared and unique mutations of the IGHV gene. Some of these unique bone marrow mutations were found in multiple subclones (subclonal heterogeneity) of the bone marrow infiltrate, while they were absent in the lymph node clones. These data suggest the presence of ongoing somatic mutation in a location independent from the lymph node GC reaction.

3. Mutational pattern of IGVH gene in the different grades of follicular lymphoma

We have found that grade 1-2 FLs (low-grade FLs), and grade 3 FLs (high-grade FLs), express Ig VH-D-JH gene sequences with different patterns of somatic mutation. The presence of ongoing somatic mutation/intraclonal diversity was detected in the low cytological grades indicating that the tumor cells are still under the influence of the mutation machinery, possibly generated through interactions with their environment in the GC or GC analogous milieu. In contrast, grade 3 FLs expressed mutated, but homogenous VH-D-JH gene sequences, suggesting that the previously active mutational mechanism is already terminated, and the neoplastic cells have become less dependent on GC-like environmental stimulation for survival, and gained post follicular features.

5. The progression and transformation of follicular lymphoma is associated with aberrant somatic hypermutation of the BCL6 gene.

We found a total of 58 mutations in the 5' noncoding region of the BCL6 gene in 7/10 cases. In five cases, the mutations were present in both the original FL and the clonally related FL or DLBCL biopsies. In two cases only the DLBCL samples contained mutations, while the BCL6 gene was unmutated in the original FL. The BCL6 mutations were identical in the first and second biopsy specimens of the 2 FL cases that did not show morphological transformation. We found considerable intraclonal sequence heterogeneity in six patients where FL underwent morphological transformation, indicating an ongoing type of somatic mutation, that may possibly be related to clonal evolution..

5. CONCLUSIONS

Main findings of the thesis are the following:

1. Thymic B-cells are follicular/postfollicular cells based on the mutation profile of IGHV and BCL6 genes.

2. The somatic mutation pattern of IGHV gene support postfollicular origin of primary mediastinal B-cell lymphoma.

3. The similar mutational pattern of IGHV and BCL6 genes indicate the common origin of thymic B-cells and primary mediastinal B-cell lymphoma.

4. Low-grade FLs demonstrate ongoing somatic hypermutation in line with follicular origin, while high-grade FLs show lack of intraclonal heterogeneity suggesting termination of ongoing hypermutation compatible with post-follicular like genotype.

5. Follicular lymphoma retains the ongoing nature of IGHV mutation during bone marrow involvement suggesting the presence of active hypermutation machinery and the presence of a GC analogous suitable microenvironment in the bone marrow.

6. The progression and transformation of follicular lymphoma may be associated with aberrant somatic hypermutation of the BCL6 gene.

6. LIST OF PUBLICATIONS

6.1. Publications related to the thesis

1. **Csernus, B**., Timár, B., Fülöp, Z., Bognár, A., Szepesi, A., László, T., Jáksó, P., Warnke, R., Kopper, L., & Matolcsy, A. (2004). Mutational analysis of IgVH and BCL-6 genes suggests thymic B-cells origin of mediastinal (thymic) B-cell lymphoma. *Leukemia & lymphoma*, *45*(10), 2105–2110.

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1: first co-authorship

3. **Csernus,** B., Timár, B., Fülöp, Z. & Matolcsy, A. (2020) Grade I, II and III Follicular Lymphomas Express Ig V_H Genes with Different Patterns of Somatic Mutation. *Pathol. Oncol. Res.* **26,** 2765–2772 (2020).

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6.2. Publications not related to the thesis

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the injured blood-spinal cord barrier. *Journal of molecular neuroscience* : MN, 21(2), 173–184.