Clonal selection in the bone marrow involvement of follicular lymphoma

PhD theses

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1. INTRODUCTION

Follicular lymphoma (FL) is the most common adult lymphoma in Europe and in the United States, accounting for up to 35-40% of non-Hodgkin lymphomas, and 75-80% of low-grade B-cell lymphomas. FLs are composed of a mixture of centrocytes and centroblasts which grow in a highly organized nodular pattern. Based on the proportion of centroblasts and centrocytes within the malignant follicles, FLs are subclassified into grade I, grade II and grade III categories. The histological subclassification or grading system of FLs has been shown to correlate with the clinical prognosis of the disease.

Although FL is a clinically indolent disease, it has remained incurable, largely due to its disseminated nature at diagnosis (nearly 80% is Ann Arbour stage III-IV.). During the clinical course of the disease the neoplastic clones invade multiple nodal and extranodal sites, including the bone marrow (BM).

According to previous reports, growth pattern, immunophenotype, and the ongoing hypermutation activity of the original neoplastic clone are retained during the expansion of neoplastic cells to adjacent GCs and distant lymph nodes

(LN). However, details of tumor cell dissemination to the BM have remained largely unknown, and the biological characteristics of the tumor clone responsible for BM infiltration has not been studied.

2. OBJECTIVES

To provide further insight into the pathways of BM involvement of FL, we tried to delineate the dissemination and migration of FL cells among the LN and the BM, and to reveal the clonal evolution of BM involvement, by answering the following main questions.

- Are the morphological / phenotypical / genotypical characteristics of tumor cells of the BM and LN the same?
- Which tumor clones participate in infiltrating the BM?
- Does clonal selection take place during BM infiltration?
- Is the BM infiltration of tumor clones a continuous process?
- During which phase of the development of LN clones does BM infiltration take place?

Our results obtained during the study raised the following additional questions.:

- Does migration of tumor cells take place among the LN and the BM ?
- Is LN involvement necessary for the development of BM manifestation?

3. METHODS

To study the development of the BM involvement of FL, we compared the cytological grades, growth pattern and immunophenotype of the tumor cells from matching LNs and BMs of 21 untreated patients with FL. Furthermore, we performed simultaneous mutational analysis of the IgV_H genes of the LN and the respective BM specimens in 3 selected cases.

The phenotype of the lymphoma cells was characterized by immunohistochemical staining using a three-step avidin-biotin immunoperoxidase method and the following monoclonal antibodies: CD20, bcl-2, Ki67, CD10, p53, CD21 és CD23.

We used the mutational pattern of immunglobulin heavy chain genes to identify and follow FL tumor clones. Genomic DNAs were extracted from tissue samples of LN biopsies and BM aspirates, using the salting out technique. IgV_H genes were amplified by PCR, they were clones sequenced, and according to the obtained sequences, the phylogenetic analysis of tumor clones was carried out.

4. RESULTS AND CONCLUSIONS

In order to characterize the pathways of BM involvement in FL, we performed comparative morphological, immunophenotypical and mutational analysis of the IgV_H genes on FL samples originating from LNs and BMs. Our results show that the cytological grade, immunophenotype and mutation pattern of IgV_H genes of FL cells are frequently different in the LNs and the matching BMs. Our results also provide evidences that the BM provides a microenvironment similar to that of LNs, where tumor cells retain the ongoing nature of the mutations of their IgV_H genes, and mutations accumulate in a way suggesting that tumor cells have been selected by antigen.

The comparative morphological analysis revealed that in the majority of cases where the cytological grades of LNs are higher than grade I., the grade of the respective BM infiltrations are lower than that of parallel LN samples. These results are consistent with previous findings showing that the BM aggregates of FL are mostly composed of small centrocytes and less frequently centroblasts.

The selection of centrocytes in the BM infiltration is also supported by our immunohistochemical studies. Ki67 and p53 immunostaining revealed positivity in fewer tumor cells in

the BM infiltrates than in the matching LN samples, in concordance with the finding that proliferative activity correlates with tumor grade.

Furthermore, tumor cells in the BM expressed CD10 less frequently than in the LN. All these findings corroborate the idea that BM infiltrates have a tendency to represent a lower grade of FL than the tumor cells of the respective LNs, and suggest that clones with different phenotypes participate in the development of BM involvement.

However, the intensive clonal selection of small, centrocyte like tumor cells in the BM infiltration of FL may suggest that the interfollicular compartment of FL, which is also composed of small centrocyte like cells, preferentially involves the BM, we and other authors have found that the microenvironment of the BM is more similar to the environment of the follicle center than that of the paracortical area. The neoplastic cells in the BM are enmeshed in CD21 and CD23 positive FDCs, both in the paratrabecular and in the follicular infiltrations, similarly to the basic structure of FL in LNs. The presence of FDCs in the BM infiltration suggests that FDCs are essential components of the FL microenvironment, presumably because tumoral-nontumoral cell interactions inhibit the apoptosis of the neoplastic B cells

and they provide the necessary antigenic stimuli for the survival of tumor cells.

The ongoing type of somatic hypermutation in the IgV_H genes of neoplastic cells is a well-known feature of FL. According to our findings, the ongoing somatic hypermutation of IgV_H genes is present in the tumor cells of the BM manifestation, likewise in the LN infiltrations. The mutation frequency in clones of the BM and LN are practically in the same range, although intraclonal divergence of the LN and BM clones were not shared, indicating that the ongoing somatic hypermutation activity is independent at the two sites. In both the BM and the LN, the distribution of mutations in the IgV_H genes suggested the presence of antigen selection. These findings also support the idea that the BM provides a microenvironment where FL cells retain their biological features.

Based on the intraclonal IgV_H gene diversity observed in FL, it is assumed that the capacity of ongoing somatic hypermutation is retained during lymphomagenesis. Thus, the sequences of IgV_H genes from different tumor clones can provide data concerning the clonal relatedness and the clonal history of FL. To gain further insight into the process of clonal selection and the clonal history of the BM involvement of FL, we performed a phylogenetic analysis of the IgV_H sequences

obtained from LN and the respective BM specimens of selected cases. In all the three cases, the topology of the genealogical trees revealed homogeneous clusters of tumor clones in the BM, branching off early in the trees. These BM clusters did not contain clones deriving from LNs, suggesting that a significant part of the BM infiltration is an early event in the clonal evolution of FL, and they probably developed independently from the tumor clones detected in the LNs. These findings are highly consistent with the idea that subclones of FL may develop primarily in the BM and these clones do not “metastasize” to the LN. The development of distinct clonotypes of tumor clones in the BM and LN are consistent with previous findings showing that FL can be segregated in two distinct tumor clones, and clonal selection may occur parallelly in at least two cell populations.

FLs are characterized by extensive tumor cell migration among follicles and interfollicular zones. In the present study, we found evidences for the trafficking of tumor cells between LNs and BMs.

According to a previously proposed hypothesis, the t(14;18) translocation occurs as an error of D-J_H or V_H-DJ_H joining at the pre-B cell stage in the BM, and can be regarded as the starting-point of FL lymphomagenesis. The pre-neoplastic B-cell with the t(14;18) translocation then enters the

circulation and homes to LNs, where a second genetic hit is provided for the neoplastic transformation by antigenic stimulation through FDCs.

Another theory suggested the so-called “importing model” to explain the BM involvement of FL. In this model, LN experienced FL cells “import” microenvironment from LNs that favors the growth of tumor cells in the BM.

The results of our present study show insight to the pathogenesis of the BM infiltration of FL and provide new observations about the pathways of its development. Our results suggest that the BM involvement of FL develops through an alternative pathway. The results of the present analysis provide evidences that the BM infiltration of FL is composed of a heterogeneous tumor cell population. We identified tumor clones in the BM which are not derivatives of LN experienced clones. This suggests that circulating pre-neoplastic B-cells carrying the t(14;18) translocation, may enter the BM (homing), or they do not enter the circulation at all, but remain in the BM. These pre-neoplastic B-cells may gain antigen stimuli through FDCs present in the BM, providing a microenvironment necessary for the survival and transformation of these cells, which thus develop into tumor cells.

All together, our findings provide evidences that the BM involvement of FL is associated with intensive clonal selection of tumor cells. The BM infiltration is composed of a heterogeneous tumor cell population, its development may take place at different stages of clonal evolution. The BM provides a microenvironment for FL cells where the biological nature and the ongoing hypermutation activity of the IgV genes of neoplastic clones are retained.

Our new idea about the development of the BM involvement of FL suggests that clonal selection of tumor cells of the LN and BM may take place through different pathways. This provides a good explanation for the systemic nature of the presentation of the disease and for the fact that patients with low tumor burden in early stages of the disease present BM involvement.

5. PUBLICATIONS

Publications in the subject of the dissertation

Á Bognár, B Csernus, Cs Bödör, L Reiniger, Á Szepesi, E Tóth, L Kopper, A Matolcsy: Clonal Selection int he bone marrow infiltration of follicular lymphoma. Leukemia 19: 1656-1662, 2005. *(IF.5,81)*

B Csernus, B Timár, Z Fülöp, **Á Bognár** , Á Szepesi, T László, P Jáksó, R Warnke, L Kopper , A Matolcsy: Mutational analysis of IgV_H and *BCL-6* genes suggests thymic B-cell origin of mediastinal (thymic) B-cell lymphoma. Leukemia & Lymphoma 45 (10), 2105-2110, 2004. *(IF.1,147)*

B Timár, Z Fülöp, B Csernus ,C Angster , **Á Bognár** , Á Szepesi , L Kopper , A Matolcsy: Relationship between the mutational status of V_H genes and pathogenesis of diffuse large B-cell lymphoma in Richer's syndrome. Leukemia 18: 326-330, 2004. *(IF. 5,81)*

Publications in different subject

L Reiniger,Cs Bodor , **Á Bognár**, Z Balogh, J Csomor, Á Szepesi, L Kopper, A Matolcsy. Richter's and prolymphocytic transformation of chronic lymphocytic leukemia are associated with high mRNA expression of activation-induced cytidine deaminase and aberrant somatic hypermutation. Leukemia.: 20(6):1089-95,2006.*(IF:5,81)*

Cs Bödör, **Á Bognár**, L Reiniger, Á Szepesi, E Tóth, L Kopper, A Matolcsy: Aberrant somatic Hypermutation and expression of activation-induced cytidine deaminase, RNA in mediastinal large B-cell lymphoma. British Journal of Haematology 129: 373-376, 2005. *(IF: 3,195)*