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New molecular markers in diagnosis of splenic lymphomas

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ABSTRACT

The splenic malignant lymphomas with indolent course present numerous diagnosis controversies; the frequent involvement of viral etiopathogeny, which can be followed up both serologically as well as on the splenectomy sample, makes these lymphomas an ideal topic for further study. The phenotypical and genetic heterogenity of the splenic lymphomas made it difficult to elucidate the molecular mechanisms which concur in initiation and growth of these neoplasms. Therefore the classification, the diagnosis and therapeutic management of these patients has been frequently controversial and inadequate. In this context, new cytogenetic and molecular findings were added to existing data.

Key words: molecular markers, splenic lymphomas

The splenic malignant lymphomas with insidious evolution present numerous diagnosis controversies; the frequent involvement of viral etiopathogeny, which can be followed up both serologically as well as on the splenectomy sample, makes these lymphomas an ideal candidate for further study. The splenic lymphocyte can be characterized immunophenotypically and by molecular biology techniques; it may constitute a biological study model regarding pathogenesis of chronic lymphoproliferations in the context of genome insertions of viral homologues of some cellular oncogenes (1,2).

The splenic lymphomas that are most frequently suitable for diagnostic confusions are (2,3):

- ***Marginal zone lymphomas*** sub-classified in:
  - *mucosa-associated lymphoid tissue (MALT)*, characterised by **t(11;18)(q21; q21)** translocation which results from the

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fusion of the apoptosis inhibitor 2 (API2), also known as c-IAP2 and MALT1 (MALT translocation gene1) (4-6). Although the fusion protein **API2-MALT1** increases the activation of the signal factor to the nucleus B, by increasing the proliferation and the resistance against the FAS-induced apoptosis, its precise role in inhibiting the apoptosis has *not* been established yet. The MALT lymphomas which present this translocation are refractory to treatment against Helicobacter pylori and also present weak response to chemotherapy (2,3).

– *marginal zone lymphomas of the spleen* present complex karyotypical changes, most frequent being **del 7q** (in the region 22-32) and mutations at the level of gene p53 (17p13) and which are associated with unfavourable prognosis and weak response to chemotherapy (7,8).

The differential diagnosis between the splenic marginal zone lymphomas and the ones with a MALT phenotype can only be made by **splenectomy sample study by using molecular techniques** (2,3).

- ***Lymphoplasmocytoid lymphomas*** present the type **t(9;14)** translocations as prediction factor mentioned in literature. These mutations lead to an over expression of the genes for PAX proteins, to an excessive activation of the transcription factors and implicitly to the proliferation of the splenic B lymphocytes by the inactivation of the p53 (4-6). The changes in the expression of the **p53 protein** are considered a predictive factor in the evolution, by influencing the chemotherapy response as well. It has been suggested that the loss of cell cycle regulation, by vicious DNA repair, which leads to the inhibition of the apoptosis in the change of the p53 expression, would lead to immunosenescence with the accumulation of memory T lymphocytes (9). The decline of the activity of the immune system increases the risk of malignant lymphoproliferation. There is conflicting data in the literature regarding the influence of the p53 mutations on the progression of the disease. On one hand it was suggested that it only influences the state of the disease and not its evolution, on the other hand other studies demonstrate tight correlation with the progression of the disease (2,3,9).
- ***Mantel phenotype lymphomas*** are characterized by another protein whose modified expression can induce progression of the cell cycle from G1 to S phase, favouring lymphomagenesis, to be more precise, the **D1 Cyclin** (10-12). Overexpression of **D1 Cyclin** appears because of **t(11; 14)(q13;q32)** translocation**.** Changes in the expression of D1 cyclin were also mentioned as a predictive factor of unfavourable prognosis in other subgroups of splenic lymphomas (2,3,13,14).
- The phenotypical and genetic heterogenity of the splenic lymphomas made it difficult to elucidate the molecular mechanisms which concur in initiation and growth of these neoplasms. Therefore the classification, the diagnosis and therapeutic management of these patients have been frequently controversial and inadequate. In this context, new cytogenetic and molecular findings were added to existing data (2,3,12,15). ❑

I. CHARACTERIZATION OF MOLECULAR MARKERS IN MALT LYMPHOMA

In 1981, Levine and his colleagues were the first to report the first chromosomal anomalies of malignant lymphomas by describing t(11;18)(q21;q21) translocations. This translocation, which is the most frequent chromosomal structure alteration of low-grade MALT lymphomas, is produced by the fusion of API-2 gene, (apoptosis inhibitor-2) located on chromosome 11, with MALT gene (lymphoma-associated translocation MALT1 gene), located on chromosome 18 (Fig. 1) (4-6). API-2 gene is one of the 5 human genes which encode proteins involved in apoptotic processes regulation. The protein codified by API-2 gene has been noticed to be intensely expressed in lymphoid and thymic cells (16). The function of the protein codified by MALT1 gene is still unknown (it seems that it represents a structural homologue of immunoglobulinic domains). The function of the MALT1 gene product in the context of the transcript is also unknown, but the proportion of MALT1 sequence in the fusion gene is variable. The chimeric protein produced by API2-MALT1 fusion transcript has a demonstrated antiapoptotic function, hence the specu-

lations on the critical role played by the abrogation of apoptosis in the development of these lymphomas. Inhibition of apoptosis due to the presence of API2-MALT1 transcript may provide a survival benefit to B lymphocytes, setting them free from the control that regulates the antigen-dependant proliferation (4-6). A significant inhibition of the apoptosis could also be in favour of developing some additional genetic anomalies which can lead to transformation of low-grade extranodal marginal zone lymphomas (MZL) in aggressive large cell lymphomas (4-6). ❑

II. CHARACTERIZATION OF MOLECULAR MARKERS IN SMZL LYMPHOMAS

Generally, the number of genetic anomalies reported for SMZL remains very small, suggesting a molecular heterogeneity of this malignant disease: (a) some t(11;14) type translocations, typical for MCL, have been reported in some SMZL cases (b) structural anomalies of 7q chromosome (7q32 deletions and 7q22 translocations), usually reported for myeloid neoplasms, have also been described in relation to splenic lymphomas, especially 7q deletions (it seems that 7q deletions in SMZL play an important role in the inactivation of p53 on the path of tumoral progression) (7,8). In some studies, observation of cytogenetic alterations has been correlated to the clinical evolution and the prognosis (17,18). Thus, the t(2;8)(p12;q24) and t(14;18)(q32;q21) translocations have been reported in the case of highly aggressive SMZL. Complex chromosomal faults including 6q, 11q, 12 and 17p have been histologically associated with a high-grade conversion, while 7q and 17p deletions have been correlated with a low survival. 17p deletions, frequently encountered in human cancers, including several NH lymphoma histotypes, also affect a region of the p53 tumour-suppression gene, which is 17p13.1 locus. Incidence of mutations and expression anomalies of p53 gene have been sporadically reported in SZML, but they seem to be associated with an increased severity of the disease and worsening prognosis. Microsatellites instabilities, which are described in relation to p53 mutations for the patients with MZL and MALT, have not been described for SZML (7,8).

The frequency of deletions has been reported in 40% of SZML, in comparison with

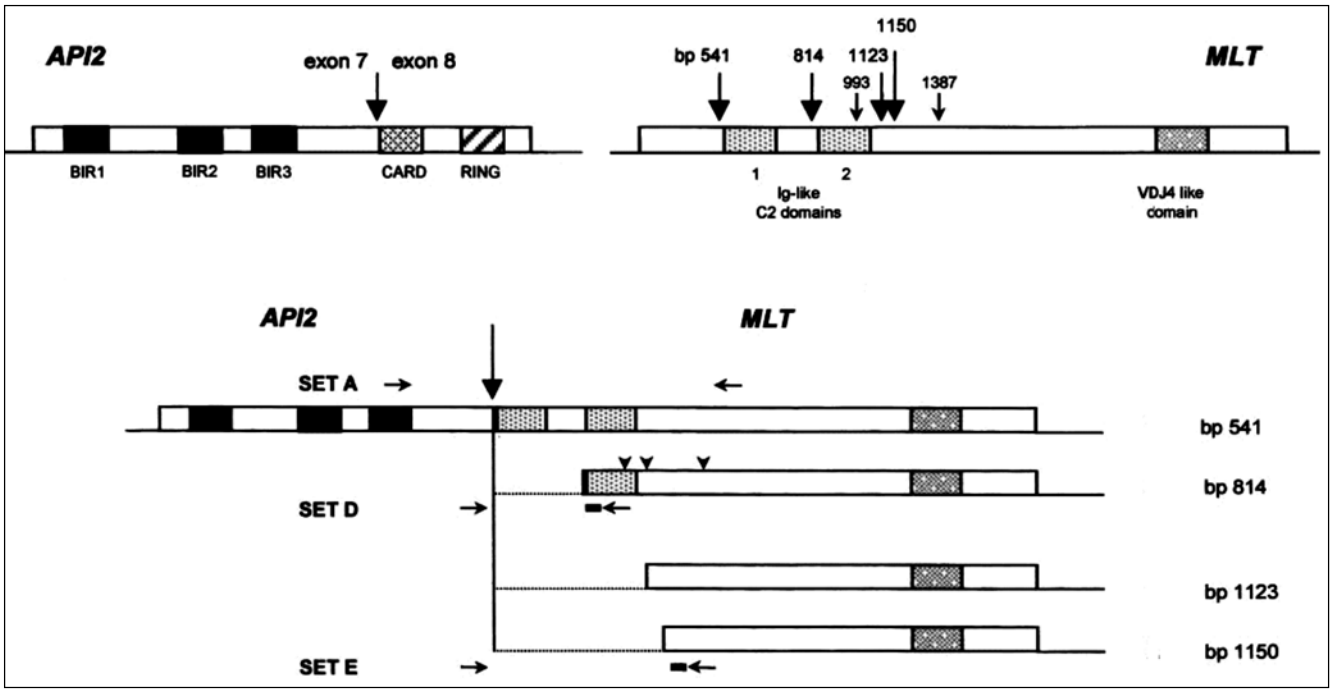

FIGURE 1. *API2-MLT* translocation. API2, MLT genes and fusion transcript types
API2 contains three repetitive domains (BIR) separated in the carboxyterminal domain (RING) by a CARD domain. The arrow indicates the breakpoint at the level of API2, observed between exon 7 and 8 in all cases. MLT gene contains 2 Ig-like C2 domains and a VDJ4 like sequence. The large arrows indicate different MLT breakpoints, the small arrows indicate the splicing sites which are used when the breakpoint occurs 814bp upstream.

only 7.7% in B-cell lymphoproliferative disorders (17,18). The deletion of D7S487 microsatellite deletion was observed most frequently (45% of SMZL); the deletions of 7q31-32 region were present in 31.2% of SZML studied cases. Yet the 7q chromosome deletions aren't entirely specific to SZML; they have also been described for other solid tumours or myeloid disorders (17,18). Frequently, observation of 7q region deletions suggests that a tumour suppressing gene, undiscovered yet, may be involved in the pathogenesis of these malignant proliferations. Future genetic studies will surely make progress in identifying the tumour suppressor from the 7q region. The relevance of this genetic anomaly in SMZL pathogeny is underlined by the fact that in different descriptions it appears to be the only genetic mutation (7,8,14). ❑

III. THE CHARACTERIZATION OF P53 GENE MUTATIONS IN SPLENIC LYMPHOMAS

In comparison to other mutations, p53 gene mutations are not as frequent in splenic lymphomas (33%), the expression of p53 protein (25%) being independent of p53 gene mutations. The frequency of p53 gene mutations reported in lymphoid malignant diseases varies between 5 to 42% (2,9). Based on the mutagen status of p53 gene and on immunohistochemically defined p53 and p21waf1 gene expressions, there are studies which describe an immunophenotype in lymphomas – the Dp53 phenotype – which is correlated in 100% of all cases with p53 gene mutations, therapeutic failure and low life expectancy and it represents about 12% of all cases. In the majority of lymphomas the instability of microsatellites could not be correlated with p53 mutations. p53 gene mutations are associated with therapeutic failure and unfavourable prognosis of many malignant diseases. p53 mutations, correlated with p53 high expression and p21 lack of expression have been suggested in fact as a marker of the aggressive tumour transformation and refractory response to treatment (9).

p53 tumour suppressing gene is the gene with the highest number of mutations in human cancers. The functions of p53 gene are essential in providing the genome stability; the gene plays the role of integrating the cellular response to DNA molecule deterioration. p53 tumour suppressing gene encodes a phosphoprotein with inhibiting properties over cancers. The development of tumours frequently implies the inactivation of p53 functions through several mechanisms, such as: losing an allele on p53 locus (17p13), deletions, insertions, punctiform mutations or silencing mechanisms started by viral or cellular proteic complexes (15). p53 mutations can be encountered anywhere in the structure of the gene, no preferential region having been described in this regard. Because of the large variety of the gene mutations and of mutant sites (over 235 codons of p53 gene), the diagnosis of p53 gene mutations is not simple; it's being reserved for fundamental research (9). ❑

IV. CHARACTERIZATION OF MUTATIONS IN MCL LYMPHOMAS

MCL associates most frequently with t(11;14)(q13;q32) type translocations, consisting of rearrangements of the BCL-1/IgH genes. This fusion transcript induces overexpression of D1 cyclin in almost 100% of all cases (10,12). Although it is not included in the WHO diagnostic criteria, this molecular marker can be very useful, especially in the differential diagnosis of other types of low-grade B-cell lymphomas. The expression level of D1 cyclin has been reported, in some studies, to be a predictive factor for unfavourable prognosis in other splenic lymphoma subgroups (10,11).

Modifications of D1 cyclin gene expression at mRNA level can be emphasized by quantitative PCR techniques like *real-time reverse transcriptase-mediated quantitative polymerase chain reaction* (RQ-PCR) (13).

Recent studies have reported that in order to formulate a molecular diagnosis of mantel cell lymphomas (MCL), D1 cyclin expression *real time* testing is much more sensitive than t(11;14) rearrangements diagnosis, because the localization of the breakpoints on chromosome 11 in t(11;14) is highly variable (13). Thus translocation identification at genomic level by PCR techniques is quite difficult and possibly because of this, it is reported in only 30-50% of all cases.

D1 cyclin is a regulator playing a critical role in the cell cycle and transcriptional processes. D1 cyclin overexpression is usually encountered in human cancers (19). TCCND1 gene generates two mRNA transcripts – D1a and D1b cyclin (10,11). Recent data indicate the fact that

D1b cyclin might be a nuclear onchogene, and that it is the only one responsible of fibroblast development. Supposedly the expression of D1 cyclin in B cells, in which the protein is physiologically absent, may be the cause of malignant transformations. The presence of D1b mRNA has been reported in two hemopathies: multiple myeloma (45-50% of all cases) and MCL (~100% of all cases where t(11;14) (q13;32) transcript is present). D1 cyclin mRNA expression can be identified with the highest sensitivity by real-time quantitative PCR (13).

The studies reported in literature indicate the fact that, considering the outline of therapeutic strategies and prognostic evaluations, the spleen NH lymphomas differential diagnosis has to be backed up by a large selection of molecular and cytogenetic research. ❑

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