Malignant Lymphomas

CARMA1 and chromosomal translocations in extranodal marginal zone B-cell lymphomas of MALT type or diffuse large B-cell lymphomas

We analyzed the configuration of the CARMA1 gene, encoding a protein that closely interacts with BCL10 and MALT1, in a series of 120 extranodal marginal zone B-cell lymphomas of MALT-type and 35 diffuse large B-cell lymphomas. Our study suggests that CARMA1 is not targeted by chromosomal translocations in these lymphoma entities.

Extranodal marginal zone B-cell lymphomas (MZBCL) of mucosal associated lymphoid tissue (MALT) type are characterized by several recurrent genetic aberrations, including trisomies of chromosomes 3, 7, 12 and 18, structural abnormalities at 1q21 and 1p34 and especially including trisomies of chromosomes 3, 7, 12 and 18, characterized by several recurrent genetic aberrations, of mucosal associated lymphoid tissue (MALT) type. In addition, they seem to target nuclear factor kappa B (NF-$\kappa$B) activation. In addition to BCL10 and MALT1 proteins, NF-$\kappa$B activation in B and T cells upon antigen receptor stimulation requires another important adaptor molecule termed CARD11/CARMA1 (caspase recruitment domain-containing guanylate kinase protein-1).

Recently, overexpression of CARMA1 was detected in gastric MZBCL of MALT type from different localizations (60 pulmonary, 40 ocular, 14 gastric, 22 salivary gland, 3 thyroid and 10 cutaneous) and 50 cases of diffuse large B-cell lymphoma (DLBCL) (15 extranodal and 35 nodal) were selected from the pathology files of the Reference Center for Lymph Node Pathology at the University of Würzburg, Germany. All tumors were carefully reviewed and classified according to the criteria of the World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues. The cases were assembled into tissue microarrays (TMA). By immunohistological staining using antibodies directed against CD20, CD5 and Ki67 it was assured, that the cores in the tissue microarrays really contained tumor.

The bacterial artificial chromosome (BAC) clones RP11-659F18, RP11-915C16, RP11-400E07, RP11-745M18 and RP11-792G24 that flank a genomic region of approximately 500 kb at the CARMA1 locus (Figure 1A) were selected according to their location, using Map Viewer at www.ncbi.nlm.nih.gov and www.ensembl.org. An interphase segregation fluorescence in situ hybridization (FISH) assay was designed by pooling RP11-659F18, RP11-915C16 and RP11-400E07, RP11-745M18 and RP11-792G24, respectively, and labeling them in different colors. The integrity of the probes was tested using metaphases from normal individuals. Segregation FISH was performed on routine paraffin sections as described previously and on tissue microarrays, adopting already established procedures for routine paraffin sections.

FISH results were obtained from 155 samples (120 MZBCL of MALT-type - 47 pulmonary, 37 ocular, 5 gastric, 18 salivary gland, 3 thyroid, 10 cutaneous - and 35 DLBCL -9 gastric and 26 nodal). The analysis of 44 samples was not possible because of insufficient hybridization or loss of tissue during the hybridization procedure. The remaining 155 lymphoma samples harbored only co-localized FISH signals, indicating the presence of an intact CARMA1 locus on 7p22 without evidence of a chromosomal break in this region (Figure 1B). Twelve cases (7 nodal DLBCL, 4 gastric DLBCL and 1 MZBCL of the lung) showed a gain of genetic material in this locus, demonstrated by more than two (three or four) co-localized FISH signals. In nine of these samples conventional karyotypic analysis had been performed previously, showing...
tetraploid karyotypes in five cases and trisomy 7 in three samples. The karyotype of the remaining case showed no gross structural alterations of chromosome 7. Altogether numerical aberrations affecting chromosome 7 were infrequent events in our series, confined mainly to DLBCL.

Constitutive NF-κB activation seems to be a unifying oncogenic event in several types of lymphoma and is triggered by various genetic and epigenetic mechanisms. Translocations deregulating BCL10 and MALT1 target NF-κB in extranodal marginal zone lymphoma, at least in a subset of cases. A recent study using global RNA interference screens identified CARMA1 expression to be crucial for survival in the activated B-cell like subtype of DLBCL and suggested that NFκB activation characteristic for this DLBCL subtype may be caused by constitutive CARMA1 signaling. Until now cytogenetic studies have not provided data on genetic aberrations involving CARMA1. However, as this gene is located on chromosome 7p22 at a subtelomeric region, it is possible that genetic alterations may have been missed using conventional G-banding analysis.

Using a FISH segregation approach we provide data regarding the genetic status of the CARMA1 locus in a large series of extranodal MZBCL and DLBCL. Our results indicate that, in contrast to BCL10 and MALT1, the CARMA1 locus is not disrupted by chromosomal breakpoints in these lymphomas. The approach we chose, however, could not exclude the presence of small genomic insertions as well as breakpoints located outside the genomic region flanked by our probes. In addition and similarly to other genes involved in lymphomagenesis, deregulation of CARMA1 may represent an end-point of other genetic alterations (e.g. mutations in essential regulatory sites) or epigenetic mechanisms (demethylation and histone acetylation). Interestingly, Oshiro and colleagues recently demonstrated a recurrent high-level amplification at the 7p22 locus in aggressive adult T-cell leukemia/lymphoma and suggested CARMA1 as a possible target gene. In our series, a genomic gain at the CARMA1 locus was identified in 12 cases. Further analyses, however, indicated that this was mostly due to underlying numerical chromosomal aberrations (trisomy 7 or tetraploidy).

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Key words: MZBCL, DLBCL, CARMA1, chromosomal translocations.

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Letters to the Editor