Chromosomal translocations involving *BCL6* in MALT lymphoma

Chromosomal translocations involving *BCL6* were detected in 7 out of 392 cases of MALT lymphoma by interphase fluorescence *in situ* hybridisation with a *BCL6* break-apart probe. With the exception of *BCL6* protein expression in 2 of the 7 cases, these *BCL6* translocation-positive MALT lymphomas were histologically and immunophenotypically identical to classic cases.

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Deregulation of *BCL6* expression by chromosome translocation and somatic mutation of its 5' non-coding region is one of the most common molecular events in B-cell lymphomagenesis. *BCL6* translocations involve not only immunoglobulin genes but also a number of non-immunoglobulin gene loci, and can occur either as a primary or secondary genetic event, largely depending on the lymphoma subtypes. These translocations bring the coding region of *BCL6* under the transcriptional control of the heterologous promoters and enhancer elements of its translocation partner genes. *BCL6* translocations are frequently seen in nodal diffuse large B-cell lymphomas (DLBCL) and in grade 3 follicular lymphomas.[1,2] At extranodal sites, *BCL6* translocations have been reported in 50% of gastric DLBCL with a residual MALT lymphoma.[3] Such translocations have also been reported in a few cases of MALT lymphoma/marginal B cell lymphoma of the stomach,[4] small intestine,[5] Waldeyer's ring, and liver.[6] However, the true incidence of *BCL6* translocation in MALT lymphoma of various sites is unknown. We investigated the presence of *BCL6* translocations in 392 cases of MALT lymphoma of the stomach (n=135), lung (n=80), salivary gland (n=63), ocular adnexa (n=57), skin (n=27), thyroid (n=15), liver (n=6) and intestine (n=9), using interphase fluorescence *in situ* hybridization (FISH) on formalin-fixed paraffin-embedded whole tissue sections (306 cases) or tissue microarray slides (86 cases). All cases were screened using a *BCL6* dual-colour break-apart probe (Abbott Laboratories Ltd, Maidenhead, United Kingdom). The positive cases were further examined for chromosome translocations involving some or all of the IGH, MALT1, BCL10 and BCL2 loci using the respective dual-colour break-apart probes, and for *IGH-BCL6* translocations using an in-house dual-color fusion probe. The cases positive for *BCL6* translocation were subjected to thorough review of histology and immunophenotype.

Seven cases (1.8%) with typical histologic and immunophenotypic features of MALT lymphoma were found to harbour a BCL6-involved chromosome translocation (Supplement table 1, Figure 1). These cases arose at various extranodal sites and none showed evidence of transformation. Of these *BCL6* translocation-positive cases, 4 (cases 2, 5-7) showed evidence of a breakage at the *IGH* locus and further FISH with a dual-colour fusion probe confirmed *IGH-BCL6* fusion, suggesting the presence of a t(3;14)(q27;q32). Long distance PCR for the *IGH-BCL6* genomic fusion was performed in case 5 where high molecular weight DNA was available,[7] and the results showed that *BCL6* was fused to the *IGH* switch region. None of the 6 cases examined showed evidence of chromosome translocations involving the *MALT1* locus. Bcl10 immunohistochemistry was carried out in 6 cases and none of them showed the strong nuclear positivity seen in cases harbouring a *BCL10* translocation. The remaining case showed no evidence of a *BCL10* translocation by FISH (Supplement table 1).

BCL6 immunohistochemistry was performed in all *BCL6* translocation-positive cases and was successful in each case as shown by nuclear staining of lymphocytes in reactive germinal centres. However, only 2 of the 7 cases showed *BCL6* staining in MALT lymphoma cells. One case (case #1) showed moderately intense nuclear *BCL6* staining in a minority of MALT lymphoma cells but, inter-

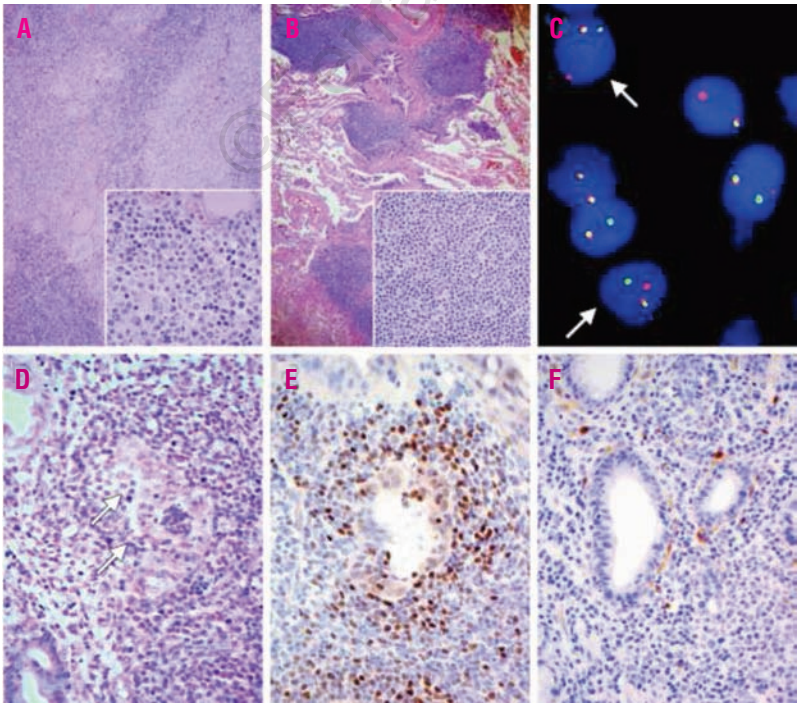

Figure 1. All cases showed the typical morphologic features of MALT lymphoma. Low and high power views of case 6 (A, thyroid) and case 7 (B, lung) are shown. All cases showed a *BCL6* translocation by interphase FISH using a *BCL6* break-apart probe (C). Case 1 (stomach) contained *BCL6*-positive, CD10-negative MALT lymphoma cells, particularly around lymphoepithelial lesions (D, H&E; E, *BCL6* immunostaining; F, CD10).

estingly, showed strong positivity in lymphoma cells in and around lymphoepithelial lesions (Figure 1). The other case (case #3) displayed weak *BCL6* staining in approximately 50% of lymphoma cells. None of the remaining 5 cases showed any *BCL6* immunostaining in MALT lymphoma cells. Irrespective of *BCL6* expression, all 7 cases were negative for the germinal centre marker CD10.

The present study provides further evidence for the existence, although rare, of *BCL6* translocations in MALT lymphomas. Based on the cases studied here, the *BCL6* translocation-positive MALT lymphomas are indistinguishable from classic MALT lymphomas on both histologic and immunophenotypic grounds with the exception of *BCL6* expression, which may be positive in cases with *BCL6* translocation. The finding of a lack of *BCL6* expression by immunohistochemistry in 5/7 cases of *BCL6* translocation-positive MALT lymphoma is intriguing. However, negative *BCL6* immunostaining has been well described in DLBCL and grade 3 follicular lymphomas carrying a *BCL6* translocation.[2,8,9] The reason for this is unclear. It is possible that in such cases the level of *BCL6* protein expression is below the threshold detectable by immunohistochemistry, but studies have also shown a lack of correlation between *BCL6* mRNA levels and *BCL6* translocation status in such cases.[10]

Given that *BCL6* is a protein of germinal centre B cells and is commonly expressed in germinal centre-derived lymphomas, it is interesting to find *BCL6* translocations in MALT lymphomas, which originate from the post-germinal centre marginal zone B cells of acquired MALT. However, neither *BCL6* translocation nor *BCL6* protein expression is associated exclusively with lymphomas with a germinal centre B cell phenotype. For example, both are found frequently in activated B cell-like DLBCL, which show features of post-germinal centre B-cells, often undergoing plasmacytic differentiation.[11] The finding of *BCL6* translocations in different lymphoma subtypes deriving from B-cells at various stages of maturation is in keeping with the diverse functions of *BCL6* during B cell development and differentiation. *BCL6*, a nuclear transcriptional repressor, plays important roles in regulating the differentiation, survival, proliferation and genetic stability of B-cells. Through these properties, in combination with the effects of other genetic changes, *BCL6* is believed to contribute to lymphoma development.

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