BCL10 gene amplification associated with strong nuclear BCL10 expression in a diffuse large B cell lymphoma with IGH-BCL2 fusion

Cytogenetic investigation of a nodal diffuse large B cell lymphoma carrying an IGH-BCL2-fusion revealed a homogeneously staining region at chromosome 1p21-22. Fluorescence in situ hybridisation (FISH) demonstrated heterogeneous BCL10 gene amplification in tumour cells. Immunohistochemistry showed heterogeneous over-expression of the protein in the nuclei of tumour cells, similar to that seen in MALT lymphoma cells with t(1;14)(p22;q32).

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t(1;14)(p22;q32) is specifically associated with MALT lymphoma and occurs in 3% of cases.[1,2] The translocation juxtaposes the *BCL10* gene to the immunoglobulin heavy chain (*IGH*) gene locus and deregulates its expression.[3] The BCL10 protein specifically links the surface antigen receptor signalling to the canonical NFκB activation pathway in both B and T cells.[4] In keeping with its physiological role, the protein is expressed primarily in the cytoplasm of normal B and T cells.[5] Intriguingly, the protein is expressed predominantly in the nuclei of MALT lymphoma cells with t(1;14).[5] Such aberrant BCL10 nuclear expression, although at a much lower level, is also seen in almost 100% of those with t(11;18)(q21;q21), and in up to 20% of MALT lymphomas lacking t(1;14) or t(11;18).[1,2] These findings suggest a role of BCL10 nuclear expression in the pathogenesis of MALT lymphoma

In general, the genes that are deregulated by chromosomal translocations involving the immunoglobulin gene locus are oncogenes, and these genes are also targeted by genomic amplification. For example, the MALT1 and FOXP1 genes that are respectively associated with t(14;18)(q32;q21) and t(3;14)(p14;q32) in MALT lymphoma and DLBCL, are also targeted by gene amplification.[6,7] Amplification of the BCL10 gene has been reported in pancreatic cancers,[8] however, it remains unknown whether the gene is also targeted for amplification in lymphoma. Here, we provide for the first time evidence that amplification of the BCL10 gene occurs also in lymphoma, although at a low frequency.

A 55 year-old male had one year of history of weight loss and presented peripheral lymphadenopathy and splenomegaly. A biopsy of inguinal lymph nodes showed a diffuse infiltration of medium-sized to large lymphoid cells (Figure 1), which expressed CD20, BCL2, BCL6, MUM1 and CD30, but not CD10. Around 80% of neoplastic cells were MIB1 positive. A diagnosis of DLBCL was made. The clinical course rapidly progressed and the patient died of the disease 10 months after initial diagnosis. Post-mortem revealed widespread of the lymphoma including involvement of the stomach, diaphragm, liver, spleen, renal, adrenal gland (left), some vertebral bodies and bone marrow.

Routine cytogenetic investigation revealed:

41~43,XY,der(1)t(1;9)(p31;q22)hsr(1)(p21~22),der(2)ins(2;?)(p11;?)add(2)(q31),add(3)(p11),-4,der(9;17)(p10;q10),der(13)t(3;13)(p11;p11),add(14)(q32),der(15)t(1;15)(p33;q23),dic(16;?)(q11;?),del(18)(q22),add(21)(p11),-22,+1~2mar[cp8]. Interphase-FISH with commercially available probes (Vysis, Downers Grove, IL) revealed evidence of an *IGH* break (17/100 cells), an *BCL2* break (12/100 cells) and a *BCL2-IGH* fusion (23/100 cells) indicating presence of t(14;18)(q32;q21). There was no evidence for a breakpoint affecting the MALT1 locus or an IGH-MALT fusion. Both metaphase and interphase FISH with YAC and BAC probes spanning and flanking the *BCL10* gene[1,3] showed high level amplification of the *BCL10* gene approximately 9% of the nuclei, which was due to the cytogenetically described hsr(1)(p21~22) (Figure 1). There was no evidence of a break at the BCL10 locus. Interestingly, the level of BCL10 gene amplification was considerably heterogeneous. Similarly, BCL10 immunohistochemistry showed heterogeneous over-expression of the protein in tumour cells, remarkably predominant in the nuclei (Figure 1).

Figure 1. A) A haematoxylin and eosin stained slide shows diffuse infiltration of medium-sized to large lymphoid cells. B) Metaphase FISH with the pooled non-chimeric YACs (876G12, 769E11 and 937C11) spanning the BCL10 locus3 and BAC probes (RP11-40K4 and RP11-1080I1 both telomeric to BCL10 are labelled in red, and RP11-1077C10 and RP11-36L4 both centromeric are labelled in green) flanking the BCL10 locus1 shows high levels of gene amplification at the BCL10 locus. Arrow: normal signal; arrowheads: amplified signals. C) Interphase FISH with BCL10 dual colour break-apart rearrangement probes1 shows markedly heterogeneous BCL10 gene amplification. D) Immunohistochemistry with mouse monoclonal BCL10 antibody shows over-expression of the protein in the nuclei of tumour cells and the extent of the protein expression is remarkably heterogeneous.

To further examine the frequency of *BCL10* gene amplification in DLBCL, we investigated 84 cases (41 extranodal and 43 nodal origin) by BCL10 immunohistochemistry, followed by interphase FISH on selected cases. Five cases (4 extranodal and 1 nodal) showed weak homogeneous BCL10 nuclear staining in majority of tumour cells, similar to that seen in MALT lymphoma with t(11;18). The remaining cases displayed negative (9 cases), weak (59 cases) or moderate (11 cases) BCL10 staining in the cytoplasm. 22 cases (15 extranodal and 7 nodal) including all 5 showing BCL10 nuclear staining were subjected to interphase FISH with *BCL10* break-apart probes and none showed any evidence for amplification or a break at the *BCL10* locus.

The remarkable correlation between the level of *BCL10* gene amplification and the extent of its protein expression in the nucleus in the index case, together with previous finding of strong BCL10 nuclear expression in MALT lymphoma cells with t(1;14), suggests that BCL10 nuclear localisation may be partially modulated by its level of expression. In keeping with this notion, the level of BCL10 expression in t(11;18) positive MALT lymphoma cells, where the protein was predominantly expressed in the nuclei, was also much higher than those in MALT lymphomas that lacked t(11;18) and expressed

the protein in cytoplasm.[9] The relative high BCL10 expression in t(11;18) positive MALT lymphomas was thought to be the result of reduced degradation by API2. API2 directly bound to BCL10 and targeted it for degradation via ubiquitination, which depended on the ubiquitin ligase (E3) activity of the C-terminal RING domain of API2.[9] In MALT lymphoma with t(11;18), one allele of the API2 gene is fused to the MALT1 gene and the resultant API2-MALT1 fusion product lacks the E3 activity, thus unable to degrade BCL10.

An association of BCL10 nuclear localisation with increased protein expression was also seen in *BCL10* transgenic mice, in which *BCL10* gene expression was driven by *IGH* enhancers. These transgenic mice showed a marked expansion of the splenic marginal zone B-cells, reminiscent of human marginal zone lymphoma.10 Remarkably, BCL10 was preferentially expressed in the nuclei of splenic marginal zone B cells.[2] Although the mechanism underlying the BCL10 nuclear expression remains elusive, growing evidence suggests that aberrant nuclear BCL10 expression might confer novel function that could play an important role in lymphoma development.

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