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Letter to the Editor

The apoptotic regulatory gene, BCL10, is mutated in sporadic mismatch repair deficient colorectal cancers

Dear Editor,

BCL10, a novel apoptotic regulatory gene, was recently cloned from a low-grade MALT lymphoma and is mutated in 45% primary lymphoid cancers and in cell lines derived from cancers of the colon, mesothelium and testicular germ cells.[1] BCL10, also described in the literature as mE10, CIPER and CLAP contains an amino terminal caspase recruitment domain, or CARD, similar to other proteins involved in the control of apoptosis. Mutations in BCL10 presumably disrupt binding of the C terminus of BCL10 to pro-caspase-9, causing abrogation of its pro-apoptotic function. BCL10 is the first CARD protein implicated in tumorigenesis. The two insertion/deletion mutations in the coding mononucleotide repeats of BCL10 in the colorectal cancer cell lines LoVo and LS513 described by Willis *et al*[1] are characteristic of the type of mutations found in mismatch repair defective colorectal cancers. These cancers are characterised by high-level microsatellite instability (MSI-H). Mutational targets of MSI-H colorectal cancers include the coding repeat sequences of CDX2,[2] the proapoptotic gene BAX,[3] genes involved in the regulation of cell growth such as the transforming growth factor β receptor type II (TGFBRII),[4] the insulin-like growth factor II receptor (IGFIIR)[5] and CHK1,[6] the transcription factors E2F4[7] and TCF-4,[8] the mismatch repair genes MSH3 and MSH6[9] and the putative mismatch repair gene MBD4.[10] The importance of defining the molecular basis of MSI-H colorectal cancers by identifying new mutation targets is emphasised by several studies showing patients with tumours displaying MSI-H have a favourable prognosis.[11,12]

Here, we analyzed a selected series of MSI-high, MSI-low and MS-stable primary colorectal cancers and microsatellite instability positive (MSI+) colorectal cell lines for mutations in BCL10. Genomic DNA extracted from frozen tissue, peripheral blood samples and cell lines was characterised for microsatellite instability at six microsatellite loci (BAT25, BAT26, 3′UTR c-myb (T)22 tract, MYCL, D2S123 and D5S346). Colorectal cancers were classified as MSI-high if three or more loci displayed microsatellite instability, MSI-low if up to two loci were MSI+ or MS-stable, if no loci displayed MSI. PCR-single strand conformation polymorphisms (PCR–SSCP) and sequencing analysis of the three BCL10 exons identified multiple mutations and polymorphism in 2 MSI-high colorectal cancers. Both MSI-high tumours (PS and AW) had 1-base pair deletions in the $(A)_8$ tract in exon 2 (136delA). The tumour (PS) also has a putative 58-2A→G splice aberration in the exon 1 acceptor site on the same allele as the 136delA mutation. A second deletion mutation in the $(T)_7$ tract in exon 3 was also detected in this tumour. Tumour (AW) had a second mutation T618A/Asp206Glu in exon 3. Neither tumour demonstrated LOH at the 1p22 BCL10 locus using the four microsatellite markers D1S236, D1S424, D1S435 and D1S497. Eight MSI+ colorectal cell lines including Co-115, HCT116, KM12C, Lisp-1, LIM1215, LIM2405, LoVo and LS174T were analyzed for frameshift mutations in the polyA and polyT tracts in exons 2 and 3 respectively using PCR and denaturing gel electrophoresis (DGE). The $(A)_8$ tract was amplified using primers 5′-GTGTATACCTGTGTGAGA-3′ and 5′-CTACTTGATGTTC-GACAAG-3′ and the $(T)_7$ tract was amplified using primer set 3.2.[1] The only additional frameshift mutation detected was found in the MSI+ cell line, LoVo (Figure 1A). Sequence analysis revealed a 1-base pair thymidine insertion at nucleotide 136 in the MSI+ cell line LoVo and not an adenine insertion as reported by Willis *et al*[1] (Figure 1B). This finding was confirmed by direct manual sequencing.

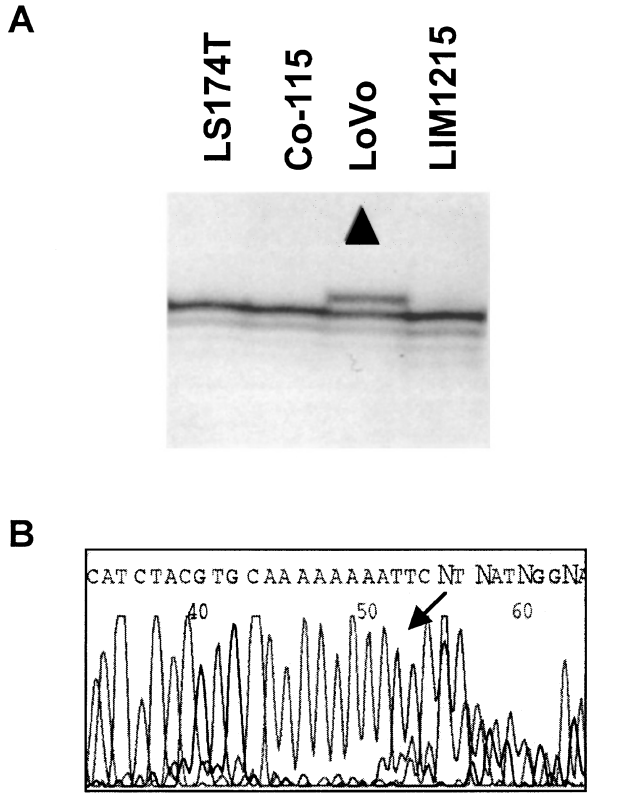

Figure 1 BCL10 frameshift mutation detected in the coding polyA tract in an MSI+ colorectal cancer cell line. (**A**) Denaturing polyacrylamide gel showing an insertion mutation in the cell line LoVo. (**B**) Direct sequence from cell line LoVo shows an insertion of one thymidine immediately following the polyA tract in exon 2

This is the first study describing BCL10 mutations in primary colorectal cancers suggesting BCL10 does play a role in the development of some MSI-high colorectal cancers, although it is not a commonly mutated gene. BCL10's pro-apoptotic function is thought to normally suppress tumour formation in a similar manner to p53 and BAX. Interestingly, tumour PS also contains a frameshift mutation in the $(G)_8$ tract in the proapoptotic gene BAX.[13] Similarly, the hMSH2 deficient cell line LoVo contains inactivating mutations in both BCL10 and BAX.[3] These results suggest that inactivating mutations in proapoptotic genes such as BCL10 and BAX are selected for during the development of MSI-high colorectal cancers underlying the importance of escape from apoptosis for this tumour subset.

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