CASE REPORT

Absence of aneuploidy and gastrointestinal tumours in a man with a chromosomal 2q13 deletion and *BUB1* monoallelic deficiency

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SUMMARY

Chromosomal instability is a potentially critical step in the development of colorectal cancer. The budding uninhibited by benzimidazole 1 (*BUB1*) gene is a highly conserved protein that plays a critical role at the spindle assembly checkpoint during cell division. *BUB1* mutations function in a dominant-negative fashion and have been implicated in causing dysfunctional kinetochore attachments, premature chromatid separation, accelerated mis-segregation of whole chromosomes and aneuploidy. *BUB1* mutations have been observed in patients with colorectal cancers. We report a remarkable case of *BUB1* haploinsufficiency owing to a 1.7 Mb deletion of chromosome 2q13 in a 54-year-old man with no prior history of carcinoma. These mutant alleles were observed in both tissue from the hand and peripheral blood. Aneuploidy was not observed on cytogenetic analysis. These findings highlight the insufficiency of *BUB1* haploinsufficiency to directly stimulate tumourigenesis, and suggest that other factors may be more critical to this process.

BACKGROUND

Clinical and basic science investigations have shown that chromosomal instability is an important step in carcinogenesis, and is more likely than microsatellite instability to be associated with colorectal cancers.^{1 2} The benzimidazole 1 *BUB1* gene encodes a highly conserved protein Bub1 (budding uninhibited by benzimidazole 1) that plays a prominent role in the functioning of the spindle assembly checkpoint, a critical checkpoint of cell division.² Bub1 localises and interacts closely with adenomatous polyposis coli (APC) as part of a mitotic checkpoint multimer complex at chromosomal kinetochores. Alterations in *BUB1* are responsible for dysfunctional kinetochore attachments, premature chromatid separation, accelerated mis-segregation of whole chromosomes and aneuploidy—all implicated in driving gastrointestinal (GI) tumourigenesis. Biallelic alterations in *BUB1* result in insufficient levels to maintain the crucial interaction with APC.³ Mutations to *BUB1* are also thought to function in a dominant-negative fashion, given that the mutant gene has been shown to confer chromosomal instability to chromosomally stable cell lines.⁴ Additionally, in mice in which expression of Bub1 is gradually reduced, spontaneous tumours developed with increased incidence in a dose-dependent fashion.⁵ In humans, haploinsufficiency of *BUB1* has been reported in a

patient with the presence of microsatellite-stable colon carcinoma at 37 years of age, in which an increased level of aneuploidy was also observed in peripheral lymphocytes.⁶ Some have advocated the clinical determination of spindle-assembly checkpoint status, primarily through *BUB1* genotyping, to guide diagnostic and therapeutic surveillance of GI neoplasias. We report a case of a man with *BUB1* haploinsufficiency with no history of carcinoma or aneuploidy.

CASE PRESENTATION

While performing an aCGH screen on diseased tissue from a series of patients with Dupuytren's disease, we encountered in a 54-year-old Caucasian male patient a 1.7 Mb deletion in chromosome 2q13 containing the *BUB1* gene. He had an unremarkable medical and surgical history notable only for Dupuytren's disease treated with surgical fasciectomy. He was married and had no children. Routine evaluation with upper GI endoscopy and colonoscopy were both unrevealing except for a small 5 mm polyp in the rectum, which was biopsied and found to be benign. The patient has nine siblings, none of whom have any history of GI problems. Both of his parents also had no history of gastrointestinal pathology and were both deceased. His father died of a cerebrovascular accident and his mother of renal failure.

INVESTIGATIONS

DNA extraction

Genomic DNA was isolated from the patient's Dupuytren's disease-affected tissue of the hand and from peripheral lymphocytes using QIAamp DNA Mini Kit (Qiagen Inc., Valencia, California, USA), according to the manufacturer's standard protocol. Genomic DNA was also isolated from the patient's father's peripheral lymphocytes using identical methods.

Oligonucleotide array comparative genomic hybridization analysis

The labelling of patient and control DNAs, hybridisation onto an oligonucleotide array slide (G4449A SurePrint G3 Human CGH 4×180K Oligo Microarray Kit, each array contains 173 341 60-mer oligonucleotide probes, Agilent Technologies Inc, Santa Clara, California USA), posthybridisation wash, image capture and signal feature extraction were performed as described earlier.⁷ Genome-wide copy number variation was analysed using Agilent's

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Figure 1 Results of array comparative genomic hybridization and Real-time quantitative polymerase chain reaction for the 2q13 region with aCGH chromosome and gene views showing a 1.7-Mb deletion (Chr2: 111 115 515—112 819 206) of chromosome 2q13 (shaded in red).

DNA Analytical (V4.0) with the built-in ADM-2 algorithm set at threshold value of 6, a cut-off value of 0.25, and a filter of six continuous probes. All base pair positions for detected genomic imbalances were designated according to the March 2006 Assembly (NCBI36/hg18) in the UCSC Human Genome browser (<http://genome.ucsc.edu/>).

Figure 2 Real-time quantitative polymerase chain reaction results of the 2q13 region. RT-qPCR was used to confirm the deletion in the patient and showed no corresponding deletion in the patient's father. Genes within the deleted intervals and the flanking regions were targeted with RT-qPCR probes in the patient (P), patient's father (F) and a normal control (C). Data represent mean $\pm$ SEM. A relative quantity of 1 and 0.5 indicates the presence of two copies and one copy in the genome, respectively.

Cytogenetic analysis

Conventional cytogenetic investigations were performed according to standard methods on cultured lymphocytes from the proband and his father. Chromosome spreads were processed for G-banding.

Real-time quantitative polymerase chain reaction assay

RT-qPCR was performed in triplicate using the M $\times$ 3000P QPCR System (Agilent Technologies Inc, Santa Clara, California, USA) to confirm copy number alterations identified by microarray analysis. The Real-time quantitative polymerase chain reaction RT-qPCR reactions were prepared using Brilliant II SYBR Green QPCR Master Mix (Agilent Technologies Inc) following the manufacturer's protocol. Five primer pairs for the targeted genes *MALL*, *BUB1* and *ZC3H6* within the deleted region and the locus in the neighbouring region and one primer pair for the reference gene *MALL* at 2q13 were designed to assess gene copy number of the 2q13 region.

On aCGH screen, the patient was noted to have a deletion on chromosome 2q13 in a region containing *BUB1* as well as several other genes including *ACOXL*, *BCL2L11*, *ANAPC1*, *MERTK* and *FBLN7* (Figure 1). The patient underwent conventional cytogenetic analyses revealing a normal karyotype and chromosomal G-banding. RT-qPCR confirmed the presence of this 1.7 Mb deletion in DNA from the skin contracture. A follow-up RT-qPCR analysis on DNA extracted from the patient's peripheral blood detected the same 2q13 deletion pattern. Combined results from aCGH and RT-qPCR involving five primer pairs (P1–P5) further defined the deletion break points between 110 337 690 and 111 111 335 in the centromeric side and between 112 819 206 and 112 820 146 in the

RT-qPCR	Primer Sets	Sequence	Position	Tm (oC)	Amplicon (bp)
Reference gene	MALL-L (forward)	CTGGCTTCAGTGAGCTTCAG	chr2:110220724-110220743	58.91	87
	MALL-R (reverse)	AAATGGCCAGTACCACTTTC	chr2:110220791-110220810	58.91	
P1	2q1-L (forward)	TGCTAAGGGAACAAGGGAAG	chr2:111111335-111111354	59.31	100
	2q1-R (reverse)	AGCAGACAACCTGGCAITTG	chr2:111111415-111111434	58.93	
P2	5'-BUB1-L (forward)	GTCAAAGTCAGGAGCGCATA	chr2:111142495-111142514	59.03	114
	5'-BUB1-R (reverse)	AAGGACCTCCTGAAGCAAA	chr2:111142589-111142608	58.91	
P3	3'-BUB1-L (forward)	GGTCTCCCTGTATCCCTGA	chr2:111114711-111114730	58.94	91
	3'-BUB1-R (reverse)	GAAAGGAAGTCATGCAGCAA	chr2:111114782-111114801	59	
P4	ZC3H6-L (forward)	GTTCTGGGTCAAGTCCAGGT	chr2:112798280-112798299	59	112
	ZC3H6-R (reverse)	GTGGTAAAGTGGGCTCTGT	chr2:112798398-112798417	59.06	
P5	2q3-L (forward)	TTGACGACGCTACATCACT	chr2:112926801-112926820	59.62	141
	2q3-R (reverse)	CAATAGTGGGCTGATTGTAGTGT	chr2:112926919-112926941	58.21

telomeric side. This clearly demonstrates a monoallelic-insufficiency of *BUB1* (Figure 2). However, the chromosome 2q13 deletion was not detected in the patient's father's peripheral blood.

OUTCOME AND FOLLOW-UP

Patient is healthy at 1 year follow-up and remains without any GI symptoms.

DISCUSSION

Our report suggests that haploinsufficiency of *BUB1* is alone insufficient to drive development of aneuploidy or colorectal cancer detectable using conventional screening methodologies. Aneuploidy was not observed in our patient with *BUB1* haploinsufficiency. Though studies have demonstrated causality between *BUB1* mutations and chromosomal instability,⁴ our observations suggest that other factors in addition to *BUB1* insufficiency may be required to promote the development of aneuploidy. The role of *BUB1* in the development of colorectal cancer remains unclear. Additionally, it has not been established whether genomic instability is the instigating factor for the adenoma–carcinoma sequence, or rather arises as a result of the tumorigenic process.¹ Thus, a more detailed understanding of the *BUB1* pathway genomic is needed before intervention can be advocated on the basis of *BUB1* haploinsufficiency. A more cautious and complete understanding of the basis of chromosomal instability of the cancer genome needs to be achieved before new diagnostic, prognostic and therapeutic modalities can carry the promise of more effective medical treatment strategies for colon cancer.

The *BUB1* gene is an exciting area of cancer biology research. Microdeletions of 2q13 involving the *BUB1* gene have been recognised as recurrent genomic aberrations in phenotypically normal family members and control individuals mediated by regional highly homologous low copy repeats or segmental duplications via non-allelic homologous recombination.⁸ Evolutionarily, chromosome 2 is the result of a telomere–telomere fusion point of two ancestral chromosomes, located to the 2q13 band.⁹ At this fusion point, an array of TTAGGG sequence repeats within and between proximal 2p and 2q can trigger DNA rearrangements. This region of segmental duplications is extremely unstable and prone to breakage, fragility and recombination.¹⁰ The significance of these chromosomal characteristics is not well understood, though the highly conserved

nature of *BUB1* argues for the critical role of Bub1 in the process of cell division. More studies are needed to better characterise the role of *BUB1* in tumourigenesis and elucidate its upstream and downstream interactions.

Learning points

- ▶ *BUB1* haploinsufficiency alone appears to be insufficient to directly stimulate tumourigenesis.
- ▶ *BUB1* genotyping may not be necessary to guide diagnostic and therapeutic surveillance of gastrointestinal neoplasias.
- ▶ *BUB1* mutations are associated with chromosomal instability, though other factors may be required to promote development of aneuploidy.

Competing interests None.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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