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Research paper

An investigation of the potential for epigenetic inactivation by transcription read-through in a sporadic colorectal cancer

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ABSTRACT

Aberrant transcription read-through of a gene promoter as a result of genetic structural rearrangements can cause the epigenetic inactivation of a neighbouring gene. All reported cases have involved copy number alterations that remove the 3′ poly(A) transcription terminator sequence of a gene leading to transcription read-through (TRT) and methylation of the gene promoter of a downstream gene. We aimed to determine whether deletion of poly (A) transcription terminator sequences was associated with the methylation of neighbouring genes in a CRC with extensive copy number alterations. We performed a high resolution CGH array and methylation analysis on a CRC specimen to identify such alterations. Analysis of the CRC using high-resolution CGH identified 6 genes with deletions in the 3′ part of the gene that encompassed the poly(A) transcription terminator sequence. Bisulphite sequencing of the promoter region of neighbouring (affected) genes at these six regions showed all candidate genes were unmethylated. Considering the fact that six TRT affected genes in a CRC with multiple deletions show no signs of hypermethylated promoters, it would be fairly appropriate to suggest that epigenetic inactivation by TRT might be a rare phenomenon in sporadic CRCs.

1. Introduction

In cancer, aberrant CpG island promoter methylation can result in the transcriptional silencing of tumor suppressor genes (TSGs) (Jones, 2012), also known as epigenetic inactivation. Transcription of a gene usually ends at a specific termination point, the poly(A) transcription termination signal, which normally prevents RNA polymerase from reading through the next gene (Fig. 1A) (Akiva et al., 2006). Deletion of the poly(A) transcription termination signal of a gene can lead to the epigenetic inactivation of a neighbouring gene by a mechanism known as transcription read-through (Hesson et al., 2010). This is known to occur in the regulation of some imprinted genes, including *Igf2r*, *UBEA3*, and *LIT1* (Wutz et al., 1997; Rougeulle et al., 1998; Mitsuya et al., 1999) and it is a known cause of α-thalassaemia (Barbour et al., 2000). Here, a deletion of the 3′ part of the *LUC7L* gene juxtaposes it to the hemoglobin alpha 2 (*HBA2*) gene causing antisense transcription read-through and methylation of the *HBA2* promoter (Fig. 1C) (Tufarelli et al., 2003). The exact mechanism by which transcription read-through leads to epigenetic inactivation is unclear but one possible mechanism is that it directly interferes with transcription of the gene on the opposite DNA strand (Tilghman, 1999). Aberrant transcription read-through has also been linked to predisposition to other disease including colorectal cancer (CRC). In several families with predisposition to CRC, epigenetic inactivation of the MutS homolog 2 (*MSH2*) gene co-segregated with an at-risk haplotype that included deletions of the poly(A) transcription termination signal of the gene *EPCAM* (epithelial cell adhesion molecule) (Kovacs et al., 2009; Ligtenberg et al., 2009; Niessen et al., 2009; Kuiper et al., 2011). *EPCAM* is highly expressed in epithelial tissues such as the colorectal mucosa and is located immediately upstream of *MSH2*. *EPCAM* deletions result in *EPCAM–MSH2* fusion transcripts and promoter methylation of *MSH2* by transcription read-through of the *MSH2* gene promoter (Ligtenberg et al., 2009).

The examples described above are caused by a structural alteration in the form of a germline CNV that removes the 3′ part of a gene (Fig. 1). Copy number alterations are frequent in the development of sporadic cancers, therefore we questioned whether deletions of the end of a gene correlated with methylation of the promoter of downstream genes. This model would be consistent with transcription read-through if the gene with the deletion is normally expressed.

In this study, we investigated a CRC specimen with extensive copy number alterations to determine whether promoters located downstream of expressed genes with deletions undergo methylation.

Abbreviations: CGH, Comparative Genomic Hybridization; CRC, Colo-Rectal Cancer; CNV, Copy Number Alteration; TRT, Transcriptional Read Through; TSG, Tumor Suppressor Gene.

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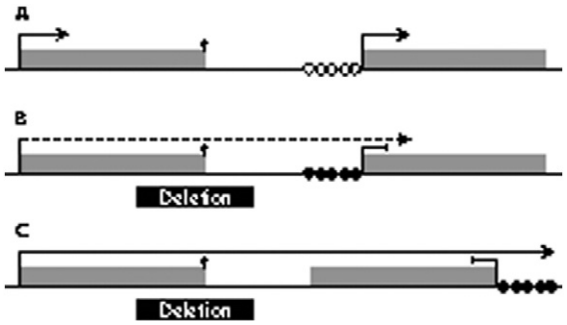

Fig. 1. Methylation of a promoter by transcription read-through. A, Normally the poly(A) transcription termination signal of a gene (asterisk) prevents RNA polymerase from transcribing through the next gene. Both genes are expressed and the promoter of the downstream gene is unmethylated (white circles). B, Deletions that include the poly(A) transcription termination signal can lead to continued transcription through the promoter of the next gene resulting in methylation (black circles) and transcriptional silencing (an example of this is described in reference (Ligtenberg et al., 2009)). C, Antisense transcription read-through of a gene promoter by the same mechanism can also cause promoter methylation and inactivation of gene expression (an example of this is described in reference (Tufarelli et al., 2003)).

2. Materials and methods

2.1. Tumour sample

A 35 mm lesion from the transverse distal colon and a biopsy of rectal mucosa was resected from a male patient using endoscopic mucosal resection (EMR) at Westmead Hospital, Sydney (ethics approval number 2009/6/4.6 and 11194) and used for Comparative Genomic Hybridisation (CGH). Histological examination using haematoxylin and eosin staining showed submucosal invasion.

2.2. Comparative genomic hybridisation (CGH)

Normal and tumour DNA were hybridized separately against normal human reference DNA using the human 720 K CGH Microarray platform (Nimblegen, Roche). Data was visualized using SignalMap software v2.0 (Nimblegen, Roche) and loci with tumour-specific log2 ratio values below −0.2 were identified as deleted.

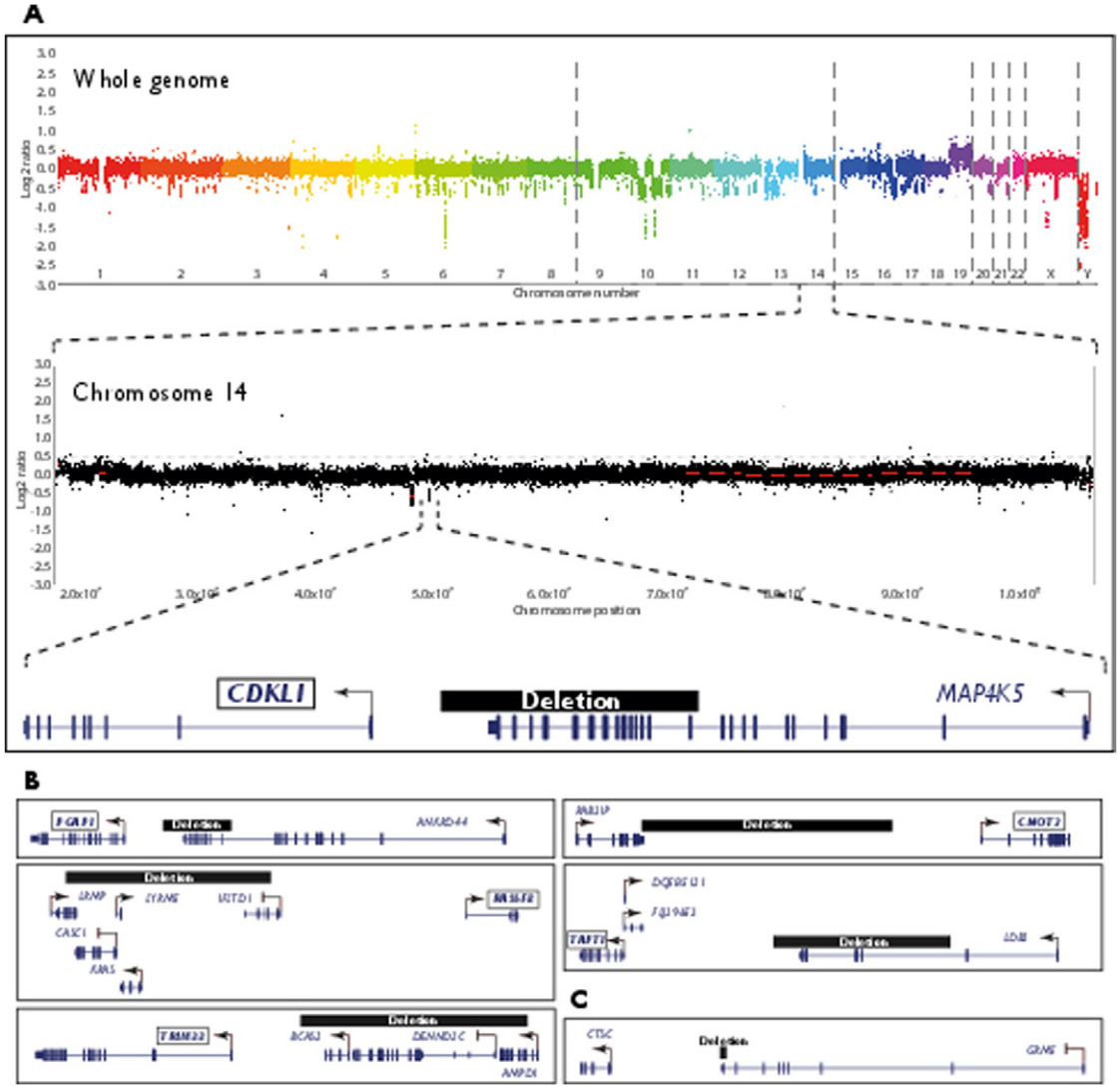

Fig. 2. CGH identifies candidate genes for inactivation by transcription read-through in a CRC. A, Whole genome copy number profile (top panel) showing widespread chromosome instability with multiple deletions across the genome of a CRC specimen. Bottom panel shows a 48 kb deletion on chromosome 14 (black horizontal bar), which removes the 3′ region of the *MAP4K5* gene. B, The genomic context of the remaining 5 candidate genes (*PGAP1*, *CNOT2*, *RASSF8*, *TAPT1* and *TRIM33*) as well as C, An example of a gene eliminated as a candidate (*CTSC*) based on the lack of expression of the gene with a terminal deletion (*GRM5*) in normal colorectal mucosa. In each panel candidate gene names are boxed. Arrows indicate gene expression status (see also Fig. 3) with blunt ended arrows indicative of transcriptionally silent genes and arrow heads indicative of expressed genes.