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Truncation of MBD4 predisposes to reciprocal chromosomal translocations and alters the response to therapeutic agents in colon cancer cells

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ABSTRACT

We previously identified a novel genomic instability phenotype of multiple reciprocal chromosomal translocations in a MLH1-defective, microsatellite unstable (MSI) colon cancer cell line (HCA7) and, further, showed that it was unlikely to be directly caused by the mismatch repair (MMR) defect in this cell line. To gain insight into the molecular basis to this novel translocation phenotype, we examined coding and splice-site nucleotide repeat tracts in DNA repair genes for mutations by direct sequencing together with RT-PCR expression analysis of the associated transcript. The material was a selected panel of 8 MSI cell lines including HCA7. A strong candidate identified through this approach was *MBD4* as it showed a homozygous truncating mutation associated with substantial loss of the transcript in HCA7 not seen in the other lines. In previous published studies, heterozygous *MBD4* mutations were observed in up to 89% of sporadic MSI microdissected colon tumor foci. Using MFISH, we show that over-expression of the truncated MBD4 (+MBD4^{tru}) in DLD1, a MSH6 defective, MSI human colon carcinoma cell line predisposed these cells to acquire structural chromosomal rearrangements including multiple reciprocal translocations after irradiation, reminiscent of those seen in HCA7. We also show that over-expression of MBD4^{tru} in DLD1 alters the colony survival after exposure to cisplatin or etoposide. These data suggest a wide role for MBD4 in DNA damage response and maintaining chromosomal stability.

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1. Introduction

Using 24-color FISH technology (SKY, spectral karyotyping and MFISH, multiplex florescent *in situ* hybridization) we have identified a novel chromosomal instability pattern, distinct from the previously described numerical chromosomal instability (CIN) by Lengauer et al. [1], in 2 out of 7 mismatch repair (MMR) defective colon cancer cell lines [2]. In both these lines we showed multiple reciprocal translocations with little numerical change or variability and, particularly in the

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line HCA7, the translocation events appeared to be ongoing, since in some metaphases additional translocations were seen. This was an unusual observation in carcinoma cells in which most reported translocations are non-reciprocal [3]. None of MMR proficient lines that we examined using the same technique showed such pattern of genomic instability [2]. An important anti-carcinogenic function of the MMR system, is its role in preventing recombination between similar, but non-identical (homeologus) sequences, with the purpose to prevent genome rearrangements resulting from interactions between repetitive elements [4,5]. We, therefore, further corrected the MMR defect in a set of isogenic cell lines derived from HCA7 and tested the effect of this manipulation on the reciprocal translocation phenotype. The results showed that MMR defect was not necessary for the development of reciprocal chromosomal translocations [6] but might be permissive in the presence of as yet unidentified defect in DNA repair?

The first aim of the present investigation was to identify the molecular defect(s) associated with the novel reciprocal translocation phenotype. A plausible sequence of events that may explain the association between multiple reciprocal translocations and MMR deficiency would be that MMR defect targets DNA repair gene(s) that contain repeat tracts which, on the background of MMR deficiency, results in the balanced translocation phenotype. In support of this hypothesis, some DNA repair genes were shown to be particular mutation targets in MMR-deficient cells [7–9]. One of the DNA repair genes found to be mutated in MMR-deficient colorectal cancer cells is *MBD4* [10,11].

MBD4 is a methyl-CpG binding DNA glycosylase involved especially in the repair of mismatches arising from deamination of methyl-C in mammalian cells. MBD4 has been shown *in vitro* to excise mismatched thymine (T) bases from oligo templates [12–14]. MBD4 can also bind to MLH1 and Fas-associated death domain (FADD) proteins [15], and the small intestine of $Mbd4^{-/-}$ mice show reduced apoptosis in response to a variety of DNA-damaging agents [15–17]. Absence of *Mbd4* in mice also increases tumorigenicity on the tumor-susceptible Apc^{min} background [18,19]. The form of mutation of *MBD4* seen naturally in human cancers (as opposed to in experimental mouse systems) is a frameshift of a polyadenine tract due to the MMR defective background. This leads to a premature truncation of the MBD4 protein. Studies of the truncated protein have shown that it acts in a dominant negative way, competitively inhibiting normal glycosylase activity of wild type MBD4, and when over-expressed in cells it increases mutation frequency [20].

MBD4 is present only in mutant form in the HCA7 cell line [20] suggesting that MBD4 could play a role in the generation of the sort of chromosomal instability seen in HCA7. We addressed this question first by comprehensively screening a number of MSI cell lines for microsatellite alterations in a large number of DNA repair genes. We then tested the effect of truncated MBD4 on chromosomal instability and response to DNA-damaging agents in cells with or without exogenously expressed mutant MBD4 protein.

2. Materials and methods

2.1. Cell lines, clones and culture

The colon cancer cell lines HCA7, LoVo, HCT116 were cultured as before [2], RKO and SW48 were cultured in EMEM (containing 2 mM L-glutamine and 10% FBS); HCT15, and LIM1215 in RPMI1640 (containing 20% FBS). DLD1 and all subclones (the latter generated as described in Bader et al. [20]) were cultured in RPMI 1640 containing 5% FBS. The endometrial cancer cell line HEC59 was cultured in DMEM/F12 containing 20% FBS. (All tissue culture reagents were from Invitrogen–GIBCO, Europe.) All cell lines have previously been reported to be defective for at least one of the MMR genes *MLH1*, *MSH2*, or *MSH6* and show MSI [21–25].

2.2. Mutation analysis of DNA repair genes

Table 1 shows the repair genes and repetitive tracts examined in this work. DNA was extracted from cell lines using DNeasy kit (Qiagen, Valencia, CA) and fragments were PCR amplified using the high fidelity polymerase “Phusion” (Finnzymes; Espoo, Finland) according to manufacturer’s protocol. The primers used are supplied in Supplementary Table. PCR products were submitted for direct sequencing using ABI3730 sequencer/genotyper (Applied Biosystems, Europe). The material for this analysis was 7 MSI colon cancer cell lines (HCA7, LoVo, RKO, HCT15, HCT116, SW48, LIM1215) and one MSI endometrial cancer cell line (HEC59). In addition, two anonymous normal samples (a lymphoblastoid line and a blood DNA) were included in all analyses as control.

2.3. Expression analysis

RNA was isolated from cell lines using RNAeasy minikit (Qiagen, Chatsworth, CA). Reverse transcription of isolated RNA was done by using Superscript RT (Life Technologies, Rockville, MD). Primers used for monitoring expression and/or exon skipping are supplied in the supplementary table. GAPDH was used as an internal control in the same reactions in every analysis. PCR amplification was performed in duplicates one for 25 and another for 30 cycles. After electrophoresis on 2% SeaKem LE Agarose (Cambrex, Rockland, ME) gels, the DNA repair gene-specific amplification products were visually inspected for possible exon skipping and reduced or lost expression relative to GAPDH.

2.4. WRN gene mutation analysis

WRN cDNA was examined for mutation in overlapping fragments using the “second amplification primers” as described by Oshima et al. [26].

2.5. γ-Irradiation

This experiment was performed on the earliest available passages of the 4 DLD1 sub-clones. Exponentially growing cells were trypsinised and 10^3 cells were exposed to 1, 3 or

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