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Instability of a dinucleotide repeat in the 3′-untranslated region (UTR) of the microsomal prostaglandin E synthase-1 (*mPGES-1*) gene in microsatellite instability-high (MSI-H) colorectal carcinoma

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ABSTRACT

DNA mismatch-repair gene mutations, with consequent loss of functional protein expression, result in microsatellite instability (MSI). Microsatellite sequences are found in coding regions and in regulatory regions of genes (i.e., 5′-UTRs and 3′-UTRs). In addition to being a surrogate marker of defective mismatch repair, deletion or insertion microsatellite sequences can dysregulate gene expression in MSI-H (microsatellite instability-high) tumors. The microsomal prostaglandin E synthase-1 (*mPGES-1*) gene product, mPGES-1, participates in prostaglandin E_2 (PGE_2) production. Moreover, mPGES-1 is often overexpressed in human colorectal tumors, and is thought to contribute to progression of these tumors. Here we identified a dinucleotide repeat, $(GT)_{24}$, in the *mPGES-1* gene 3′ untranslated region (3′-UTR), and analyzed its mutation frequencies in MSI-H and microsatellite stable (MSS) tumors. The $(GT)_{24}$ repeat exhibited instability in all MSI-H tumors examined (14), but not in any of the MSS tumors (13). In most cases, $(GT)_{24}$ repeat instability resulted in insertion of additional GT units. We also determined *mPGES-1* mRNA levels in MSI-H and MSS colorectal cancer cell lines. Three of four previously designated "MSI-H" cell lines showed higher *mPGES-1* mRNA levels compared to MSS cell lines; correlations between elevated *mPGES-1* mRNA levels and microsatellite $(GT)_{24}$ repeat characteristics are present for all six cell lines. Our results demonstrate that *mPGES-1* is a target gene of defective mismatch repair in human colorectal cancer, with functional consequence.

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1. Introduction

mPGES-1, the protein product of the microsomal prostaglandin E synthase gene (*PTGES*, *mPGES-1*), converts prostaglandin H_2 (the product of both cyclooxygenase 1 and cyclooxygenase 2) to prostaglandin E_2 (PGE_2). Elevated PGE_2 levels are postulated to play a role in colorectal cancer pathogenesis. Increased mPGES-1 protein levels occur in human colorectal adenomas and adenocarcinomas, suggesting *mPGES-1* may play a role in early stages of colon tumor development. (Yoshimatsu et al., 2001; Kamei et al., 2003) *mPGES-1* overexpression in human colorectal cancer (CRC) cell lines leads to increased cell proliferation and increased PGE_2 production, while global *mPGES-1* gene deletion reduced significantly the total number of intestinal polyps in $Apc^{\Delta 14/+}$ mice. (Kamei et al., 2003; Nakanishi et al., 2008) In related studies, *mPGES-1* global deletion reduced the number of aberrant crypt foci, number of polyps, and the size of those tumors that did form in a carcinogen-induced colon cancer mouse model. (Nakanishi et al., 2008, 2011; Sasaki et al., 2012) Taken together, these observations provide strong evidence for a role of *mPGES-1* in colon carcinogenesis.

Mismatch-repair (MMR) deficiency, due to mutation or loss of expression of one or more of the DNA mismatch repair genes, occurs in both familial and sporadic human CRCs. In familial CRCs such as Lynch syndrome, MMR-deficiency is caused by germline inactivating mutations in the *hMLH1*, *hMSH2*, *hMSH6*, and *hPMS2* DNA repair genes; in sporadic CRCs epigenetic modifications such as promoter hypermethylation of the *hMLH1* gene account for the MMR-deficiency. (Marra and Boland, 1995; Herman et al., 1998) As a result of MMR-deficiency, microsatellites (mono, di-, tri-, and tetranucleotide repeats) located in both intergenic regions and in either the coding or regulatory regions of genes may undergo instability, resulting in insertion or deletion of these repeating units. Microsatellite instability (MSI) is seen in ~15% of sporadic CRCs and in most tumors associated with Lynch syndrome. (Marra and Boland, 1995) Instability of microsatellites located in the 5′-untranslated regions (5′-UTR), coding regions, and 3′-untranslated regions (3′-UTR) of genes has been associated with altered gene expression, suggesting MSI represents a mechanism for dysregulating gene function. (Parsons et al., 1995; Baranovskaya et al., 2009; Paun et al., 2009; Yuan et al., 2009; Kim et al., 2013) In this study, we identified a dinucleotide repeat region located in the 3′-UTR of the *mPGES-1* gene and investigated whether the *mPGES-1* gene is a target of defective MMR in human colorectal cancer.

2. Materials and methods

2.1. Microsatellite stable (MSS) and microsatellite instability-high (MSI-H) tumor samples

Twenty-seven de-identified tumor DNA samples, 13 MSS and 14 MSI-H, and their matched normal mucosa were examined in this study. All samples were analyzed previously for their MSI status with the National Cancer Institute (NCI)-recommended reference panel of five microsatellite markers (*BAT25*, *BAT26*, *D2S123*, *D5S346* and *D17S250*) in our clinical laboratory (Boland et al., 1998). Tumor samples exhibiting novel length alleles (i.e., insertion or deletion mutations) at two or more of the five markers were characterized as MSI-H; tumors exhibiting no length changes at all five markers were characterized as MSS (Boland et al., 1998).

2.2. Colorectal cancer cell lines

Four reported MSI-H (HCA7, HCT116, LoVo, and LS174T) and two reported MSS (Caco2 and SW620) CRC lines were used in this study (Di Pietro et al., 2005; Jung et al., 2009; Williams et al., 2010). All cell lines were maintained in Dulbecco's modified eagle's medium (DMEM) at 37 °C and 5% CO_2.

2.3. $(GT)_{24}$ repeat genotyping and microsatellite instability analysis

For tumor samples, DNA was isolated from the microdissected MSS/MSI-H tumors and their matched normal mucosa, using the standard protocols in our clinical laboratory. For CRC lines, DNA was isolated using the Qiagen DNeasy mini kit (Qiagen, Carlsbad, CA). The $(GT)_{24}$ repeat region located in the 3′-UTR of the *mPGES-1* gene was amplified with the following primers: forward primer 5′-GAAACTGCAAATGTCCCCTTGAT-3′ and the reverse primer 5′-CACATCTCAGGTCACGGGTCTA-3′ (6-FAM labeled). The primers are expected to amplify a PCR fragment of 109 bp if there is no expansion or contraction of the repeat (see Figure 1). The PCR amplification conditions used included 35 cycles with a 55 °C melting temperature, and a 30 s extension. Amplified fluorescent PCR products were mixed with formamide and GeneScan™ ROX™ size standard, denatured, and subjected to capillary electrophoresis on an ABI 3130xI Genetic Analyzer. Data were analyzed with GeneMapper Fragment Analysis Software (Applied Biosystems). Each tumor $(GT)_{24}$ repeat microsatellite profile was compared to the profile for its matched normal mucosa. Tumors were characterized as MSI when the tumor sample exhibited a PCR product that demonstrated an elongation and/or contraction when compared to the PCR product from the matched mucosal sample.

2.4. Quantitative real time RT-PCR for mPGES-1 expression

Total RNA was isolated from the six CRC lines, using the Qiagen RNeasy mini kit (Qiagen, Carlsbad, CA). Complementary DNA (cDNA) was synthesized using the iSCRIPT cDNA synthesis kit (BioRad, Hercules, CA), using 50 ng of cDNA per reaction as template. Quantitative real time RT-PCR was performed using SYBR green PCR master mix (Applied Biosystems). The *mPGES-1* mRNA levels were normalized to β−actin levels and the relative expression of *mPGES-1* mRNA levels was determined by the comparative CT method (Livak and Schmittgen, 2001).