HHS Public Access

Author manuscript

*Nat Med*. Author manuscript; available in PMC 2012 August 01.

Published in final edited form as:

*Nat Med*. ; 18(2): 224–226. doi:10.1038/nm.2608.

# Prostaglandin $E_2$ promotes intestinal tumor growth via DNA methylation

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## Abstract

Although DNA methylation is one of the critical ways for silencing tumor suppressor and DNA repair genes during tumor initiation and progression, the mechanisms underlying DNA methylation in cancer remain unclear. Here we show that prostaglandin $E_2$ ($PGE_2$) silences certain tumor suppressor and DNA repair genes via DNA methylation to promote tumor growth. These findings uncover a previously unrecognized role for $PGE_2$ in the promotion of tumor progression.

Evidence for the link between inflammation and cancer comes from epidemiologic and clinical studies showing that use of nonsteroidal anti-inflammatory drugs (NSAIDs) reduces the relative risk for developing colorectal cancer (CRC) by 40–50%. NSAIDs exert one of their anti-inflammatory and anti-tumor effects by targeting a prostaglandin-endoperoxide synthase 2 (PTGS2). The PTGS2-$PGE_2$ signaling plays a key role in CRC progression[1,2]. The observations showing a positive association between PTGER2 and CpG island methylator phenotype (CIMP) in CRC and an inverse correlation between NSAIDs use and CIMP in CRC[3,4] prompted us to postulate that $PGE_2$ may promote tumor growth by affecting DNA methylation machinery in CRC.

We first examined the correlation between the levels of PTGS2, $PGE_2$, and DNA methyltransferases (DNMTs) in human CRC and found that the $PGE_2$ levels and PTGS2 expression are positively correlated with *DNMT1* and *DNMT3B* expression in CRC specimens (Supplementary Fig. 1). We found that $PGE_2$ treatment reversed the effect of a PTGS2 inhibitor celecoxib on downregulation of DNMT1 and DNMT3B in HT-29 cells (Supplementary Fig. 2a), indicating that $PGE_2$ regulates DNMT expression. Indeed, $PGE_2$



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**Author Contributions**

R.N.D., D.W., and D.X. designed this research project; D.X. performed most of the experiments; S.H.K. contributed to establish the DNMT1 and DNMT3B knockdown stable cell lines and H.K. conducted the DNA methylation analysis for human tissues samples; D.X., and D.W. conducted the data analyses; D.W. wrote the manuscript with D.X.'s help; and R.N.D. supervised the project.

**Competing financial interests**

All authors do not have any competing financial interests.

directly upregulated DNMT1 and DNMT3B protein expression (Fig. 1a) but not other DNMTs (**data not shown**) in three human CRC cell lines.

Based on the observations that the CGI hypermethylation is detected in the promoters of certain tumor suppressor and DNA repair genes in human CRC[5,6], we examined and found that $PGE_2$ enhanced the CGI methylation in the promoters of cannabinoid receptor 1 (*CNR1*) and O-6-methylguanine-DNA methyltransferase (*MGMT*) (Fig. 1b) as well as *CDKN2B* and MutL homolog 1 (*MLH1*) genes (Supplementary Fig. 2b,c) in LS-174T cells. *CNR1* is silenced by CGI methylation in human CRC and acts as tumor suppressor *in vivo*[7]. $PGE_2$ also increased CGI methylation in the promoters of *BAX*, *CHEK2*, *NOTCH1*, *CAV1*, *NHS*, *MYOD1*, and *TMEFF2* (**data not shown**). As expected, $PGE_2$ downregulated the expression of CNR1 and MGMT (Fig. 1c) as well as CDKN2B and MLH1 (Supplementary Fig. 2d) at both mRNA and protein levels in LS-174T cells. Subsequently, we found that only a PTGER4 antagonist (ONOAE-208) blocked the effect of $PGE_2$ on DNMT1 and DNMT3B expression but not a PTGER1 antagonist (SC19220) or a PTGER1-3 antagonist (AH6809) (Fig. 1d). Moreover, knockdown of DNMT1 or DNMT3B by shRNAs attenuated the $PGE_2$-induced downregulation of CNR1, MGMT, CDKN2B, and MLH1 in LS-174T cells (Fig. 1e,f and Supplementary Fig. 2e). Collectively, these results demonstrate that $PGE_2$ silences certain tumor suppressor and DNA repair genes by enhancing their promoter CGI methylation via a PTGER4-DNMT pathway *in vitro*.

Our *in vitro* studies were confirmed *in vivo*. Treatment of $Apc^{Min/+}$ mice with $PGE_2$ increased Dnmt1 and Dnmt3b protein expression in colonic tumor epithelial cells (Fig. 2a) and accelerated intestinal adenoma growth (Fig. 2b,c). Moreover, $PGE_2$ enhanced the CGI methylation of *Cnr1* and *Mgmt* (Fig. 2d) as well as *Cdkn2b* and *Mlh1* (Supplementary Fig. 3a) in the colonic tumor epithelial cells isolated from $Apc^{Min/+}$ mice. As expected, $PGE_2$ also downregulated the expression of Cnr1, Mgmt, Cdkn2b, and Mlh1 at both the mRNA and protein levels in the colonic tumor epithelial cells from $Apc^{Min/+}$ mice (Fig. 2e and Supplementary Fig. 3b,c). Importantly, treatment of $Apc^{Min/+}$ mice with 5-aza-2'-deoxycytidine (5-Aza-dC) reversed the effect of $PGE_2$ on promoting adenoma growth (Fig. 2f) and inducing the CGI methylation of *Cdkn2b* (Supplementary Fig. 4a), demonstrating that $PGE_2$ accelerates intestinal adenoma growth via regulating CGI methylation. Intriguingly, combined treatment with both celecoxib and 5-Aza-dC more effectively reduced the tumor burden in $Apc^{Min/+}$ mice than either agent alone (Fig. 2g and Supplementary Fig. 4b). Furthermore, treatment of $Apc^{Min/+}$ mice with $PGE_2$ reversed the effects of celecoxib on inhibiting small intestinal adenoma growth (Supplementary Fig. 4c), demonstrating that the tumor inhibitory effect of celecoxib depends on $PGE_2$. Collectively, these results suggest that $PGE_2$ promotes intestinal tumor growth by silencing tumor suppressor and DNA repair genes via its effects on CGI methylation.

Our *in vitro* and *in vivo* results are of potential clinical relevance because the levels of $PGE_2$, *PTGS2*, *DNMT1*, and *DNMT3B* are positively associated with CGI methylation in the *CNR1*, *MGMT*, and *MLH1* promoters in human CRC specimens, respectively (Supplementary Fig. 5a). The correlation of these genes to the CGI methylation of each individual gene was also significant except for MLH1 (Supplementary Table 1). The correlation of *MLH1* to $PGE_2$ and *DNMT1* didn't reach significance although we observed

positive trends. Moreover, the expression levels of *CNR1*, *MGMT*, and *MLH1* are negatively correlated with their respective levels of CGI methylation (Supplementary Fig. 5b).

Dysregulation of DNMT expression is associated with human cancer progression[8,9]. Particularly, DNMT3B expression is positively associated with CIMP in CRC[10] and colorectal adenomas[11], while overexpression of DNMT1 is also associated with CIMP in CRC[12]. One study revealed similar profiles of DNMT3B-methylated genes between mouse colon and human CRC[13]. In $Apc^{Min/+}$ mice, modulation of Dnmt3b expression affected colon adenoma growth[14,15]. Consistent with these findings, our results provide the first evidence that $PGE_2$ promotes intestinal adenoma growth by silencing certain tumor suppressor and DNA repair genes via induction of DNMT1 and DNMT3B. Although Dnmt3b was not responsible for CGI methylation at the *Mgmt* locus in $Apc^{Min/+}$ mice[15], Dnmt1 may mediate $PGE_2$-enhanced CGI methylation in the *Mgmt* promoter in our studies. Our data further showed that inhibition of CGI methylation by 5-Aza-dC dramatically suppressed $PGE_2$-induced intestinal adenoma growth in $Apc^{Min/+}$ mice, suggesting demethylating agents could serve as anti-tumor agents. However, these agents have been reported to activate oncogenes and promote cancer cell proliferation, migration, and invasion in some *in vitro* studies[16–18]. Moreover, global and locus-specific hypomethylation are associated with a poor prognosis of some CRC patients[19,20]. Therefore, further investigation is needed before considering demethylating agents as a possible treatment for CRC.

In summary, our findings not only significantly improve our understanding of the intricate roles of $PGE_2$ in cancer progression and of how DNA methylation machinery is regulated in cancer but also provide a rationale for considering the development of a novel combination treatment employing PTGS2 inhibitors and demethylating agents for prevention and possibly future therapy in the appropriate subsets of patients.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

We want to thank D. Menter and P. Yang for their suggestions and their help in $PGE_2$ measurement. This work is supported, in part, by the NIH MERIT award R37 DK47297, RO1 DK62112, NCI P01 CA77839, and CPRIT RP100960. We also thank the National Colorectal Cancer Research Alliance (NCCRA) for its generous support (RND) and a cancer prevention fellowship (DX) supported by the NCI grant R25T CA57730 PI: Shine Chang, Ph.D.

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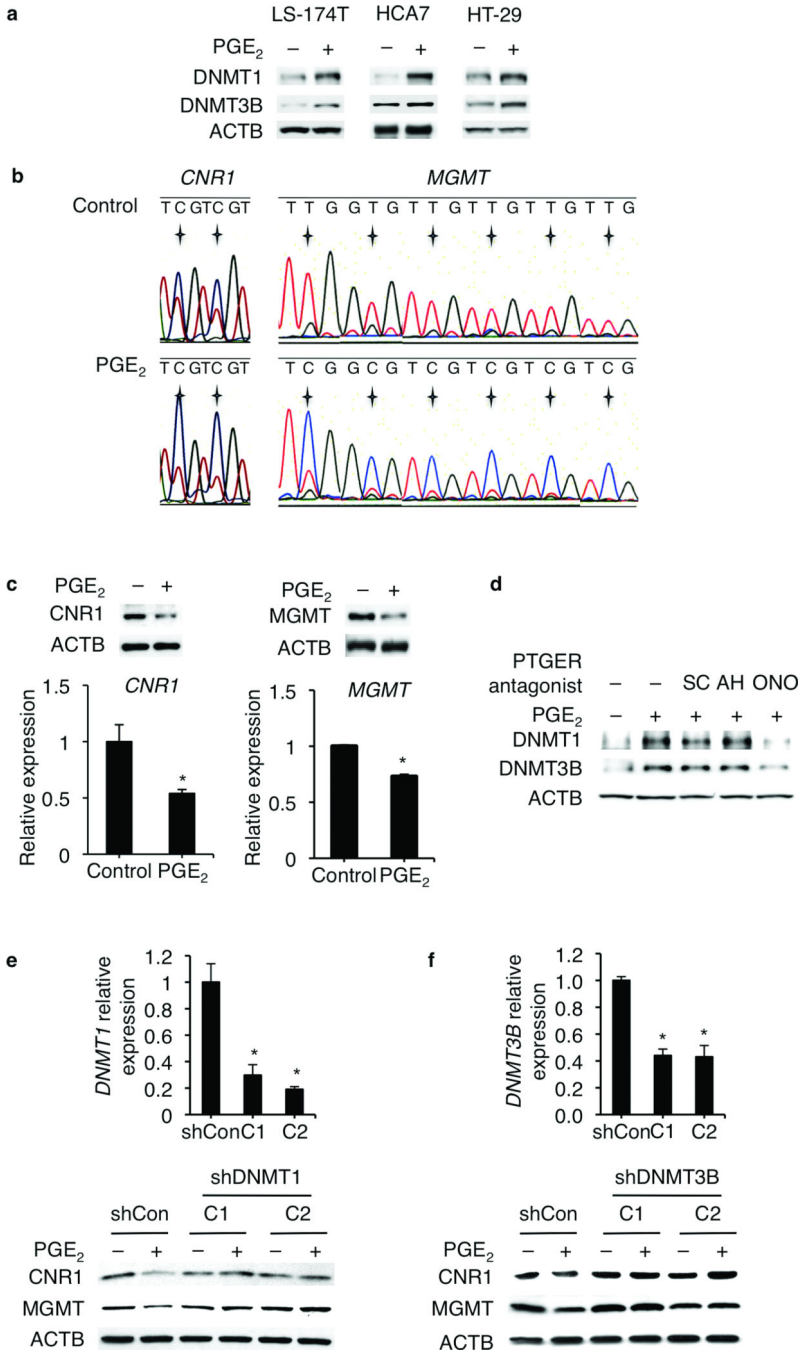


**Figure 1.**

$PGE_2$ silences certain tumor suppressor and DNA repair genes by enhancing their promoter CGI methylation in human CRC cell lines. (**a**) $PGE_2$ increased DNMT1 and DNMT3B protein expression in LS-174T, HCA7, and HT-29 cells. (**b**) Bisulfite PCR sequencing analysis showed that $PGE_2$ increased CGI methylation in the promoters of *CNR1* and *MGMT* in LS-174 cells. For *CNR1* promoter, a region (−370 to −160) that contains 24 CpGs was examined. Two representative CpGs were presented. For *MGMT* promoter, a region (+27 to +342) that contains 29 CpGs was examined. Six representative CpGs were

presented. The asterix indicates the locations of CpGs. (**c**) $PGE_2$ downregulated the expression of CNR1 (CB1) and MGMT at both protein (upper panels) and mRNA (lower panels) levels in LS-174T cells. Error bars indicate s.d. * $P < 0.05$ (two-tailed unpaired Student's *t* test). (**d**) Blockade of PTGER4 (EP4) attenuated the upregulation of DNMT1 and DNMT3B by $PGE_2$ in LS-174T cells. SC19220 (SC): PTGER1 (EP1) antagonist; AH6809 (AH): PTGER1-3 (EP1-3) antagonist; ONOAE-208 (ONO): PTGER4 (EP4) antagonist. (**e,f**) Knockdown of DNMT1 or DNMT3B by shRNAs attenuated $PGE_2$-induced downregulation of CNR1 (CB1) and MGMT in LS-174T cells. Knockdown efficiency was examined by Q-PCR in two clones (C1 and C2) along with a non-silencing shRNA transfected control (shCon) (upper panels). Error bars indicate s.d. * $P < 0.05$ (two-tailed unpaired Student's *t* test). CNR1 (CB1) and MGMT protein expression was examined by western blotting in these two clones (C1 and C2) and control ShCon cells (lower panels).

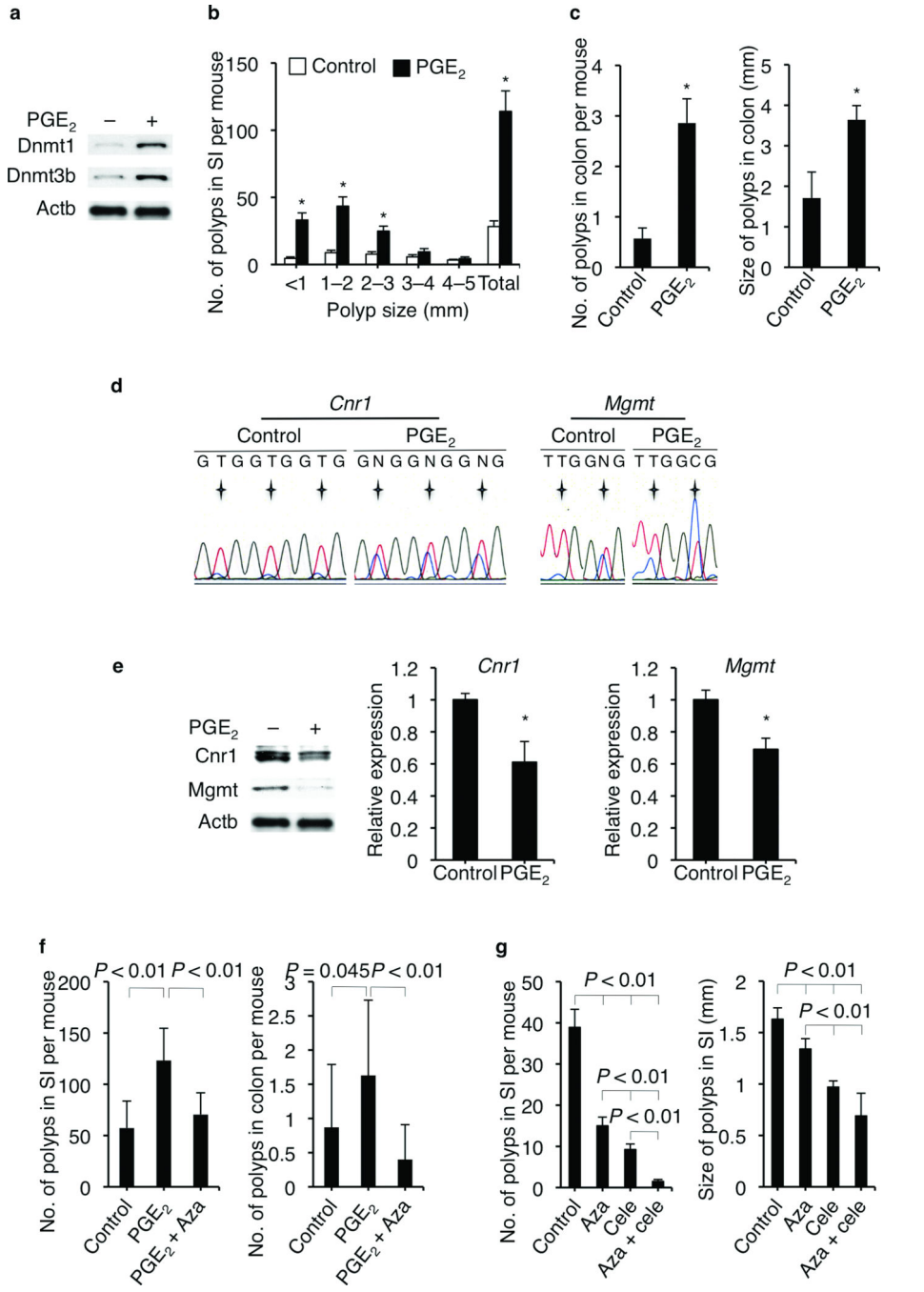


**Figure 2.**

$PGE_2$ promotes intestinal tumor growth via upregulating CGI methylation in $Apc^{Min/+}$ mice. (**a**) Treatment of $Apc^{Min/+}$ mice with $PGE_2$ increased Dnmt1 and Dnmt3b protein expression in the colonic tumor epithelial cells. (**b,c**) $PGE_2$ increased intestinal polyp number and size in $Apc^{Min/+}$ mice. Error bars indicate s.e.m ($n$ = 7 for each group). * $P$ <0.05 (Wilcoxon Rank Sum test). No.: number; SI: small intestine. (**d**) Treatment of three $Apc^{Min/+}$ mice with $PGE_2$ increased the promoter CGI methylation of *Cnr1* and *Mgmt* in the colonic tumor epithelial cells as compared to three $Apc^{Min/+}$ mice treated with vehicle. For *Cnr1* promoter,

a region (−369 to −34) that contains 35 CpGs was examined. Three representative CpGs were presented. For *Mgmt* promoter, a region (−458 to −243) that contains 6 CpGs was examined. Two representative CpGs were presented. Asterix indicates the locations of CpGs. (**e**) Treatment of $Apc^{Min/+}$ mice with $PGE_2$ decreased the expression of Cnr1 and Mgmt at both protein levels (left panel) and mRNA levels (middle and right panels) in the colonic tumor epithelial cells. One representative result from three mice was shown. Error bars indicate s.d. * $P < 0.05$ (two-tailed unpaired Student's *t* test). (**f**) Inhibition of CGI methylation by 5-Aza-dC attenuates $PGE_2$-induced tumor growth in male $Apc^{Min/+}$ mice. Error bars indicate s.e.m. ($n = 15$ for each group. Wilcoxon Rank Sum test). (**g**) Combination treatment with celecoxib and 5-Aza-dC more efficiently inhibited tumor growth. Error bars indicate s.e.m. ($n = 12$, 14, 14, and 7, respectively. Wilcoxon Rank Sum test).