Short Communication

β-catenin negatively regulates expression of the prostaglandin transporter PGT in the normal intestinal epithelium and colorectal tumour cells: a role in the chemopreventive efficacy of aspirin?

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BACKGROUND: Levels of the pro-tumorigenic prostaglandin PGE$_2$ are increased in colorectal cancer, previously attributed to increased synthesis through COX-2 upregulation and, more recently, to decreased catabolism. The functionally linked genes 15-prostaglandin dehydrogenase (15-PGDH) and the prostaglandin transporter PGT co-operate in prostaglandin degradation and are downregulated in colorectal cancer. We previously reported repression of 15-PGDH expression by the Wnt/β-catenin pathway, commonly deregulated during early colorectal neoplasia. Here we asked whether β-catenin also regulates PGT expression.
METHODS: The effect of β-catenin deletion in vivo was addressed by PGT immunostaining of β-catenin$^{-/lox}$-villin-cre-ERT2 mouse tissue. The effect of siRNA-mediated β-catenin knockdown and dnTCF4 induction in vitro was addressed by semi-quantitative and quantitative real-time RT-PCR and immunoblotting.
RESULTS: This study shows for the first time that deletion of β-catenin in murine intestinal epithelium in vivo upregulates PGT protein, especially in the crypt epithelium. Furthermore, β-catenin knockdown in vitro increases PGT expression in both colorectal adenoma- and carcinoma-derived cell lines, as does dnTCF4 induction in LS174T cells.
CONCLUSIONS: These data suggest that β-catenin employs a two-pronged approach to inhibiting prostaglandin turnover during colorectal neoplasia by repressing PGT expression in addition to 15-PGDH. Furthermore, our data highlight a potential mechanism that may contribute to the non-selective NSAID aspirin's chemopreventive efficacy.
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Recent studies in the *Lancet* (Algra and Rothwell, 2012; Rothwell *et al*, 2012a, b) have reinforced interest in the potential value of using NSAIDs, such as aspirin, in colorectal cancer chemoprevention. Over much of the last few decades, a major focus of colorectal cancer chemoprevention has been inhibition of COX-2, an inducible cyclooxygenase isoform that is upregulated at the medium/large adenoma stage, and to which the elevated levels of the protumorigenic prostaglandin PGE$_2$ have previously been attributed (Rigas *et al*, 1993; Eberhart *et al*, 1994; Elder *et al*, 2002). However, several lines of evidence suggest a potential role for COX-1 in colorectal neoplasia. For example, tumorigenesis in animal models can be suppressed by deletion of either *COX-1* or *COX-2* genes (Chulada *et al*, 2000), or by administration of a COX-1-selective inhibitor (Kitamura *et al*, 2002), though the precise role of COX-1 in intestinal neoplasia is unclear. Our recent paper in

Gut (Smartt *et al*, 2012) highlighted a mechanism by which COX-1 could contribute to early colorectal cancer development. We showed that β-catenin, activated at the earliest stages of colorectal neoplasia, can repress expression of the colorectal tumour suppressor and prostaglandin catabolising enzyme 15-prostaglandin dehydrogenase (15-PGDH). Here, we now show that β-catenin also represses expression of a second gene crucial for prostaglandin catabolism, the prostaglandin transporter PGT. PGT (aka SLCO2A1) mediates uptake of secreted prostaglandins, enabling their oxidative inactivation by cytoplasmic 15-PGDH (Nomura *et al*, 2004). Like 15-PGDH, downregulation of PGT has been reported in both human colorectal tumours and in pre-malignant adenomas in APC$^{min/+}$ mice, and this was suggested to result, at least in part, from epigenetic silencing (Holla *et al*, 2008). However, we hypothesised that β-catenin may also have a role in repression of PGT expression in colorectal cancer, as we reported previously for 15-PGDH. Here we show that modulating β-catenin expression or function increases PGT expression, both in the intestinal epithelium *in vivo*, and in colorectal adenoma and carcinoma cells *in vitro*. Our data suggest that β-catenin co-ordinately regulates prostaglandin turnover through the

negative regulation of both PGT and 15-PGDH expression, and highlight a potential mechanism that could contribute to the chemopreventive efficacy of the non-selective NSAID aspirin.

MATERIALS AND METHODS

The colon carcinoma-derived cell lines HCT15, HCT116, HT29, LOVO, LS174T, SW480 and SW620, obtained from the American Type Culture Collection (Rockville, MD, USA); the HCA7 colony 29 (HCA7) colon carcinoma cell line, a kind gift of Susan Kirkland (Kirkland, 1985); the dox-inducible dnTCF4-expressing LS174T/dnTCF4 cell line, a kind gift from Hans Clevers (van de Wetering *et al*, 2002); and colon adenoma-derived cell lines PC/AA/C1, PC/AA/C1/SB10C, PC/BH/C1, S/AN/C1 and S/RG/C2 derived in this laboratory (Paraskeva *et al*, 1984, 1989; Williams *et al*, 1990) were maintained as described previously (Paraskeva *et al*, 1984; Moore *et al*, 2009), except for PC/AA/C1 and PC/AA/C1/SB10C, which were maintained on conditioned medium (Williams *et al*, 1990). Immunohistochemical staining using a rabbit polyclonal PGT antibody (kind gift of Michel Fortier, Quebec, Canada) and inducible intestinal *β*-catenin ablation in mice were carried out and validated as previously described (Smartt *et al*, 2012), as was intestinal epithelial cell fractionation (see also Mariadason *et al*, 2005). RNA interference, immunoblotting and mRNA preparation were also performed as previously described (Smartt *et al*, 2012). Semi-quantitative RT-PCR was carried out as in Chell *et al* (2006), with minor modifications, and quantitative real-time RT-PCR was carried out as described previously (Moore *et al*, 2009), using PGT primers from Qiagen (Crawley, West Sussex, UK).

RESULTS

Preliminary studies suggested that, similar to 15-PGDH, PGT also shows an expression gradient in the human intestinal epithelium, being highest in the differentiated luminal/villus compartment (Figure 1A). A similar PGT expression gradient was also observed in the murine small intestinal epithelium, both at the mRNA (Figure 1B) and protein level (Figure 1C). As Wnt/*β*-catenin transcriptional activity is known to be highest in the proliferative crypt compartment (van de Wetering *et al*, 2002; Kosinski *et al*, 2007), we hypothesised that PGT may also be repressed by *β*-catenin in intestinal epithelial cells. Importantly, the gradient of PGT expression was lost following inducible *β*-catenin ablation (Figure 1D; Fevr *et al*, 2007). Elevated PGT expression was particularly noticeable in crypt epithelia (compare Figure 1F with E), where *β*-catenin activity is normally highest (Batlle *et al*, 2002; van de Wetering *et al*, 2002). This increase is consistent with previously published microarray analysis (1.67-fold increase, Fevr *et al*, 2007), confirming that *β*-catenin represses PGT expression in the normal murine intestinal epithelium.

We then hypothesised that *β*-catenin may also repress PGT expression in human colorectal cancer cells, as is the case for 15-PGDH. Indeed, in line with this hypothesis, basal PGT levels are reportedly very low to undetectable in most colorectal carcinoma cell lines (Holla *et al*, 2008). We confirmed and extended this observation to include cell lines derived from sporadic (S/RG/C2, S/AN/C1) and familial adenomatous polyposis patient (PC/AA/C1, PC/BH/C1) adenomas and one *in-vitro*-transformed adenoma cell line (PC/AA/C1/SB10C) (Figure 2A). Indeed, in our hands, PGT mRNA was only readily detectable in LoVo carcinoma-derived and PC/BH/C1 adenoma-derived cells under basal conditions. Notably, siRNA-mediated knockdown of *β*-catenin increased PGT mRNA expression in both adenoma- and carcinoma-derived cell lines (Figure 2B and C). In addition, PGT mRNA levels were consistently increased in the colorectal carcinoma cell line LS174T/dnTCF4, 48 h following induction of dnTCF4, a dominant-negative form of this *β*-catenin-binding partner that is unable to bind *β*-catenin
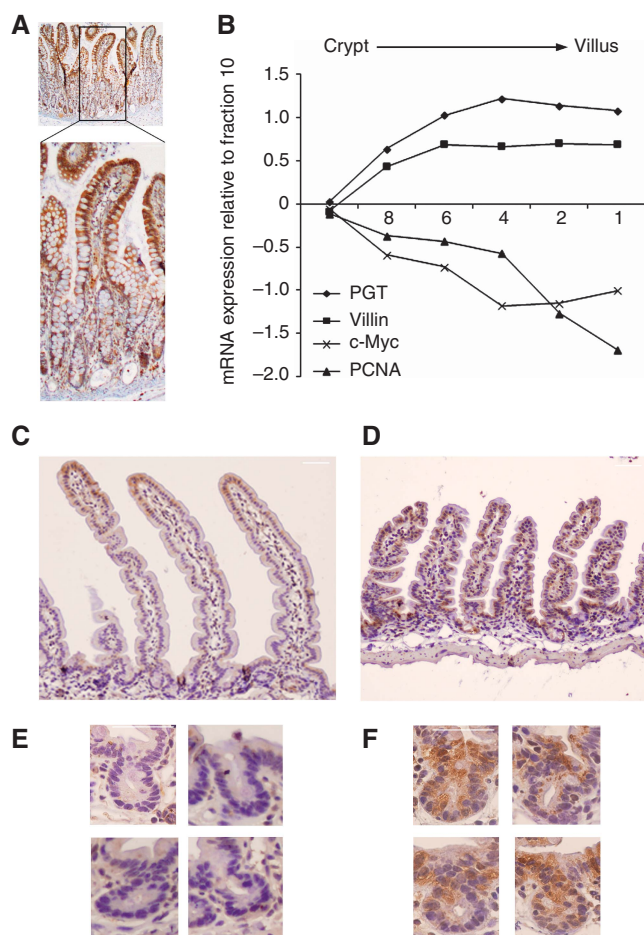

Figure 1 (**A**, **B**) Gradient of PGT protein and mRNA expression with differentiation along the crypt–villus axis in intestinal epithelium. (**A**) PGT immunohistochemistry shows high PGT expression in the differentiated human villus epithelium. Scale bar represents 50 *μ*m. (**B**) Differential fractionation of the murine intestinal epithelium followed by microarray gene expression analysis (Mariadason *et al*, 2005) shows that, like the differentiation marker villin, PGT mRNA expression increases along the crypt–villus axis. In contrast, expression of the proliferation marker PCNA and the Wnt/*β*-catenin target c-Myc both decrease along the crypt–villus axis. Data represents expression relative to fraction 10 in fractions 10, 8, 6, 4, 2 and 1. This gradient of PGT expression is confirmed at the protein level by immunohistochemistry (see **C**). (**C**–**F**) Inducible *β*-catenin ablation increases crypt epithelial PGT expression levels. Deletion of *β*-catenin was induced in *β*-catenin⁻ᐟˡᵒˣ-villin-creERT2 mice by tamoxifen injection (**D**, **F**); injected *β*-catenin⁺ᐟˡᵒˣ-villin-creERT2 mice (**C**, **E**) were used as a control. PGT staining shows PGT expression throughout the crypt–villus axis in the absence of *β*-catenin (**D**), particularly evident in crypt and lower villus epithelium (**F** as compared with **E**) (representative examples from 96 h post induction). Scale bars represent 50 *μ*m.

(Figure 2D and E). Hence, taken together, these data suggest that *β*-catenin represses PGT expression in both the normal intestinal epithelium and in colorectal cancer.

DISCUSSION

In this article, we have identified a novel link between two important pathways in colorectal neoplasia, Wnt/*β*-catenin and PGE₂ signalling, through the negative regulation of PGT expression. Therefore, similar to 15-PGDH, PGT expression could be widely downregulated at a very early stage of colorectal neoplasia in sporadic tumours as well as those arising in the context of a
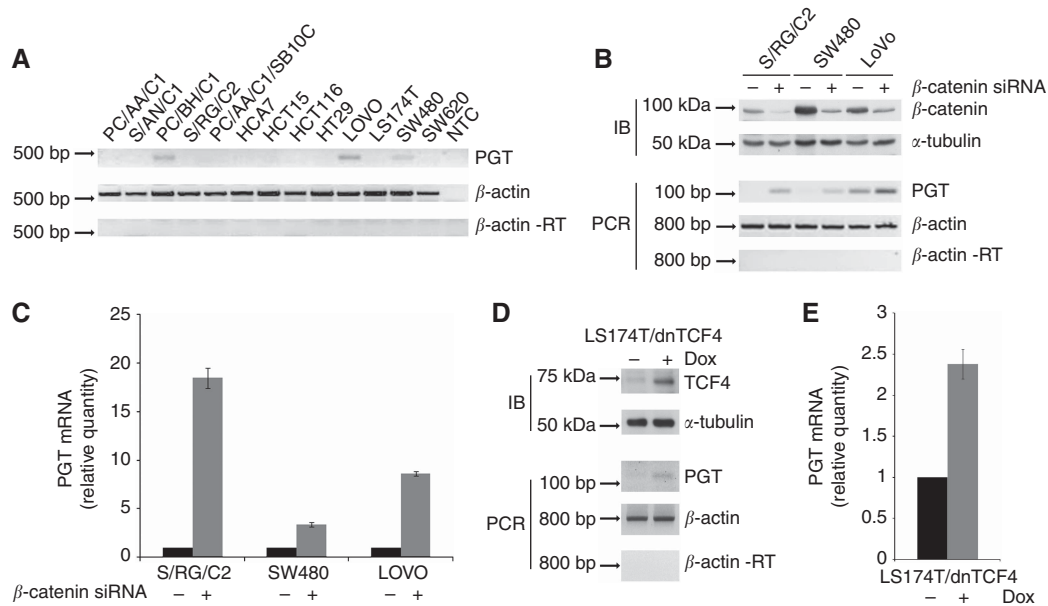
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Figure 2 (**A**) PGT mRNA expression screen in colorectal adenoma- and carcinoma-derived cell lines. PC/AA/C1, S/AN/C1, PC/BH/C1 and S/RG/C2 are adenoma-derived cell lines, PC/AA/C1/SB10C is an in-vitro-transformed adenoma-derived cell line and the remainder are carcinoma-derived. Semi-quantitative real-time PCR analysis confirmed that most colorectal carcinoma-derived cell lines express low levels of PGT with the exception of LoVo. Interestingly, a similar pattern was seen in our adenoma-derived cell lines, with mostly very low expression with the exception of PC/BH/C1 cells (NTC = no template control, −RT = no reverse transcriptase enzyme control). (**B**, **C**) β-catenin siRNA knockdown increases PGT expression. (**B**) Immunoblotting (IB) demonstrating that transient β-catenin siRNA transfection effectively reduced β-catenin protein levels in S/RG/C2, SW480 and LoVo cell lines (48 h). Semi-quantitative PCR analysis (PCR) revealed a marked and consistent increase in PGT mRNA levels in S/RG/C2, SW480 and LoVo cells following β-catenin siRNA-mediated knockdown (48 h). (**C**) Quantitative real-time PCR analysis confirms the increase in PGT mRNA in **B**. (**D**) Induction of dnTCF4 increases PGT expression immunoblotting demonstrates effective induction in LS174T/dnTCF4 cells of dnTCF4 with 1 μg ml⁻¹ doxycycline (dox; 48 h). Semi-quantitative PCR analysis (PCR) demonstrates a consistent increase in PGT mRNA levels in LS174T/dnTCF4 cells following dox-mediated induction of dnTCF4 (48 h). (**E**) Quantitative real-time PCR analysis confirms the increase in PGT mRNA apparent in **D**. Results shown are representative of a minimum of three independent repeat experiments. α-tubulin and β-actin serve as loading controls.

germline APC mutation. Downregulation of PGT, in addition to 15-PGDH, may constitute a very effective means of increasing local PGE₂ levels as impaired PGT-mediated prostaglandin import would be expected to directly increase extracellular prostaglandin concentrations available to interact with cell surface receptors.

The ability of β-catenin to downregulate both 15-PGDH and PGT, two crucial regulators of prostaglandin catabolism, points towards the importance of elevated COX-1-derived prostaglandin levels during early colorectal neoplasia before COX-2 upregulation, and

may go some way to explain the particular efficacy in colorectal cancer chemoprevention of the non-selective NSAID aspirin.

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