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?HJM Smartt¹, A Greenhough¹, P Ordóñez-Morán², M Al-Kharusi¹, TJ Collard¹, JM Mariadason³, J Huelsken², AC Williams¹ and C Paraskeva^{*,1}¹Cancer Research UK Colorectal Tumour Biology Research Group, School of Cellular and Molecular Medicine, University Walk, University of Bristol, Bristol BS8 1TD, UK; ²Cancer Stem Cell Laboratory, École Polytechnique Fédérale de Lausanne, Station 19 (SV 2521), Lausanne CH-1015, Switzerland; ³Ludwig Institute for Cancer Research, Austin Hospital, Melbourne, VIC, Australia

BACKGROUND: Levels of the pro-tumorigenic prostaglandin PGE₂ 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 PGE₂ 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 SLC02A1) 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

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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. 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^{-/-lox}-villin-creERT2 mice by tamoxifen injection (D, F); injected β -catenin^{+/-lox}-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

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 apparent in B. (D) Induction of dnTCF4 increases PGT expression immunoblotting demonstrates effective induction in LS174T/dnTCF4 cells of dnTCF4 with $1 \mu\text{g ml}^{-1}$ 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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