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 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 isozyme 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 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 Apcmin−/− 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/A/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 inductive 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; Kowalski 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 (Batle 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 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 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
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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