LGR5 promotes survival in human colorectal adenoma cells and is upregulated by PGE₂: implications for targeting adenoma stem cells with NSAIDs


Cancer Research UK Colorectal Tumour Biology Group, School of Cellular and Molecular Medicine, Medical Sciences Building, University Walk, University of Bristol, UK

*To whom correspondence should be addressed. Tel: +0117 331 2072; Fax: +0117 331 2091; Email: c.paraskeva@bristol.ac.uk

Cyclooxygenase-2 is overexpressed in the majority of colorectal tumours leading to elevated levels of prostaglandin E₂ (PGE₂), promoting many hallmarks of cancer. Importantly, PGE₂ is reported to enhance Wnt/β-catenin signalling in colorectal carcinoma cells and in normal haematopoietic stem cells where it promotes stem cell function. Although Wnt signalling plays a crucial role in intestinal stem cells, the relationship between PGE₂ and intestinal stem cells is unclear. Given that the key intestinal cancer stem cell marker LGR5 (leucine-rich G-protein coupled receptor 5) is a Wnt target and PGE₂ enhances Wnt signalling, the focus of this study was to investigate whether PGE₂ regulated LGR5 expression in colorectal adenoma cells and whether LGR5 was important for tumour cell survival. PGE₂ upregulated LGR5 protein in adenoma (RG/C2) and carcinoma (DLD-1) cell lines. LGR5 knockdown induced cell death in RG/C2 and AA/C1 adenoma cells, suggesting that LGR5 has an important survival-promoting role in adenoma cells. Indeed, we detected LGR5 protein expression in 4 of 4 human adenoma cell lines. Furthermore, LGR5 small interfering RNA inhibited the survival-promoting effects of PGE₂ in RG/C2, suggesting that PGE₂ promotes adenoma cell survival, at least in part, by increasing LGR5 expression. These studies, therefore, show the first link between PGE₂ and LGR5 in human colorectal adenoma and carcinoma cells and demonstrate a survival-promoting role of LGR5. As non-steroidal anti-inflammatory drugs (NSAIDs) cause adenomas to regress in FAP patients, these studies could have important implications for the mechanism by which NSAIDs are chemopreventive, as lowering PGE₂ levels could reduce LGR5 expression and survival of LGR5⁺ adenoma stem cells.

Introduction

Colorectal cancer (CRC) is an excellent example of the multistage process of carcinogenesis (1), and is the second most common cause of cancer deaths in much of the industrialized world. Most CRCs are derived from colorectal adenomas in what is often referred to as the adenoma carcinoma sequence (1). There is significant evidence from clinical trials and experimental data that the cyclooxygenases (COX-1 and COX-2) are important targets for CRC prevention and therapy (2,3). Although COX-2 expression levels are low in normal intestinal epithelial cells, COX-2 is overexpressed in a subset of colorectal adenomas and in 80–90% of colorectal carcinomas (4,5), indicating that COX-2 has a key role in tumorigenesis. Overexpression of COX-2 is suggested to account for the increased levels of its pro-tumorigenic product PGE₂ observed in colorectal neoplasia (6). COX-2/PGE₂ signalling can promote most, if not all, of the hallmarks of cancer (7). Importantly, both non-selective and COX-2 selective non-steroidal anti-inflammatory drugs (NSAIDs) can cause adenoma regression in FAP patients (8,9); hence, targeting this pathway using COX-inhibitory NSAIDs is currently one of the most promising approaches to CRC prevention and possibly treatment (10). However, the mechanisms by which tumour regression is induced are not currently fully understood. In order to maximize efficacy of chemoprevention and chemotherapy and minimize potential side effects, a thorough understanding of the mechanisms involved is required.

As well as displaying COX-2 overexpression, the majority of CRCs show aberrant activation of the Wnt/β-catenin signalling pathway. This is frequent via mutation/loss of the intestinal epithelial gatekeeper gene APC, which is believed to be a crucial initiating event leading to deregulated growth (11,12). Wnt/β-catenin signalling is vitally important to colorectal intestinal epithelial cells due to its role in maintaining the stem cell compartment (13).

Given the major role of the PGE₂ and Wnt/β-catenin signalling pathways in colorectal carcinogenesis, it is of significant interest that recent studies have shown that PGE₂ enhances Wnt/β-catenin signalling both in colorectal carcinoma cells (14,15) and in normal haematopoietic stem cells where it promotes stem cell function (16). Because Wnt/β-catenin signalling has a key role in intestinal stem cell biology, this suggests a potentially important role for PGE₂ in intestinal stem cell biology.

Up until relatively recently, research on intestinal stem cells has been hampered by a lack of normal and cancer stem cell (CSC) markers. Recent studies have identified a stem cell population in the base of both murine small and large intestinal crypts, marked by expression of the Wnt/β-catenin target gene Lgr5 (leucine-rich G-protein coupled receptor 5) (17). Lgr5 is a seven-transmembrane receptor (18) with a role in enhancing Wnt responsiveness (19,20). Deleting APC in the Lgr5-positive stem cell region in a normal murine crypt leads to macroscopic adenoma formation, whereas deletion of APC in Lgr5-negative cells does not (21), suggesting that the Lgr5⁺ cells are the origin of mouse intestinal adenomas. Furthermore, Lgr5 is also expressed in a subpopulation of cells in murine intestinal adenomas (both small and large intestine) (21,22). Indeed, a recent study using lineage tracing provided evidence that Lgr5 marks a subpopulation of mouse adenoma cells that drive the growth of established intestinal adenomas (23). A previous study has suggested that LGR5 can serve as a CSC marker (24) and recent research has shown that LGR5 is also functionally important in CRC stem cell behaviour (25). For example, primary human CRC cells revealed a clear subpopulation of LGR5-expressing cells (25). Overexpression of LGR5 enhanced clonogenicity, whereas knockdown of LGR5 inhibited clonogenic growth of the CSCs (22). Taken together, these data suggest that the Wnt target gene LGR5 is a candidate CSC marker.

Although Wnt signalling plays a crucial role in intestinal stem cells (13), the relationship, if any, between PGE₂ and intestinal stem cells is currently unclear. For example, whether PGE₂ regulates the expression of intestinal stem cell markers and if this is important for tumour cell survival.

Because PGE₂ enhances Wnt/β-catenin signalling and LGR5 is a Wnt target, it raises the exciting possibility that PGE₂ has a role in intestinal tumour stem cell survival by regulating the stem cell marker, LGR5. In this study, we show for the first time that PGE₂ upregulates LGR5 protein in PGE₂-responsive colorectal adenoma and carcinoma cells. Furthermore, our studies using a small interfering RNA (siRNA) approach also showed a pro-survival role for LGR5 in human adenoma cells and that PGE₂ promotes adenoma survival, at least in part, through LGR5. As NSAIDs cause adenomas to regress in FAP patients, at least in part through inhibiting PGE₂,

Abbreviations: COX, cyclooxygenase; CRC, colorectal cancer; CSC, cancer stem cell; FAP, familial adenomatous polyposis; LGR5, leucine-rich G-protein coupled receptor 5; NSAID, non-steroidal anti-inflammatory drug; PGE₂, prostaglandin E₂; siRNA, small interfering RNA.
these studies could have important implications for the mechanism by which NSAIDs are chemopreventive and cause adenoma regression, as lowering PGE$_2$ levels could reduce LGR5 expression and survival of LGR5$^+$ adenoma stem cells.

Materials and methods

Cell lines and cell culture

The human colorectal adenoma-derived cell lines RG/C2, BH/C1, AN/C1 and AA/C1 and the transformed adenoma cell lines RG/GV and AA/C1/SB10C (10C) were derived in this laboratory and maintained as described previously (26–29). The HCA7 colony 29 (HCA7) colorectal carcinoma-derived cell line was a kind gift of Susan Kirkland (30) and the other colorectal carcinoma-derived cell lines were obtained from the American Type Culture Collection (Rockville, MD) and maintained as described previously (31).

Treatments

RG/C2 cells were treated with PGE$_2$ (Sigma–Aldrich, Poole, UK), as described previously (32). CRC cells were grown in standard growth conditions (10% batch tested fetal bovine serum in Dulbecco’s modified Eagle’s medium), whereas adenoma cell lines were routinely grown in 20% fetal bovine serum Dulbecco’s modified Eagle’s medium and switched to 10% fetal bovine serum Dulbecco’s modified Eagle’s medium for PGE$_2$ treatment. Subconfluent (~30% confluent) cells were treated with PGE$_2$ at the appropriate concentrations and time points as illustrated in the figures.

RNA interference

Cells were transiently transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) as described previously (33), with 20nM of siRNAs targeting β-catenin (Ambion, Huntingdon, Cambridgeshire, UK) or LGR5 (Dharmacon, Lafayette, CO), the latter in the form of a smartpool unless otherwise stated.

Overexpression studies

The LGR5-FLAGFLAGHAHA (LGR5-FH) construct was a kind gift of Hans Clevers (19) and was transiently transfected in suspension into LS174T cells using Lipofectamine 2000 according to the manufacturer’s protocol. LS174T cells were from a 58-year-old female Dukes type B adenocarcinoma of the colon and obtained from the American Type Culture Collection.

Fig. 1. Validation of the LGR5 antibody for western blotting analysis (A–E). Validation of the LGR5 antibody used in this study. (A) Western blotting demonstrates that both LGR5 and HA antibodies recognize transiently expressed exogenous FLAG-HA-tagged LGR5 in LS174T. Note FLAG-HA-tagged LGR5 runs above the endogenous LGR5 doublet apparent in control LS174T cells in the LGR5 increased exposure blot. (B) Quantitative PCR confirms effective siRNA-mediated knockdown of LGR5 mRNA in RG/C2 cells. The LGR5 transcript level is relative to the Control siRNA, which is given an arbitrary value of 1. (C) Sequencing the amplification product confirmed 100% identity to human LGR5 gene (data not shown). (D) The ~100kDa signal detected with a candidate LGR5 western blotting antibody is reduced by 20nM LGR5 siRNA using both smartpool (SP) siRNA and the four individual siRNA sequences (1–4) of the deconvoluted pool at 48 h post-transfection. (E) Quantitative PCR demonstrates a reduction by 20nM β-catenin siRNA of the mRNA levels of LGR5, a Wnt/β-catenin target gene. The LGR5 transcript level is relative to the Control siRNA, which is given an arbitrary value of 1. (E) Western blotting confirms effective β-catenin knockdown and a reduction in the candidate LGR5 band. α-Tubulin was used as a loading control. **p < 0.01.
Western blotting

Western blotting was performed as described previously (32) using LGR5 (ab75850; Abcam, Cambridge, UK), HA (MMS-101R, Covance, UK), β-catenin and α-tubulin (Cell Signaling Technology, Danvers, MA) antibodies. The LGR5 antibody was validated by siRNA and using the LGR5-FLAGFLAGHAHA (LGR5-FH) construct as shown in the Results.

Cell yield and cell death determination

Cell counts were made in triplicate using a Neubauer Haemocytometer counting chamber. The attached cells were counted as a determination of cell yield and the detached cells as a measurement of cell death as described previously in detail (32).

RNA extraction and quantitative PCR analysis

Total RNA extraction and comparative quantitative real-time PCR were performed as described previously (31) using LGR5, TATA-binding protein (TBP) and hypoxanthine-guanine phosphoribosyltransferase (HPRT) primers (QIAGEN, Crawley, West Sussex, UK). LGR5 transcript levels were normalized to the housekeeping genes TBP and HPRT.

Statistical analysis

Statistical analysis was carried out using SPSS statistical software for Windows (version 19, SPSS Chicago, IL). Student’s t-test and analysis of variance were used to determine differences among means where appropriate. The experiments were repeated three times in triplicate and the results presented as a mean of the three separate experiments unless stated otherwise. *P < 0.05, **P < 0.01, ***P < 0.001 and NS = not significant.

Results

LGR5 antibody validation for western blotting

In order to carry out LGR5 expression studies and to develop an in vitro model to study the biological role of LGR5 in human adenoma cells, we sought to identify an LGR5 antibody suitable for the detection of endogenous human LGR5 by western blotting.

Initial screening experiments (data not shown) identified a promising commercial LGR5 antibody (Abcam ab75850). This antibody detected a protein product migrating as a doublet of ~100kDa, consistent with the predicted size of LGR5. To validate the antibody, a FLAG and HA double-tagged LGR5 construct (LGR5-FH) (19) was transiently expressed in LS174T cells. Both an HA antibody and the LGR5 western blotting antibody detected a doublet of the same size in LGR5-FH-expressing cells (Figure 1A). Similar results were found with the LS174T-LGR5 cell line that stably expresses LGR5 (results not shown).

To further confirm the specificity of the antibody for endogenous LGR5, we silenced LGR5 using siRNA in the adenoma-derived RG/C2 cell line that resulted in a ~60% decrease in LGR5 mRNA (Figure 1B). Parallel western blots showed a band at the expected size that was markedly reduced in intensity both with a pool of four siRNA sequences and with each sequence of the deconvoluted smart-pool (Figure 1C). Decreased LGR5 mRNA and protein levels were also observed following β-catenin knockdown, confirming that LGR5 is a Wnt/β-catenin target gene in RG/C2 adenoma cells (Figure 1D and E). Similar results were obtained in the carcinoma-derived cell lines LoVo and SW620 (Supplementary Figure 1, available at Carcinogenesis Online). In HCT116 cells where LGR5 is silenced by DNA methylation (34), LGR5 protein was not detected by western blotting (see below).

LGR5 is expressed in colorectal adenoma- and carcinoma-derived cell lines

Having identified a suitable LGR5 antibody for western blotting, we examined LGR5 expression in colorectal adenoma and carcinoma cell lines, as well as in two in vitro models of tumour progression. An mRNA expression screen demonstrated a wide range of LGR5 mRNA expression levels (Figure 2A), with relatively low expression in several carcinoma-derived cell lines. HCT116, in agreement with previous studies did not express detectable LGR5 mRNA, consistent with LGR5 being silenced by DNA methylation in these cells (34).

Interestingly, an extended screen of LGR5 protein expression correlated broadly but not exactly with mRNA expression levels, suggesting possible post-transcriptional regulation (Figure 2B). Notably, all four adenoma-derived cell lines screened expressed readily detectable levels of LGR5 protein and expression was retained in in vitro transformed variants. In contrast, carcinoma-derived cell lines had variable expression levels. Of the nine carcinoma-derived cell lines examined, four had very low to undetectable LGR5 protein (Figure 2B). As expected, in HCT116 where LGR5 is silenced by DNA methylation (34), LGR5 protein was not detected by western blotting. It is also interesting to note that the two metastasis-derived cell lines, SW620 and particularly LoVo, have higher LGR5 expression levels than all of the carcinoma cell lines derived from primary cancers (Figure 2B).

PGE₂ upregulates LGR5 protein expression in PGE₂-responsive cell lines

An important aim of the study was to investigate whether PGE₂ regulates expression of the Wnt target and stem cell marker LGR5 (17). We were particularly interested in whether PGE₂ regulates LGR5 expression in adenoma cells because animal models suggest that LGR5⁺ cells may be the target for adenoma formation (21,23). Furthermore, LGR5 is expressed in all four adenoma cell lines tested here as well as in mouse and human adenomas in vivo (21,35,36). We, therefore, treated the adenoma cell line RG/C2 with PGE₂ and showed that it increased cell yield and reduced cell death (Figure 3A) as reported previously (29,32). Importantly, PGE₂ treatment also...
upregulated LGR5 protein levels in RG/C2 cells as detected by western blotting (Figure 3B). PGE₂ was found to upregulate LGR5 protein in the parental adenoma cell line from which RG/C2 cells were derived, S/RG (data not shown) showing that sensitivity to regulation of LGR5 by PGE₂ is retained with in vitro passage. Another cell line responsive to growth stimulation by PGE₂ is the colorectal carcinoma cell line DLD-1 (Figure 3C); this also showed increased LGR5 expression following PGE₂ treatment (Figure 3C). Conversely, the adenoma cell line AA/C1, which is relatively unresponsive to growth stimulation by PGE₂ (Figure 3D), did not show LGR5 upregulation in response to PGE₂ (Figure 3D). These results reveal a novel regulation of LGR5 by PGE₂ in the PGE₂-sensitive colorectal tumour cell lines.

PGE₂ upregulates LGR5 protein in RG/C2 by a β-catenin-independent pathway

Because PGE₂ can enhance β-catenin/T-cell factor activity (14–16) and LGR5 is a Wnt target gene (37–39), we next asked whether PGE₂ increases LGR5 expression through a transcriptional mechanism. Interestingly, contrary to expectations, PGE₂ did not increase LGR5 mRNA at 24, 48 or 72 h (Figure 4A) despite LGR5 protein upregulation occurring from 24h onwards (Figures 3B and 4B), suggesting a β-catenin/T-cell factor-independent mechanism. To investigate this further, we also asked whether lowering β-catenin levels using siRNA would inhibit the ability of PGE₂ to upregulate LGR5. As expected, β-catenin siRNA reduced basal LGR5 protein levels (Figure 4C). In order to compare the extent of LGR5 upregulation by PGE₂ in the presence and absence of β-catenin siRNA, we carried out densitometric analysis to determine the fold increase in LGR5 protein levels with PGE₂ treatment. The results show that PGE₂ treatment led to an ~2-fold increase in LGR5 protein with or without β-catenin siRNA (Figure 4C). As expected, PGE₂ did not increase LGR5 mRNA in the presence of either control or β-catenin siRNA (Figure 4D). Hence, these data suggest that PGE₂ can increase LGR5 protein expression through a β-catenin-independent pathway.

LGR5 promotes cell survival in adenoma cells

This study shows for the first time that PGE₂ upregulates LGR5 protein in colorectal adenoma cells. However, although recent studies suggest
that an LGR5+ subpopulation of cells in mouse adenomas drives tumour growth (23), the functional significance of LGR5 expression in human adenomas is currently unclear. It is therefore important to determine whether PGE2 might convey a survival advantage in human adenoma cells through regulating LGR5 protein expression. This is a potentially important question, given that the mechanism(s) by which NSAIDs cause adenoma regression in FAP patients remain unclear and in particular whether NSAIDs through blocking PGE2 could inhibit LGR5 CSC survival. Interestingly, LGR5 siRNA knockdown in RG/C2 cells reduced attached cell yield and increased cell death (Figure 5A). Similar results were observed in a second adenoma cell line, AA/C1 (Figure 5B), suggesting that LGR5 expression promotes colorectal adenoma cell survival. We next addressed whether the regulation of LGR5 protein by PGE2 was biologically significant by determining whether LGR5 knockdown compromised the ability of PGE2 to enhance RG/C2 cell survival. Indeed, PGE2 was no longer able to significantly enhance RG/C2 survival following LGR5 siRNA knockdown as a significant decrease in cell death was no longer observed (Figure 5C and D). Hence, our studies suggest that LGR5 has an important pro-survival role in human colorectal adenoma cells and that PGE2, at least in part, promotes survival in adenoma cells through LGR5.

**Discussion**

Despite considerable advances in our understanding of its molecular pathology, CRC remains a major cause of cancer death in the industrialized world. Increasing attention has focussed on both targeting PGE2 and LGR5 as potential therapeutic targets.
Fig. 5. LGR5 siRNA reduces survival in colorectal adenoma-derived cell lines. (A and B) LGR5 siRNA-mediated knockdown by 20 nM LGR5 smartpool (SP) siRNA decreases cell yield and increases cell death at 72 h in (A) RG/C2 and (B) AA/C1 adenoma-derived cell lines. LGR5 knockdown efficiency was confirmed by western blotting. (C) LGR5 siRNA-mediated knockdown reduces the sensitivity of RG/C2 to PGE2 treatment as it prevents PGE2 from significantly reducing cell death in RG/C2 cells. (D) LGR5 western blotting confirms upregulation of LGR5 protein by PGE2 and effective LGR5 knockdown. α-Tubulin was used as a loading control. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ and NS= not significant.
the earlier stages of tumorigenesis for chemoprevention, for example, through inhibiting PGE₂ production with NSAIDs (2) and, more recently, the possible targeting of resistant ‘cancer stem cell-like’ subpopulations of cells potentially responsible for relapse after therapy (40,41). The fact that NSAIDs cause adenoma regression in FAP patients (8,9), at least in part through inhibiting PGE₂, raised the interesting possibility that PGE₂ may have a role in maintaining adenoma stem cells.

Both the Wnt/β-catenin and COX/PGE₂ signalling pathways are important in driving colorectal tumorigenesis (7,11) and recent studies have suggested an important link between the two. For example, PGE₂ enhances Wnt/β-catenin signalling in colorectal carcinoma cells (14,15) and in normal haematopoietic stem cells where it promotes stem cell function (16). Although Wnt signalling plays a crucial role in intestinal stem cells, the relationship between PGE₂ and intestinal stem cells was yet to be determined.

Given the importance of targeting the early premalignant stages of tumorigenesis for chemoprevention, and the fact that the key intestinal CSC marker LGR5 is a Wnt target and PGE₂ enhances Wnt signalling, the focus of this study was to investigate whether PGE₂ could regulate LGR5 expression in colorectal adenoma cells and whether LGR5 was important for adenoma cell survival. In this study, using an siRNA approach, we showed for the first time a pro-survival role for LGR5 in human colorectal adenoma cells, which may explain its widespread expression in adenoma-derived cell lines reported here and in mouse and human adenomas in vivo (21,35,36).

We also showed that PGE₂ increases the level of LGR5 protein expression in PGE₂-responsive adenoma and carcinoma cells. Furthermore, our studies showed that PGE₂ promotes adenoma survival, at least in part, through LGR5, which is consistent with PGE₂ having an important role in intestinal stem cell behaviour.

Given the link between PGE₂ and Wnt signalling in colorectal carcinoma cells (14,15) and in normal haematopoietic stem cells (16) in our recent studies (42), we hypothesized that PGE₂ may also have a functional role in intestinal normal and/or tumour stem cell biology (42). In these studies, we showed that β-catenin can repress expression of the prostaglandin catabolizing enzyme 15-hydroxyprostaglandin dehydrogenase in colorectal tumour cell lines, including adenoma-derived cells, and that reducing β-catenin expression decreases PGE₂ levels. We suggested that PGE₂ may be elevated at the very earliest stages of colorectal neoplasia, when β-catenin is first activated, even before COX-2 is upregulated in the epithelial cells, which could explain the chemoprevention efficacy of non-selective NSAIDs (42). We also showed that β-catenin suppresses 15-hydroxyprostaglandin dehydrogenase in the base of normal murine intestinal epithelial crypts (42).

Indeed, some recent publications have added significant support for a role for PGE₂ in normal and/or intestinal tumour stem cell biology. Firstly, work using intestinal organoid culture models has shown that PGE₂ can support normal intestinal stem cell growth in vitro and hence could be part of the intestinal stem cell niche signals in vivo (24,43). Furthermore, one very recent report, published during the preparation of this article, suggests that cancer-stimulated mesenchymal stem cells create a carcinoma stem cell niche via PGE₂ signalling. In this model, mesenchyme-derived PGE₂ stimulates stemness-associated characteristics in LoVo colorectal carcinoma cells, such as upregulation of the putative stem cell marker aldehyde dehydrogenase (44).

Taken together with animal model data suggesting that Lgr5+ stem cells may be the origin of intestinal adenomas (21), and recent data suggesting that Lgr5+ adenoma cells constitute a CSC population (23), our data showing that PGE₂ upregulates LGR5 and LGR5 has a survival role in human colorectal adenoma cell lines has potentially important implications for the prospects for NSAIDs in CRC chemoprevention. One of the very striking observations in the field of chemoprevention was that the non-selective NSAID sulindac causes adenoma regression in FAP patients (8) and that this has also been observed with the COX-2 selective Celecoxib (9). Although the mechanism of adenoma regression may be, in part, through the induction of apoptosis, the precise mechanism remains unclear. The fact that the NSAIDs cause adenomas to regress in vivo does suggest that they act on the cancer (in this case adenoma) stem cells. It is interesting to speculate that the NSAIDs are causing tumour regression, at least in some adenomas, by blocking the survival-promoting effects of PGE₂ on LGR5+ adenoma stem cells. Furthermore, because many human CRCs express LGR5 (25) and that PGE₂ can drive the creation of a carcinoma stem cell niche by mesenchymal stem cells (44), supports the view that NSAIDs may be useful not only in chemoprevention but also as an adjuvant to therapy by blocking the ability of PGE₂ to support adenoma and carcinoma stem cell survival.

Supplementary material
Supplementary Figure 1 can be found at http://carcin.oxfordjournals.org/

Funding
Cancer Research UK Programme Grant (C19/A11975); Citrina Foundation; John James Bristol Foundation; Wellcome Trust; Oman government PhD scholarship (to M.R.A.-K.).

Acknowledgement
The LGR5-FLAGFLAGHA (LGR5-FH) construct and the LS174T-LGR5 cell line, which stably expresses LGR5 was a kind gift of Hans Clevers.

Conflict of Interest Statement: None declared.

References
PGE\textsubscript{2}/LGR5-mediated survival in adenoma cells


Received October 31, 2012; revised January 8, 2013; accepted January 12, 2013