Gastrin stabilises β-catenin protein in mouse colorectal cancer cells

DH Song¹, JC Kaufman¹, L Borodyansky¹, C Albanese², RG Pestell² and M Michael Wolfe*¹

¹Section of Gastroenterology, Boston University School of Medicine, Boston Medical Center, 650 Albany Street, Boston, MA 02118, USA; ²Department of Oncology and the Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC, 20057, USA

As gastrin may play a role in the pathophysiology of gastrointestinal (GI) malignancies, the elucidation of the mechanisms governing gastrin-induced proliferation has recently gained considerable interest. Several studies have reported that a large percentage of colorectal tumours overexpress or stabilise the β-catenin oncoprotein. We thus sought to determine whether gastrin might regulate β-catenin expression in colorectal tumour cells. Amidated gastrin-17 (G-17), one of the major circulating forms of gastrin, not only enhanced β-catenin protein expression, but also one of its target genes, cyclin D1. Furthermore, activation of β-catenin-dependent transcription by gastrin was confirmed by an increase in LEF-1 reporter activity, as well as enhanced cyclin D1 promoter activity. Finally, G-17 prolonged the τ/2 of β-catenin protein, demonstrating that gastrin appears to exert its mitogenic effects on colorectal tumour cells, at least in part, by stabilising β-catenin.

British Journal of Cancer (2005) 92, 1581 – 1587. doi:10.1038/sj.bjc.6602509 www.bjcancer.com
Published online 29 March 2005 © 2005 Cancer Research UK

Keywords: MC-26 cells; G-17; β-catenin; cyclin D1

Gastrin was originally described as a gastrointestinal (GI) regulatory peptide whose principal function was to stimulate postprandial gastric acid secretion. However, in addition to its recognised role in the physiological regulation of acid secretion, another biological property attributed to gastrin is its trophic effects. A prospective study by Thorburn et al (1998) suggested that hypergastrinemia is associated with an increased risk for colorectal cancer (CRC), and numerous studies have demonstrated that gastrin stimulates the growth of malignant colorectal adenocarcinomas (Wang et al, 1996; Malecka-Panas et al, 1997; Baldwin & Shulkes, 1998; Nakata et al, 1998; Koh et al, 1999; Stepan et al, 1999; Smith & Watson, 2000). Transgenic mice overexpressing progastrin and glycine-extended gastrin demonstrate enhanced colonic proliferation (Wang et al, 1996; Koh et al, 1999), and conversely, gastrin-deficient mice manifest decreased colonic proliferation (Koh et al, 1997). Repression of the gastrin gene in human colon cancer cells by antisense gastrin RNA yields a significant growth inhibition of these cells, suggesting that gastrin expression may be required for colon tumour progression (Singh et al, 1996). Although these studies all suggest a role for gastrin in the pathogenesis of CRC, little is known regarding the factors and mechanisms involved in mediating the trophic properties of this important peptide.

Overwhelming evidence derived from studies involving primary colorectal tumours suggests that hereditary and sporadic origins have implicated aberrations of the adenomatous polyposis coli (APC) tumour suppressor gene and β-catenin oncoprotein in the pathogenesis of CRC (Kinzler & Vogelstein, 1996; Mirabelli-Primdahl et al, 1999; Miyaki et al, 1999; Samowitz et al, 1999). Although multiple mechanisms may induce the neoplastic growth of colorectal tumours, β-catenin appears to play a pivotal role in this process. Under normal conditions, β-catenin degradation ensures tightly regulated cytoplasmic levels of this protein. Adenomatous polyposis coli appears to regulate the degradation of β-catenin protein by recruiting β-catenin into the negative regulatory complex for phosphorylation by glycogen synthase kinase 3β (GSK3β) in the N-terminus (Dominguez et al, 1995; Yost et al, 1996; Korinek et al, 1997) and subsequent proteasomal degradation (Aberle et al, 1997). Intricate interactions among other β-catenin binding partners also serve to facilitate the degradation of β-catenin to maintain a delicate balance (Ikeda et al, 1998; Kishida et al, 1998; Li et al, 1999; Farr et al, 2000). In contrast to the proteasomal degradation of β-catenin, which normally serves as a negative regulator of tumorigenesis, a positive regulator of β-catenin and tumorigenesis has also been identified. Protein kinase CK2 (formerly known as casein kinase 2), a serine/threonine kinase that is overexpressed in many malignancies, has been shown to phosphorylate β-catenin in the midportion of the protein and enhance its stability (Song et al, 2000, 2003a).

When β-catenin escapes its negative regulatory mechanisms, it translocates into the nucleus and functions as a critical transcriptional coactivator of the T-cell factor/lymphocyte enhancer binding factor (TCF/LEF), which activates oncogenes, such as c-myc (He et al, 1998) and cyclin D1 (Shtutman et al, 1999). Thus, activating mutations or stabilisation of β-catenin represent a critical process in the growth of the human CRC. Many β-catenin target genes have also been demonstrated as important factors in the pathogenesis of CRC. In particular, cyclin D1 was upregulated in human colorectal tumours and was associated with altered
β-catenin expression (Wang et al., 2002). Moreover, increased levels of both β-catenin and cyclin D1 were found in a clinical analysis of tissue samples obtained from CRC patients (Utsunomiya et al., 2001) and in the colonic tissue extracts of mice when hyperproliferation/hyperplasia was induced (Sellin et al., 2001).

Interestingly, an association between gastrin and β-catenin was not made until Koh et al. (2000) identified gastrin as a downstream target gene of β-catenin/TCF transcription. Because these factors are important contributors to CRC growth, we sought to determine whether additional relationships might exist between gastrin and β-catenin. We have previously demonstrated the trophic properties of gastrin in mouse colorectal tumour cells (MC-26), in which the peptide caused a significant incorporation of [3H]thymidine at 24 and 48 h (Yao et al., 2002). Furthermore, when MC-26 cells were injected subcutaneously into BALB/C mice and treated with amidated gastrin-17 (G-17) by continuous infusion, the weight and volume of resulting tumour tissues were significantly greater than in untreated controls (Yao et al., 2002).

In the present study, to determine whether the trophic properties of G-17 might involve modulation of β-catenin, MC-26 cells were treated with various concentrations of G-17, and various aspects of β-catenin expression were examined. We observed that G-17 not only enhanced the expression of total cellular β-catenin, but also increased nuclear β-catenin accumulation and β-catenin-dependent LEF-1 activity. Furthermore, cyclin D1 protein levels and promoter activity were enhanced by G-17. Finally, treatment with G-17 prolonged the half-life of β-catenin protein, suggesting that one of the major mechanisms by which G-17 might induce its trophic effects is through stabilisation of the multifunctional oncogenic β-catenin protein. The results of our studies are consistent with the presence of a vicious cycle between gastrin and β-catenin that would favour an environment for uncontrolled, aggressive CRC growth.

**MATERIALS AND METHODS**

**Cell culture and treatments**

We utilised MC-26 mouse CRC cells, which were maintained in Dulbecco’s modified Eagle’s Medium (DMEM; Gibco Laboratories, Grand Island, NY, USA), supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin. Amidated G-17 (Peninsula/Bachem, Belmont, CA, USA) was added to the culture medium (20–100 nM) for 2–4 h, and 1 μM of the gastrin-specific receptor antagonist L365,260 (kindly provided by Dr L Iverson, Oxford, UK) was used in conjunction with G-17 in the indicated experiments. Cycloheximide, a de novo protein synthesis inhibitor, was used at a final concentration of 10 μg ml⁻¹, either alone or in combination with 20 or 50 nM G-17 for the indicated times (0, 3, 6, and 24 h).

**Northern analysis**

Total RNA was extracted using the Qiagen RNeasy kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer’s instructions. Each RNA sample (10 μg) was loaded onto a formaldehyde-containing agarose gel and transferred via capillary action overnight onto a Hybond-N nylon membrane (Amersham Pharmacia, Piscataway, NJ, USA) in 10 × SSC buffer. Transferred membrane was crosslinked and prehybridised prior to the addition of the labelled probe. Approximately 1 kb fragment of β-catenin cDNA and 3.2 kb fragment of actin cDNA were excised and used as probes. Both of the probes were labelled with [32P]dCTP for 30 min, purified with Quick Spin sephadex G-25 columns (Roche, Basel, Switzerland), boiled, and incubated with prehybridised membrane overnight at 65°C. Labelled membranes were washed four times, twice in 2 × SSC/0.1% SDS and twice in 0.2 × SSC/0.1% SDS, before exposing to a film. The membrane was briefly stripped with boiling 0.1% SDS and washed 3 × with 2 × SSC before addition of another probe.

**Western analysis**

Total protein was extracted, as previously described (Song et al., 2000, 2003a). For nuclear protein extraction, a protocol described by Dignam et al. (1983) was followed. Briefly, cells were washed twice in 1 × phosphate-buffered saline (PBS) and scraped in the presence of 200 mM EDTA in PBS. Through Dounce homogenation and differential centrifugation, nuclear proteins were separated from cytoplasmic fractions in the presence of protease inhibitors. Protein quantification was performed using the BCA protein assay.

Western blotting analyses were performed, as previously described (Song et al., 2000, 2003a), using antibodies against β-catenin (Transduction Laboratories, Lexington, KY, USA) and cyclin D1 (Pharmingen, Chicago, IL, USA). Ponceau S (Sigma, St Louis, MO, USA) staining and immunoblots with either a monoclonal β-actin antibody (Sigma) for whole-cell lysates or polyclonal Sp1 antibody (Santa Cruz, Santa Cruz, CA, USA) for nuclear extracts were used to confirm equal loading of Western blot membranes.

**Reporter assays**

MC-26 cells (1–2 × 10⁵) were plated 1 day prior to transfection. For transient transfection experiments, subconfluent cells were incubated with DNA and FUGENE-6 liposome reagent (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer’s instructions. Both LEF-1 and cyclin D1 (minimal and full-length cyclin D1 promoter-luciferase constructs; Albanese et al., 2003) experiments utilised the FUGENE-6 transfection reagent to deliver the target plasmids. In addition, renilla was cotransfected with the firefly reporter plasmids to normalise for transfection efficiency. Total DNA was balanced with the addition of empty vector when multiple plasmids were used. At 24 h after the transfection, the cells were incubated with 20 and 50 nM G-17 for 4 h, harvested, and assayed for firefly luciferase reporter and for renilla activity. Briefly, 10 μl of protein extract was first assayed with 50 μl of firefly luciferase substrate (Promega, Madison, WI, USA) in a luminometer for 10 s. Subsequently, in the same tube, 50 μl renilla substrate (Promega) were added and measured. Samples were assayed in duplicate and the luciferase counts were normalised to renilla measurements.

**In vitro kinase assay**

Equal amounts of protein extracted from MC-26 cells (10 μg) were assayed for CK2 kinase activity, as previously described (Song et al., 2000, 2003a). Briefly, each sample was assayed in duplicate with and without CK2-specific synthetic peptide, RRREEETEEE (Promega), for 20 min at 37°C with 5 μCi [γ-32P]ATP. Radioactive counts were blotted onto p81 filter circles, washed 4 × in 150 mM H₂PO₄, and analysed on an automated liquid scintillation counter. Buffer controls with and without the substrate peptide (background control) were also measured and subtracted from the final radioactive counts.

**Statistical analysis**

The two-way Student’s t-test was performed for paired comparisons. Statistical significance was assigned if P < 0.05.
RESULTS

Gastrin-17 enhances β-catenin protein levels in MC-26 cells

To determine the potential role of gastrin in modulating β-catenin, mRNA and protein levels were measured by Northern and Western blot hybridisations, respectively, in MC-26 cells that were transiently treated with G-17. Although incubation of MC-26 cells in the presence of G-17 did not alter the concentration of β-catenin transcripts (Figure 1A), total protein level of β-catenin was significantly enhanced by 20 and 50 nM G-17 following both 2 and 4 h of incubation (Figure 1B). Furthermore, coincubation with L365,260, a gastrin receptor (CCK2) antagonist, attenuated the upregulation of β-catenin by greater than 50%, suggesting that the increase in β-catenin was specific (Figure 1C). An induction of β-catenin was consistently detected, and the results were reproduced on four separate occasions. Although the magnitude of the change in total β-catenin protein varied within individual experiments, a 3–4-fold increase in total β-catenin protein levels was detected when bands were quantified by densitometry and normalised to β-actin levels (Figure 1D).

As mentioned above, nuclear accumulation of β-catenin represents a key event in CRC progression. To examine whether G-17 can enhance nuclear β-catenin in MC-26 cells, cells were treated with G-17 for 4 h and nuclear extracts were prepared. In all, 20 and 50 nM G-17 induced approximately a two-fold increase in nuclear β-catenin levels (Figure 1E), suggesting that G-17 promotes nuclear translocation of β-catenin. Expression of Sp1, a ubiquitously expressed transcription factor, was used as a loading control for nuclear extracts.

Gastrin-17 increases LEF-1-dependent transcriptional activity

To examine whether the increase in nuclear β-catenin protein is also associated with the activation of LEF-1, LEF-1-dependent reporter assays were performed. The pGL3-LEF-1 luciferase construct (kindly provided by Dr R Grosschedl, Munich, Germany) contains eight repeats of the LEF binding site that is activated only in the presence of an exogenous LEF-1 construct. The level of LEF-1-dependent transcription is also dependent on nuclear β-catenin levels, as β-catenin is a known coactivator for TCF/LEF transcription factors. As we speculated that an increase in β-catenin protein by G-17 might be functionally important for the transcriptional activation of its target genes, LEF-1-dependent reporter assays were performed in the absence and presence of G-17. We observed that 20 and 50 nM G-17 induced a concentration-dependent increase in LEF-1-dependent transcriptional activity (P<0.005) (Figure 2). In addition, the effects of gastrin on cyclin D1, one of the target genes of β-catenin-dependent transcription, were analysed. In response to the inclusion of G-17 in the culture medium, both cyclin D1 protein levels and promoter activity were increased (Figure 3). Specifically, 50 nM G-17 significantly enhanced the activity of the full-length cyclin D1 promoter (−1745)

Figure 1 (A) Gastrin does not affect β-catenin mRNA levels, as demonstrated by Northern blot analysis. MC-26 cells were incubated for 4 h in the presence of increasing concentrations of G-17 (10–100 nM; upper panel). 28S and 18S ribosomal RNAs are indicated on the left, and actin was used as a loading control (lower panel). (B) Gastrin increases β-catenin protein levels, as demonstrated by Western blot analysis. Compared to untreated samples, 2 and 4 h of treatment with 20 and 50 nM G-17 caused a significant increase in β-catenin protein levels (upper panel). β-Actin was used as a loading control (lower panel). (C) The addition of 1 μM L365,260, a gastrin-specific receptor antagonist, attenuated β-catenin induction by G-17 (upper panel, lane 5), indicating that the increase in β-catenin was gastrin-specific. (D) Average per cent change of β-catenin when normalised to β-actin levels, as measured by densitometry, of four independent experiments. Densitometry units were measured within each individual experiment and compared to control values, which were designated 100%, and per cent change in response to G-17 treatment was calculated. Data represent mean per cent of control±s.e. (n = 4). *P<0.005, **P<0.01. (E) G-17 (20 and 50 nM) enhanced nuclear β-catenin levels (upper panel). Sp1, a nuclear protein, was used as a loading control (lower panel).
when compared to either the empty or minimal promoter (−66) (Figure 3B, \( P \leq 0.01 \)).

**Gastrin-17 stabilises β-catenin protein by increasing its half-life in MC-26 cells**

Since we observed that G-17 did not change the number of β-catenin transcripts but increased β-catenin protein levels, protein stability of β-catenin was next examined. To determine this, MC-26 cells were incubated with cycloheximide, a de novo protein synthesis inhibitor, either in the absence or presence of 20 and 50 nM G-17. Because β-catenin is normally degraded by proteasomes, the addition of cycloheximide would enable the pool of translated cytoplasmic protein to be degraded at its natural rate. Total protein was extracted at 0, 3, and 6 h, and β-catenin protein levels were measured by Western analysis. In the presence of cycloheximide alone, nearly 50% of β-catenin was degraded by 3 h (Figure 4A and C). However, coincubation with either 20 or 50 nM G-17 stabilised β-catenin and prolonged its half-life. Even after 6 h of treatment, β-catenin protein levels were largely unchanged in cells cultured in the presence of G-17 (Figure 4A). Specifically, the half-life of β-catenin in the presence of 50 nM G-17 was approximately 24 h, whereas nearly complete degradation of β-catenin protein was detected with cycloheximide alone at 24 h (Figure 4B and C). An approximate three-fold difference in β-catenin levels was detected between control conditions and following incubation in the presence of 50 nM G-17 at 24 h, suggesting that G-17 modulates β-catenin by stabilisation of the protein (Figure 4C).

To delineate the mechanism by which gastrin might cause stabilisation of β-catenin, we examined two known regulators of β-catenin. Specifically, GSK3β, an upstream negative regulator of β-catenin that promotes proteasomal degradation of β-catenin, and protein kinase CK2, a positive regulator, were examined. No consistent effect on GSK3β kinase activity could be demonstrated in response to the incubation of MC-26 cells in media containing various concentrations of G-17 (data not shown). In contrast, 20 and 50 nM G-17 caused a marked increase in endogenous CK2 kinase activity (Figure 5A). Moreover, coincubation of 20 nM G-17 with apigenin, a purportedly selective CK2 inhibitor, attenuated total β-catenin protein levels compared to 20 nM G-17 alone, suggesting that G-17 may utilise CK2 to regulate β-catenin (Figure 5B). However, the addition of apigenin to media containing increasing concentrations of G-17 did not abolish the induction of β-catenin (Figure 5B).

**DISCUSSION**

Both gastrin and various components of the β-catenin-dependent signaling pathway have been implicated in the pathogenesis of CRC (Nusse, 2002). However, a functionally relevant association between gastrin and β-catenin was not made until Koh *et al.* (1997, 2000) demonstrated that gastrin-deficient APC (min−/−) mice produced fewer polyps than APC (min−/+ ) mice overexpressing gastrin. Furthermore, these investigators showed that β-catenin enhanced gastrin promoter activity, thus identifying gastrin as one of its numerous downstream targets. However, the possibility of a positive feedback relationship between gastrin and β-catenin expression has not been examined previously.

Utilising transplantable mouse CRC cells (MC-26) that express functional gastrin receptors, we have previously demonstrated the trophic properties of gastrin (Yao *et al.*, 2002). We hypothesised that one of the mechanisms by which gastrin might exert its trophic properties may involve the multifunctional β-catenin protein. We consistently observed that gastrin increases β-catenin protein levels. However, despite our attempts to maintain consistency, such as plating equal amounts of cells 1 day prior to each individual experiment, we nevertheless did observe some variability in the basal expression (untreated) of β-catenin during the performance of different experiments. This variability may be due in part to the role of β-catenin in cell–cell adhesion, which,
depending on cell density, may contribute to the variability in basal β-catenin expression.

Further examination of MC-26 cells in the present study suggests that gastrin prolongs the half-life of β-catenin by increasing its stability. Thus, it appears that β-catenin enhances gastrin expression, and conversely, β-catenin protein expression is stabilised by gastrin, completing a vicious cycle that may contribute to neoplastic cell survival and growth. Moreover, data presented in this study provide further evidence for the complex nature of the oncogenic process by suggesting that gastrin utilises multiple pathways in regulating β-catenin. In the present study, we observed that gastrin stimulated CK2 activity and that gastrin-stimulated β-catenin expression was partially attenuated in the presence of the CK2 selective inhibitor apigenin. Inhibition of CK2 activity did not abolish gastrin-mediated effects on β-catenin, suggesting that gastrin signalling possesses both CK2-dependent and -independent properties. It is plausible that this important regulatory peptide controls β-catenin through multiple regulators, as β-catenin itself is known to have numerous modulators (Dominguez et al, 1995; Yost et al, 1996; Korinek et al, 1997; Ikeda et al, 1998; Kishida et al, 1998; Li et al, 1999; Farr et al, 2000; Song et al, 2000, 2003a).

The existence of a pathological vicious cycle (a positive feedback loop) involving β-catenin, as we postulate, would serve to enhance the survival and continued growth of CRC cells by selectively upregulating various oncogenic factors. A recent study has suggested the existence of another pathological vicious cycle involving β-catenin in CRC. In addition to its effects on gastrin and on the expression of other target genes, Hovanes et al (2001) reported that LEF-1, one of the transcriptional partners of β-catenin, is likewise a target gene of β-catenin/TCF-dependent transcription.

The upregulation of β-catenin expression by gastrin was also associated with the enhancement of the critical cell cycle regulator, cyclin D1. Consistent with our current findings, we have previously reported that gastrin enhanced cyclin D1 protein and cyclin D1 promoter activity in the human gastric adenocarcinoma cell line AGS-B (Song et al, 2003b). However, in contrast to the present study, we did not observe an increase in β-catenin protein expression in AGS-B cells incubated in the presence of gastrin. Several possibilities may explain these disparate results, including interspecies variations. Another possibility is the fact that AGS-B cells have been engineered to overexpress the gastrin receptor, which could potentially favour a direct increase in cyclin D1 by gastrin rather than utilising β-catenin as a mediator of transcription. Furthermore, overexpression of the receptor may have modulated other components that could affect β-catenin stability. Despite our observation in the present study that both β-catenin
and cyclin D1 expression were enhanced by gastrin, it is nevertheless possible that the increase in cyclin D1 may have occurred independently of β-catenin-dependent transcription. Along these lines, gastrin has been previously shown to stimulate the expression of c-myc, another target of β-catenin, in intestinal epithelial cells (IEC-6) (Wang et al., 1995). Although we did not examine c-myc levels in this study, it is certainly possible that gastrin may involve not only c-myc and cyclin D1, but also multiple β-catenin target genes and pathways in exerting its growth potential, whether directly or indirectly. Another possibility is simply the fact that every immortal cell line possesses slightly different characteristics that produce disparate results. For example, unlike AGS-B cells (Song et al., 2003b), in AGS-E cells, a related human gastric adenocarcinoma cell line overexpressing the gastrin receptor, G-17 induction of cyclin D1 transcription was mediated through both β-catenin and CREB pathways (Pradeep et al., 2004).

In conclusion, the results of the present studies demonstrate for the first time that gastrin enhances β-catenin protein by prolonging its half-life. Furthermore, these studies support our hypothesis that a positive feedback mechanism exists between gastrin and β-catenin. Although further studies will be required to elucidate fully the mechanisms governing gastrin-induced cellular proliferation, our results suggest that through aberrant over-expression of β-catenin, malignant cells appear to amplify various signals via positive feedback between molecules as a means for potentiating tumorigenesis and proliferation.

**ACKNOWLEDGEMENTS**

We thank Dr R Grosschedl for providing plasmids used in this study. We thank Drs DC Seldin, B Rana, and members of the Wolfe laboratory for helpful discussions. This work was supported by NIH grants to MMW (RO1DK53158), to CA (RO3 AG20337, ACS IRG 97-152-11, RO1 CA075503-06), and to RGP (R01CA70896, R01CA75503, R01CA86072, RO1 CA86071). RGP is the Diane Belfer Faculty Scholar in Cancer Research and is a recipient of the Weil Caulier Irma T Hirschl Career Scientist award, and he receives support from the Breast Cancer Alliance Inc. and The Susan Komen Breast Cancer Foundation.

**REFERENCES**

Aberle H, Bauer A, Stappert J, Kispert A, Kemler R (1997) Beta-catenin is a target for the ubiquitin–proteasome pathway. **EMBO J** 16: 3797–3804

Albanese C, Wu K, D'Amico M, Jarrett C, Joyce D, Hughes J, Hulit J, Sakamaki T, Fu M, Ben-Ze'ev A, Bromberg JF, Lamberti C, Verma U, Gaynor RB, Byers SW, Pestell RG (2003) IKKalpha regulates mitogenic signaling through transcriptional induction of cyclin D1 via Tcf. **Mol Biol Cell** 14: 585–599

Baldwin GS, Shulkes A (1998) Gastrin, gastrin receptors and colorectal carcinoma. **Gut** 42: 581–584

Dignam JD, Lebovitz RM, Roeder RG (1983) Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. **Nucleic Acids Res** 11: 1475–1489

Domínguez I, Itoh K, Sokol SY (1995) Role of glycogen synthase kinase 3 beta as a negative regulator of dorsoventral axis formation in Xenopus embryos. **Proc Natl Acad Sci USA** 92: 8498–8502

Farr III GH, Ferkey DM, Yost C, Pierce SB, Weaver C, Kimelman D (2000) Interaction among GSK-3β, GSK, axin, and APC in Xenopus axis specification. **J Cell Biol** 148: 691–702

He TC, Sparks AB, Rago C, Hermeking H, Zawel L, de Costa LT, Morin PJ, Vogelstein B, Kinzler KW (1998) Identification of c-MYC as a target of the APC pathway [see comments]. **Science** 281: 1509–1512

Hovanes K, Li TW, Mungua JE, Truong T, Milovanovic T, Lawrence Marshall J, Holcombe RF, Waterman ML (2001) Beta-catenin-sensitive isoforms of lymphoid enhancer factor-1 are selectively expressed in colon cancer. **Nat Genet** 28: 53–57

Ikeda S, Kishida S, Yamamoto H, Murai H, Koyama S, Ikikuchi A (1998) Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3beta and beta-catenin and promotes GSK-3beta-dependent phosphorylation of beta-catenin. **EMBO J** 17: 1371–1384

Kinzler KW, Vogelstein B (1996) Lessons from hereditary nonpolyposis colorectal cancer. **Cell** 83: 159–170

Kishida S, Yamamoto H, Ikeda S, Kishida M, Sakamoto I, Koyama S, Ikikuchi A (1998) Axin, a negative regulator of the Wnt signaling pathway, directly interacts with adenomatous polyposis coli and regulates the stabilization of beta-catenin. **J Biol Chem** 273: 10823–10826

Koh TJ, Bulitta CJ, Fleming JV, Dockray GJ, Varro A, Wang TC (2000) Gastrin is a target of the beta-catenin/TCF-4 growth-signaling pathway in a model of intestinal polyposis. **J Clin Invest** 106: 533–539

Koh TJ, Dockray GJ, Varro A, Cahill RJ, Dangler CA, Fox JG, Wang TC (1999) Overexpression of glycine-extended gastrin in transgenic mice results in increased colonic proliferation. **J Clin Invest** 103: 1119–1126

Koh TJ, Goldenring JR, Ito S, Mashimo H, Kopin AS, Varro A, Dockray GJ, Wang TC (1997) Gastrin deficiency results in altered gastric differentiation and decreased colonic proliferation in mice. **Gastroenterology** 113: 1015–1025

Korinek V, Barker N, Morin PJ, van Wichen D, de Weger R, Kinzler KW, Vogelstein B, Clevers H (1997) Constitutive transcriptional activation by a beta-catenin–Tcf complex in APC–/- colon carcinoma [see comments]. **Science** 275: 1784–1787

Li L, Yuan H, Weaver CD, Mao J, Farr III GH, Sussman DJ, Jonkers J, Kimelman D, Wu D (1999) Axin and Frat1 interact with dvl and Gsk, bridging Dvl to Gsk in Wnt-mediated regulation of LEF-1. **EMBO J** 18: 4233–4240

Malecka-Panas E, Fligiel SE, Jaszewski R, Majumdar AP (1997) Differential responsiveness of proximal and distal colonic mucosa to gastrin. **Peptides** 18: 559–565

Mirabeli-Prindahl L, Gryfe R, Kim H, Millar A, Luceri C, Dale D, Holowaty E, Bapat B, Gallinger S, Redston M (1999) Beta-catenin mutations are specific for colorectal carcinomas with microsatellite instability but occur in endometrial carcinomas irrespective of mutator pathway. **Cancer Res** 59: 3346–3351

Miyaki M, Iijima T, Kimura J, Yasuno M, Mori T, Hayashi Y, Koike M, Shiota N, Iwama T, Kuroki T (1999) Frequent mutation of beta-catenin in colorectal tumors is not inversely related to tumor stage. **Jpn J Cancer Res** 90: 1296–1300

Pradeep A, Sharma C, Sathyanarayana P, Albanese C, Fleming JV, Wang TC, Wolfe MM, Baker KM, Pestell RG, Rana B (2004) Gastrin-mediated activation of cyclin D1 transcription involves beta-catenin and CREB pathways in gastric cancer cells. **Oncogene** 23: 3689–3699

Samowitz WS, Powers MD, Spirio LN, Nollet F, van Roy F, Slattery ML, Malecka-Panas E, Fligiel SE, Jaszewski R, Majumdar AP (1997) Gastrin is a target of the beta-catenin/TCF-4 growth-signaling pathway in human gastric adenocarcinoma. **Gastroenterology** 113: 1015–1025

Selbin JH, Umar S, Xiao J, Morris AP (2001) Increased beta-catenin expression and nuclear translocation accompany cellular hyperproliferation in vivo. **Cancer Res** 61: 2891–2896

Shutman M, Zhirinsky J, Simcha I, Albanese C, D'Amico M, Pestell R, Ben-Ze'ev A (1999) The cyclin D1 gene is a target of the beta-catenin/LEF-1 pathway. **Proc Natl Acad Sci USA** 96: 5522–5527

Singh P, Owlia A, Varro A, Dai B, Rajaraman S, Wood T (1996) Gastrin gene expression is required for the proliferation and tumorigenicity of human colon cancer cells. **Cancer Res** 56: 4111–4115

Smith AM, Watson SA (2000) Gastrin and gastrin receptor activation: an early event in the adenoma–carcinoma sequence. **Gut** 47: 820–824

Song DH, Dominguez I, Mizuno J, Kaut M, Mohr SC, Seldin DC (2003a) CK2 phosphorylation of the armadillo repeat region of beta-catenin potentiates Wnt signaling. **J Biol Chem** 278: 24018–24025
Song DH, Rana B, Wolfe JR, Crimmins G, Choi C, Albanese C, Wang TC, Pestell RG, Wolfe MM (2003b) Gastrin-induced gastric adenocarcinoma growth is mediated through cyclin D1. *Am J Physiol Gastrointest Liver Physiol* **285**: G217 – G222

Song DH, Sussman DJ, Seldin DC (2000) Endogenous protein kinase CK2 participates in Wnt signaling in mammary epithelial cells. *J Biol Chem* **275**: 23790 – 23797

Stepan VM, Sawada M, Todisco A, Dickinson CJ (1999) Glycine-extended gastrin exerts growth-promoting effects on human colon cancer cells. *Mol Med* **5**: 147 – 159

Thorburn CM, Friedman GD, Dickinson CJ, Vogelman JH, Orentreich N, Parsonnet J (1998) Gastrin and colorectal cancer: a prospective study. *Gastroenterology* **115**: 275 – 280

Utsunomiya T, Doki Y, Takemoto H, Shiozaki H, Yano M, Sekimoto M, Tamura S, Yasuda T, Fujiwara Y, Monden M (2001) Correlation of beta-catenin and cyclin D1 expression in colon cancers. *Oncology* **61**: 226 – 233

Wang HL, Wang J, Xiao SY, Haydon R, Stoiber D, He TC, Bissonnette M, Hart J (2002) Elevated protein expression of cyclin D1 and Fra-1 but decreased expression of c-Myc in human colorectal adenocarcinomas overexpressing beta-catenin. *Int J Cancer* **101**: 301 – 310

Wang JY, Wang H, Johnson LR (1995) Gastrin stimulates expression of protooncogene c-myc through a process involving polyamines in IEC-6 cells. *Am J Physiol* **269**: C1474 – C1481

Wang TC, Koh TJ, Varro A, Cahill RJ, Dangler CA, Fox JG, Dockray GJ (1996) Processing and proliferative effects of human gastrin in transgenic mice. *J Clin Invest* **98**: 1918 – 1929

Yao M, Song DH, Rana B, Wolfe MM (2002) COX-2 selective inhibition reverses the trophic properties of gastrin in colorectal cancer. *Br J Cancer* **87**: 574 – 579

Yost C, Torres M, Miller JR, Huang E, Kimelman D, Moon RT Qj(1996) The axis-inducing activity, stability, and subcellular distribution of beta-catenin is regulated in *Xenopus* embryos by glycogen synthase kinase 3. *Genes Dev* **10**: 1443 – 1454