Foxl1 Controls the Wnt/β-Catenin Pathway by Modulating the Expression of Proteoglycans in the Gut*

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Foxl1 is a winged helix transcription factor expressed in the mesenchyme of the gastrointestinal tract. Foxl1 null mice display severe structural defects in the epithelia of the stomach, duodenum, and jejunum. Here we addressed the molecular mechanisms by which Foxl1 controls gastrointestinal differentiation. First we showed that the abnormalities found in the epithelia of the null mice are the result of an increase in the number of proliferating cells and not a change in the rate of cell migration. Next we investigated the regulatory circuits affected by Foxl1. We focused on the Wnt/β-catenin signaling pathway as a possible target of Foxl1 as it has been shown to play a central role in gastrointestinal proliferation. We demonstrated that Foxl1 activates the Wnt/β-catenin pathway by increasing extracellular proteoglycans, which act as co-receptors for Wnt. Thus we establish that Foxl1 is involved in the regulation of the Wnt/β-catenin pathway, providing a novel link in mesenchymal/epithelial cross-talk in the gut. Moreover, we provide the first example implicating proteoglycans in the regulation of cellular proliferation in the gastrointestinal tract.

The epithelium of the gut is renewed continuously by division of stem cells and proliferation and migration of their descendants. This increase in cell number is balanced by apoptosis and extrusion of the senescent cells into the gut lumen (1, 2). The process of gut differentiation is regulated by a number of factors including hormones, growth factors, cytokines, cell-cell, and cell-matrix interactions (2–4). We have demonstrated previously that targeted inactivation of the transcription factor Foxl1 (previously Fkhb8) results in a dramatically altered gut epithelium, characterized by branched and elongated glands in the stomach and lengthened villi in the jejunum (5). These findings provided the first genetic evidence for a regulatory cascade between the mesenchyme and the epithelium in the mammalian gut. Thus far, the molecular mechanisms of Foxl1 action in the gastrointestinal tract have not been elucidated.

Among the signaling systems that might be regulated by Foxl1 is the Wnt/β-catenin pathway. This pathway plays a central role in the regulation of gastrointestinal proliferation, and mutations in this pathway have been found in greater than 80% of colorectal cancers (6, 7). The Wnt/β-catenin pathway consists of many components (for review, see Ref. 8). The secreted Wnt proteins bind to the cell-surface frizzled receptors, which contain seven transmembrane domains. In the off state of the pathway, a multiprotein complex containing glycosynthetase-3β phosphorylates β-catenin, followed by ubiquitination and subsequent degradation of β-catenin by the proteosome. As a consequence, the cytoplasmic and nuclear concentrations of β-catenin are low. In contrast, in the on state of the pathway, Wnt binding to the frizzled receptors activates the cytoplasmic phosphoprotein dishevelled, which leads to inhibition of β-catenin phosphorylation through glycosynthetase-3β by a still unknown mechanism. Hypophosphorylated β-catenin cannot be ubiquinated and therefore accumulates in the cytoplasm and subsequently translocates to the nucleus, where it forms a transcriptional activator by associating with HMG-box DNA-binding proteins (TCF and LEF factors). Activation of β-catenin/TCF target genes including cyclin D1 ultimately results in increased proliferation of the Wnt target cells. The elucidation of the Wnt/β-catenin pathway has provided an elegant explanation as to why mutations found in components of the pathway, like APC and β-catenin, lead to cancer in humans.

Recent evidence indicates that the Wnt pathway can also be modulated by heparan sulfate proteoglycans (HSPG)9 (9–13). HSPGs are abundant on the cell surface and in the extracellular matrix surrounding gastrointestinal cells. HSPGs act as low-affinity co-receptors for several signaling molecules and thereby facilitate ligand-receptor interactions (14). The Drosophila HSPG daily was shown by genetic means to regulate wingless signaling, which is the fly homologue of the mammalian Wnt/β-catenin pathway (10, 15). Recently, it was demonstrated through a gene targeting experiment that the HSPG Syndecan-1 is required for Wnt-1 induced mammary tumorigenesis in mice, pointing to the importance of the HSPGs as co-receptors for Wnt proteins in epithelial proliferation in mammals.

In the present study, we show that the abnormal gastrointestinal architecture in Foxl1 mutant mice is caused primarily by an increase in epithelial proliferation. This altered proliferation rate is correlated with an activated Wnt/β-catenin pathway as demonstrated by increased nuclear translocation of

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1 The abbreviations used are: HSPG, heparan sulfate proteoglycans; BrdUrd, bromodeoxyuridine; PBS, phosphate-buffered saline; bis-tris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol.

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β-catenin. We show that an increase in HSPG levels is associated with this activation of the Wnt/β-catenin signaling pathway, providing the first evidence for the involvement of HSPGs in gastrointestinal proliferation.

EXPERIMENTAL PROCEDURES

Plasmid Construction—A fragment of the mouse Syndecan-1 cDNA was amplified by reverse transcriptase-polymerase chain reaction. Reverse transcriptase reactions were performed as described previously (16) with the use of RNA extracted from mouse small intestine. The following primer pair was used for amplification: Sdc-1A 5'-CTTCT-TCTCGACTTGTGCT-3' and Sdc-1B 5'-GGCCTGTAACCTTG-GCTGA-3'. Polymerase chain reaction amplification was carried out for 32 cycles with denaturation at 95 °C for 45 s, annealing of the primers at 60 °C for 45 s, and reaction extension at 72 °C for 90 s. A 220-base pair fragment extending from positions 61 to 281 of Sdc-1 was subcloned into the EcoRV site of Bluescript KS (Stratagene, La Jolla CA).

Proliferation Assay—60-day-old wild type or Foxl1 mutant mice (F1 hybrids of 129SvEv and C57Bl/6 strains) were injected with 120 mg/kg BrdUrd and 12 mg/kg 5-fluoro-deoxyuracil 1.5 h prior to sacrifice. Tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and 5-μm sections were applied to probe-on plus slides and prepared for immunofluorescence. Sections were dewaxed and rehydrated in water. Slides were immersed in 0.01 M citrate acid buffer (pH 6.0) and microwave to boil for 7 min, then cooled, washed in PBS, and incubated for 20 min at 37 °C in 10% normal goat serum (Vector). Mouse monoclonal anti-BrdUrd (1:500, Roche Molecular Biochemicals) was diluted in 10% normal goat serum applied on the slides and incubated overnight at 4 °C in a moist chamber. After washing with PBS, fluorescein isothiocyanate-conjugated goat anti-mouse antibody (1:25, Roche Molecular Biochemicals) was diluted in 10% normal goat serum applied on the BrdUrd slides, and incubated for 45 min at 37 °C in a moist chamber. Tissue sections were counterstained with 0.01% Evan’s blue as described previously (17) and mounted with Vectashield (Vector). Images were captured on a Nikon Microphot FX microscope. BrdUrd-labeled
nuclei were counted manually in a blinded fashion and care was taken only to evaluate glands or crypts that were sectioned longitudinally.

**Indirect Immunofluorescence**—Frozen sections of stomach from 60-day-old wild type or Foxl1 mutant mice were embedded in O.C.T. (Miles Tek) and sectioned as described elsewhere (17). Tissues were fixed in ethanol (10 min at −20 °C) before immunostaining, washed in PBS, and incubated for 20 min at room temperature in protein blocking agent (ImmunoTech). Mouse monoclonal anti-β-catenin (1:500, Transduction Laboratories) and rat monoclonal anti-Syndecan-1 (1:1000, Research Diagnostics Inc.) antibodies were diluted in PBS containing 10% nonfat dry milk, applied on the slides, and incubated 1 h at room temperature in a moist chamber. Fluorescein isothiocyanate-conjugated goat anti-mouse IgG or fluorescein isothiocyanate-conjugated goat anti-rat IgG (1:25, Roche Molecular Biochemicals), were diluted in 10% nonfat dry milk and incubated for 1 h at room temperature in a moist chamber. Tissue sections were counterstained with 0.01% Evan’s blue as described previously (17) and mounted with Vectashield (Vector).

**Protein Analysis**—Total, nuclear, and cytoplasmic proteins were isolated from stomach and jejunum of 75-day-old Foxl1 mice as described previously (18). For β-catenin Western blot analysis, 15 μg of nuclear and cytoplasmic extracts were separated by SDS-polyacrylamide gel electrophoresis and transferred to an Immobilon-P membrane for 75 min at 4 °C. For Syndecan-1 Western blot analysis, 50 μg of total protein extract was separated by a Nupage 4–12% bis-tris gel (Invitrogen) and transferred to an Immobilon-P membrane for 75 min at room temperature. The membranes were blocked with 5% nonfat dry milk in PBS and 0.1% Tween overnight at room temperature. The membrane was then incubated 3 h with the primary antibody. The following antibodies were used: α-tubulin mouse monoclonal IgG, raised against the C-terminal region of sea urchin protein (Sigma) diluted 1:1000, YY1 mouse monoclonal IgG, raised against the full-length human protein (Santa Cruz Biotechnology) diluted 1:100, β-catenin mouse monoclonal IgG, raised against the C-terminal region of mouse protein (Transduction Laboratories) diluted 1:1000, and CD138 (Syndecan-1/plasma cells) rat monoclonal IgG, raised against the core protein region of mouse protein (Research diagnostic, Inc.) diluted 1:500. The membrane was washed five times for 10 min in PBS with 0.1% Tween and incubated with the secondary antibody anti-mouse/horseradish peroxidase (Amersham Pharmacia Biotech) or for Syndecan-1, anti-rat/horseradish peroxidase (Santa Cruz Biotechnology) for 45 min at room temperature. The membrane was then washed five times for 10 min in PBS with 0.1% Tween and developed with the ECL-Plus Western blotting kit (Amersham Pharmacia Biotech).

**RNA Analysis**—RNA was extracted from gastrointestinal tissues using Tri-reagent-LS (Molecular Research Center) following the manufacturer’s instructions. Ribonuclease (RNase) protection assays were performed as described previously (5). Transcripts were synthesized using T3 RNA polymerase to yield probes that protect a 220-nucleotide region for Syndecan-1. As a control for total RNA integrity, a 156-nucleotide riboprobe for TATA box binding protein was synthesized using T7 RNA polymerase.

## RESULTS

**Increased Proliferation, Not Altered Cell Migration, Causes Epithelial Dysgenesis in Foxl1 Mice**—Mice lacking the winged helix transcription factor Foxl1 (previously Fkh6) show dramatic alterations in the architecture of the gastrointestinal tract (5). Foxl1 mutant mice exhibit delayed formation of gastric glands and intestinal villi during fetal development. Later in life, these mice show thickening of the gastric mucosa with branching of the gastric glands and formation of mucin-filled cysts. In addition, the villi in the small intestine are lengthened and the crypt compartment is dramatically expanded. The lengthening of the villi in the intestine and the thickening of the gastric mucosa in these mice could, in principle, be caused by an increased rate of epithelial cell proliferation and/or a decreased rate of cell migration. We addressed this question by in vivo pulse labeling of cells in S-phase with bromodeoxyuridine (BrdUrd). To assess the rate of epithelial cell migration, mice were sacrificed 48 h post-injection. By this time, labeled cells have migrated up the crypt-villus axis as ordered cohort of cells, and the distance from the crypt to the leading edge of the gastric mucosa in these mice could, in principle, be caused by an increased rate of epithelial cell proliferation and/or a decreased rate of cell migration. We addressed this question by in vivo pulse labeling of cells in S-phase with bromodeoxyuridine (BrdUrd). To assess the rate of epithelial cell migration, mice were sacrificed 48 h post-injection. By this time, labeled cells have migrated up the crypt-villus axis as ordered cohort of cells, and the distance from the crypt to the leading edge of the
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The Wnt/β-Catenin Pathway Is Activated in the Foxl1 Null Mice—Having established that increased epithelial proliferation, not altered cell migration, is the cause for the gastrointestinal malformations in Foxl1 mutant mice, we investigated the regulatory pathways that might be impacted by Foxl1. We hypothesized that Foxl1 normally limits the signaling of a pathway that, when active, leads to epithelial cell proliferation. We considered the Wnt/β-catenin signaling pathway as a possible target of Foxl1 as it has been shown to play a central role in gastrointestinal proliferation and cancer (7, 19–22). Activation of the Wnt/β-catenin pathway leads to accumulation of β-catenin in the nucleus where it participates in transcription of pro-proliferative genes such as c-myc and cyclin D1 through its interaction with the TCF/LEF factors (23–25). To test our hypothesis that the Wnt/β-catenin pathway is chronically activated in Foxl1 mutant mice we used indirect immunofluorescence to determine nuclear localization of β-catenin as an indicator of the activated state of the Wnt pathway. Overall β-catenin levels observed at low magnification, which mainly reflect β-catenin contained in the adherens junctions, were comparable in the stomach of wild type and Foxl1 mutant mice (Fig. 2, A and C). However, at higher magnification we found a dramatic change in the subcellular localization of β-catenin (Fig. 2, B and D). Foxl1 mutant mice had a larger number of nuclei with β-catenin staining, indicating that the Wnt/β-catenin pathway had indeed been activated in these cells. A similar increase in nuclear β-catenin staining was also seen in the jejunum of Foxl1 mutant mice, confirming the correlation between increased proliferation and increased nuclear localization of β-catenin (data not shown). To quantify the observed increase in nuclear β-catenin, we performed Western blot analysis on cytoplasmic and nuclear extracts from Foxl1 mutant mice and their littermate controls (Fig. 3A). We found a significant increase in nuclear β-catenin in the stomach and jejunum (2- and 2.3-fold, respectively) of Foxl1 mutant mice (Fig. 3B) confirming the immunofluorescence results, while cytoplasmic β-catenin was unchanged. Thus, the Wnt/β-catenin pathway is activated in Foxl1 mutant mice, explaining, at least in part, the increased rate of epithelial proliferation observed in the gastrointestinal tract of these mice. We have therefore established the first link between a mesenchymal factor and the Wnt/β-catenin pathway in the gastrointestinal tract.

Increased Proteoglycan Level in Foxl1 Mutant Mice—Regulation of the Wnt/β-catenin pathway in epithelial cells of the gut by the mesenchymal transcription factor Foxl1 necessitates that Foxl1 acts by an indirect mechanism. We have shown previously that, Foxl1 does not control the levels of the Wnt ligand itself, as mRNA levels of Wnt2 and Wnt5a are not altered in Foxl1 mutant mice (5). However, the Wnt/β-catenin pathway can be modified by levels of extracellular proteoglycans, which act as co-receptors to direct Wnt molecules to their high-affinity receptors, the frizzled proteins (10–12). We hypothesized that Foxl1 could affect the levels of proteoglycans and thus impact on the interaction of the Wnt proteins with the frizzled receptor on the epithelial cell surface, thereby modulating the Wnt/β-catenin pathway indirectly. We employed indirect immunofluorescence to immunolocalize and determine the levels of two proteoglycans, Syndecan-1 and Perlecan, which are expressed abundantly in the gut (12, 26, 27). As shown in Fig. 4, Syndecan-1 protein levels were dramatically increased in both stomach (Fig. 4B) and jejunum (Fig. 4D) of Foxl1 null mice compared with their control littermates (Fig. 4, A and C). Similar observations were made for Perlecan (data not shown). To confirm these findings with quantitative assays, we performed Western blot analysis for Syndecan-1 on total protein extracts of Foxl1 mutant mice and littermate controls.

marked cells provides an accurate measure of the distance traveled by the cells labeled at the time of injection. No difference in the rate of cell migration was found between Foxl1 mutant mice and their control littermates (data not shown). To determine the rate of epithelial proliferation, mice were sacrificed shortly (1.5 h) post-injection when only actively cycling cells (S-phase) would be labeled. As shown in Fig. 1, the number of proliferating cells in the epithelium is dramatically increased in the stomach (Fig. 1B) and jejunum (Fig. 1D) of Foxl1 mutant mice. Furthermore, the proliferating cells were found to be scattered throughout the glands in the mutant stomach (Fig. 1B) in contrast to the wild type littermates (Fig. 1A), where the dividing cells were found to be restricted to the neck region of the glands. In the jejunum of wild type mice, the proliferating cells were localized to the base of the crypt (Fig. 1C). In contrast, proliferating cells in the mutant were also detected in the upper part of the crypt near the crypt/villus junction where cells normally acquire their final differentiated phenotype (Fig. 1D). These results demonstrate that epithelial cell proliferation but not migration is controlled by the transcription factor Foxl1.

FIG. 3. Quantitative analysis of nuclear and cytoplasmic β-catenin levels in stomach and jejunum of Foxl1 mice. Cytoplasmic and nuclear extracts (15 μg) from stomach and jejunum of 75-day-old wild type control and Foxl1 mutant animals were analyzed for β-catenin levels by immunoblotting (A). An increase of nuclear β-catenin was observed in both stomach and jejunum of the null animals when compared with the controls. Cytoplasmic β-catenin was not significantly different between control and mutant animals. α-Tubulin served as a loading control for the cytoplasmic extracts and the nuclear transcription factor YY1 served as the loading control for nuclear extracts. B, the levels of nuclear β-catenin in wild type and mutant mice were evaluated by comparing six mutant mice and six wild-type littermate controls. Western blots were performed and the results were scanned and quantified on ImageQuant software (IQMac v1.2). These results demonstrate statistically significant increases in nuclear β-catenin in the Foxl1−/− mice of 2-fold in the stomach and a 2.3-fold increase in the jejunum (*, p < 0.01 and **, p < 0.002).
We found that Syndecan-1 was strongly increased in Foxl1 mutant mice supporting the observations made with the immunofluorescence. These results demonstrate that Foxl1 normally acts to limit the expression of Perlecan and Syndecan-1 in the gastrointestinal tract. To determine if the proteoglycans are direct transcriptional targets of Foxl1, we analyzed their steady state mRNA levels by RNase protection assay. As shown in Fig. 5B, there was no significant difference in the mRNA levels of Syndecan-1 between the mutant and the wild type mice in both stomach and jejunum. The same result was observed for Perlecan (data not shown). Thus, we conclude that Foxl1 regulates proteoglycans expression at the post-transcriptional level.

The Foxl1 mutant mice represent the first mammalian genetic model confirming embryological data on the importance of mesenchymal to epithelial signaling in the gastrointestinal tract. Subsequently, the mesenchymal transcription factor Nkx-2.3 was also shown to affect epithelial differentiation in the gut (28). However, until now the molecular mechanism of Foxl1 action has remained elusive. In this study, we have established epithelial /H9252-catenin as an indirect target for Foxl1. This activation of the Wnt/H9252-catenin pathway in the Foxl1 null mice occurs without an increase in Wnt expression. Rather, the efficacy of Wnt signaling is increased by a dramatic up-regulation of extracellular heparan sulfate proteoglycans which act as co-receptors for Wnt, funneling Wnt molecules to the signaling receptors, the frizzled proteins. Increased signaling through the frizzled receptors on the basolateral surface of the epithelial cells results in activation of the cytoplasmic protein disheveled, followed by inhibition /H9252-catenin phosphorylation via glycogen synthetase-3β and accumulation of both cytoplasmic and nuclear /H9252-catenin. It is the nuclear /H9252-catenin that in a complex with the TCF/LEF DNA-binding proteins activates pro-proliferative target genes like cyclin D1 and c-myc, which ultimately
results in the increased proliferation of gut epithelial cells observed in Foxl1 null mice.

Previous genetics studies have demonstrated that HSPGs can play a crucial role in morphogenesis, growth regulation, and tumor suppression (13). It was first demonstrated in Drosophila that dysregulation of the proteoglycan Dally affects wingless signaling and the morphogenesis of numerous tissues (10). In mammals, it was shown recently that a mutation in the HSPG Glypican-3 is responsible for a tumor-susceptibility syndrome (29), while Syndecan-1 is necessary for Wnt-1-dependent mammary tumorigenesis in mice (12). In this study, we have shown for the first time evidence which implicates HSPGs in the control of proliferation of the gastrointestinal tract. It follows that HSPGs may also play a role in colorectal cancer in humans. Thus, it may be of clinical importance to investigate if patients with colorectal cancer show any alterations in the levels of Foxl1 or HSPGs. Future studies in our laboratory will be directed at establishing whether there is a genetic interaction between Foxl1 and the Wnt/APC/β-catenin pathway in gastrointestinal tumorigenesis. The results presented in this paper suggest that mutations in APC and Foxl1 should act synergistically, i.e. that the numbers of tumors will be increased or the onset of tumor formation will be accelerated in mice carrying mutations for both genes. Conversely, our findings predict that epithelial cell proliferation would be normalized in double mutants for both Foxl1 and Syndecan-1. Experiments aimed at verifying these predictions are currently in progress.

In this study, we demonstrate that the activation of the Wnt pathway in Foxl1 null mice is due to an increase in proteoglycan protein levels in the gut since Wnt expression itself is unchanged (5). The increased Syndecan-1 and Perlecans expression in the Foxl1 mutant mice creates a microenvironment favorable to activation of the Wnt/APC/β-catenin pathway, which leads to an increase in epithelial proliferation. Thus we have established a novel link between mesenchymal and extracellular events and the control of the proliferation of epithelial cells in the gastrointestinal tract. At present, the molecular events that result in increased HSPG expression in Foxl1 null mice remain unknown, as the mRNA levels for both Syndecan-1 and Perlecans are unchanged in the absence of the Foxl1 transcription factor. We are currently investigating the possibility that the expression of matrix metalloproteinases or their inhibitors, the TIMP proteins (tissue inhibitors of metalloproteinases) is controlled by Foxl1.

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REFERENCES

1. Beaulieu, J. F. (1997) Prog. Histochem. Cytochem. 31, 1–78
2. Gordon, J. I., and Hermiston, M. L. (1994) Curr. Opin. Genet. Dev. 6, 795–803
3. Pignatelli, A., Karayiannakis, A. J., Noda, M., Efthathiou, J., and Kmiot, W. (1997) in The Gut as a Model in Cell and Molecular Biology (Halter, F., Winton, D., and Wright, N. A., eds) Kluwer, Norwell, MA
4. Podolsky, D. K. (1993) Am. J. Physiol. 264, G179–186
5. Kaestner, K. H., Silberg, D. G., Traber, P. G., and Schutz, G. (1997) Genes Dev. 11, 1585–1595
6. Smith, K., Bui, T. D., Poulsom, R., Kaklamanis, L., Williams, G., and Harris, A. L. (1999) Br. J. Cancer 81, 496–502
7. Morin, P. J. (1999) Bioessays 21, 1021–1030
8. Seidensticker, M. J., and Behrens, J. (2000) Biochim. Biophys. Acts 1495, 168–182
9. Cumberledge, S., and Reichman, F. (1997) Trends Genet. 13, 421–423
10. Tsuda, M., Kamimura, K., Nakato, H., Archer, M., Staatz, W., Fox, B., Humphrey, M., Olsen, S., Putch, T., Kaluza, V., Siegfried, E., Stal, L., and Selleck, S. B. (1999) Nature 400, 276–280
11. Perrimon, N., and Berensfield, M. (2000) Nature 404, 725–728
12. Alexander, C. M., Reichman, F., Hinko, M. T., Lincecum, J., Becker, K. A., Cumberledge, S., and Bernfield, M. (2000) Nat. Genet. 25, 329–332
13. Selleck, S. B. (2000) Trends Genet. 16, 206–212
14. Couchman, J. R., and Woods, A. (1996) J. Cell. Biochem. 61, 578–584
15. Lin, X., and Perrimon, N. (1999) Nature 400, 281–284
16. Duncan, S. A., Nagy, A., and Chan, W. (1997) Development 124, 279–287
17. Beaulieu, J. F., Vachon, P. H., and Chartrand, S. (1991) Anat. Embryol. 183, 363–369
18. Stein, B., Rahmsdorf, H. J., Steffen, A., Liflin, M., and Herrlich, P. (1989) Mol. Cell. Biol. 9, 5169–5181
19. Miller, J. R., and Moon, R. T. (1996) Genes Dev. 10, 2527–2539
20. Cadigan, K. M., and Nusse, R. (1997) Genes Dev. 11, 3286–3305
21. Dale, T. C. (1998) Biochem. J. 329, 209–223
22. Gumbiner, B. M. (1998) Curr. Opin. Genet. Dev. 8, 430–435
23. Clevers, H., and van de Wetering, M. (1997) Trends Genet. 13, 485–489
24. Tetsu, O., and McCormick, F. (1999) Nature 398, 422–426
25. He, T. C., Sparks, A. B., Ragu, C., Hermeking, H., Zawel, L., da Costa, L. T., Morin, P. J., Vogelstein, B., and Kinzler, K. W. (1998) Science 281, 1509–1512
26. David, G. (1998) FASEB J. 7, 1023–1030
27. Rapraeger, A. C., Krufta, A., and Olwin, B. B. (1991) Science 252, 1705–1708
28. Fahl, O., Zweigerdt, R., and Arnold, H. H. (1999) Development 126, 2215–2225
29. Cano-Gauci, D. F., Song, H. H., Yang, H., McElriny, C., Choo, B., Shi, W., Pullano, R., Piscione, T. D., Grisaru, S., Soon, S., Sediakova, L., Tanwell, A. K., Mak, T. W., Yeger, H., Lockwood, G. A., Rosenberg, N. D., and Flihus, J. (1999) J. Cell. Biol. 146, 255–264