The Rac Activator Tiam1 Is a Wnt-responsive Gene That Modifies Intestinal Tumor Development*

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Mutations in the canonical Wnt signaling pathway leading to its activation are known to cause the majority of intestinal tumors. However, few genes targeted by this pathway have been demonstrated to affect tumor development in vivo. Here we show that Tiam1, a selective Rac GTPase activator, is a Wnt-responsive gene expressed in the base of intestinal crypts and up-regulated in mouse intestinal tumors and human colon adenomas. Moreover, by comparing tumor development in APC mutant Min (multiple intestinal neoplasia) mice expressing or lacking Tiam1, we found that Tiam1 deficiency significantly reduces the formation and growth of polyps in vivo. However, invasion of malignant intestinal tumors is enhanced by a lack of Tiam1. In line with this, knock-down of Tiam1 reduced the growth potential of human colorectal cancer cells and their ability to form E-cadherin-based adhesions, a prerequisite for local invasion of tumor cells. Our data indicate a novel cross-talk between Tiam1-Rac and canonical Wnt-signaling pathways that influences intestinal tumor formation and progression.

The lumen of the adult mammalian intestine is lined with an absorptive and secretory epithelium that undergoes continuous self-renewal. The epithelium is organized into two functional domains: one comprises proliferating progenitor cells located physically within crypts, and the other, comprises terminally differentiated specialized cells predominantly occupying the villi in the small intestine and the mucosal surface in the colon. In the adult intestinal mucosa, the canonical Wnt signaling pathway (reviewed in Ref. 1) maintains the undifferentiated, proliferative crypt phenotype (2), controls cell positioning along the crypt-villus axis (3), and regulates aspects of Paneth cell maturation (4). The canonical Wnt signaling pathway is initiated by extracellular Wnt glycoproteins binding transmembrane receptors. Ligand binding induces a cascade of protein-protein interactions, the ultimate consequence of which is stabilization and accumulation of cytosolic β-catenin. This protein, known also for its role in cadherin-mediated intercellular adhesion, relays the signal to the nucleus, where, through direct binding to TCF family transcription factors, it induces target gene expression; TCFs provide DNA-binding sequence specificity, and β-catenin provides essential transactivation domains (1).

Mice deficient for TCF4 die near birth and lack proliferative cells specifically in intestinal crypt precursor regions (5). Forced expression in the mucosa of dickkopf1, a secreted inhibitor of canonical Wnt signaling, blocks crypt formation and self-renewal in adult mice and impairs cell positioning (6, 7). In contrast, aberrant activation of the Wnt pathway results in intestinal tumor formation (reviewed in Ref. 8). Germline mutations in humans abolishing the function of APC, a key inhibitor of the canonical Wnt pathway, are responsible for an inherited tumor syndrome referred to as familial adenomatous polyposis (9). Carriers develop multiple benign colorectal adenomatous polyps, a fraction of which generally progress to adenocarcinomas. Similar mutations are found in the majority of sporadic colorectal tumors (8). In sporadic tumors with wild type APC, β-catenin is frequently found to harbor activating gain-of-function mutations (10). A nonsense mutation in APC was also revealed to be responsible for the multiple intestinal neoplasia found in the mouse model of the same name (11). Tiam1 is a guanine nucleotide exchange factor that selectively activates the Rho-like GTPase Rac (12). In turn, Rac regulates actin polymerization, cell adhesion and motility, and also cell survival and cell cycle progression (reviewed in Refs. 13 and 14). We have previously shown that Tiam1 expression is transcriptionally regulated during epithelial to mesenchymal transitions (15, 16). In addition, Tiam1 activity is regulated by post-translational modifications and protein-protein interactions (reviewed in Ref. 17). Potentially oncoproteins could use these mechanisms to regulate Tiam1/Rac signaling. Tiam1 is a potent modifier of oncogenic Ras-induced skin tumor initiation, promotion, and progression (18). Given the clinical significance of human colorectal tumors and the need to identify genes that influence intestinal tumor development, we decided to examine the role of Tiam1 in intestinal tumorigenesis. Our findings indicate a novel cross-talk between Tiam1/Rac signaling and the canonical Wnt-signaling pathway, with Tiam1 itself appearing to be a significant component of the Wnt-regulated genetic program implicated in intestinal tumorigenesis.

MATERIALS AND METHODS

Antibodies, Immunohistochemistry, and Immunofluorescence—Immunohistochemistry was performed on paraffin-embedded tissue sections (4 μm) using an anti-DH Tiam1-specific rabbit polyclonal antibody (19), antibodies against lysozyme (DAKO), and Ki-67 (Novocastra) as previously described (2, 3, 18). Periodic acid Schiff staining, to visualize goblet cells, was performed as standard (6). Human colon tumors were collected from subtotal colectomy specimens of familial adenomatous polyposis patients and patients with sporadic colon adenomas. For immunofluorescence, the cells grown on glass...
The images were recorded with a Leica TCS-NT confocal laser scanning microscope.

Cell Culture, Constructs, and Transfection—DLD1 cells carrying a doxycycline-inducible dominant negative TCF4 or P44 isoform of TCF1 (i.e. TCF proteins lacking the β-catenin interaction domain) (2) were cultured in RPMI with 5% fetal bovine serum. The doxycycline-inducible siRNA5 system and sequences of Tiam1 siRNA oligonucleotides used to inducibly down-regulate Tiam1 were previously described (15, 20). Rat intestinal epithelial (RIE) cells (from ATCC) were grown in Dulbecco’s modified Eagle’s medium with 5% fetal bovine serum, supplemented with 0.1 IU/ml insulin. RIE cells were infected with a retroviral expression vector encoding Wnt1 or an oncogenic form of β-catenin (S33Y), and protein was harvested 48 h later.

Protein and RNA Analysis—Tiam1 protein levels were analyzed by Western blotting using anti-Tiam1 polyclonal antibodies either from Santa Cruz Biotechnology, Inc. or the anti-DH Tiam1-specific rabbit polyclonal antibody (19). Northern blot analysis was performed as previously described (19). Protein levels of cyclin D1 were analyzed using an anti-cyclin D1 monoclonal antibody (Upstate Biotechnology, Inc.), Myc using the 9E10 anti-Myc monoclonal antibody, TCF1 using the anti-TCF1 7H3 monoclonal antibody (Upstate Biotechnology, Inc.), TCF4 using the anti-TCF4 6H5–3 monoclonal antibody (Upstate Biotechnology, Inc.), and Rac using an anti-Rac1 monoclonal antibody (BD Biosciences).

Growth Curves—DLD1 cells were plated in triplicate in 12-well plates at a density of 4 × 10^4 cells/well. The cells were grown in the presence or absence of doxycycline, which was refreshed every 48 h. Cell proliferation was determined from total protein amount in a dye binding assay. Briefly, the cells were fixed daily in 10% trichloroacetic acid and later stained with 1% bromphenol blue. Bromphenol blue was eluted with 10 mM Tris, and the absorbance was measured in a spectrophotometer at a wavelength of 590 nm. The readings were normalized for the final concentration of bromphenol blue in control cells.

Mice—Tiam1Δ/Δ mice generated as described in Ref. 18 on a C57BL/6 background as well as C57BL/6 Tiam1Δ/Δ mice were crossed with Balb/c Min/+ mice. Animals with a mixed C57BL/6/Balb/c background were used throughout the experiments. The mice were genotyped, and those carrying the Min allele were analyzed for intestinal tumor development (Tiam1Δ/Δ/Min/+, n = 37; Tiam1Δ/Δ/Min/+, n = 79; and Tiam1Δ/Δ/Min/+, n = 27). All of the mice analyzed were sacrificed at week 14 after birth. The entire small intestine as well as the colon were coiled into a Swiss roll-like configuration, fixed in formalin, and paraffin-embedded. The sections through the widest part of the roll were stained with hematoxylin/eosin, and a pathologist scored the number and grades of tumors blindly. For determining the size of a gland, a graticule was used to measure the largest dimension of all lesions from eight randomly selected mice of each genotype (Tiam1Δ/Δ/Min/+, Tiam1Δ/Δ/Min/+, Tiam1Δ/Δ/Min/+), of the same gender and age at sacrifice. In total we measured the size of 315 lesions from Tiam1Δ/Δ/Min/+ mice, 297 lesions from Tiam1Δ/Δ/Min/+ mice, and 134 lesions from Tiam1Δ/Δ/Min/+ mice.

RESULTS

Immunohistochemical staining of the intestine of wild type mice revealed Tiam1 expression in cells at the base of small intestine crypts (Fig. 1A). The cells occupying these positions include Paneth cells and neighboring progenitor cells. The Paneth cells are terminally differentiated secretory cells that control the intestinal bacterial flora. Interestingly, both Paneth and progenitor cells possess high levels of nuclear β-catenin (3), a hallmark of active canonical Wnt signaling, suggesting that Tiam1 might be a Wnt-responsive gene. As expected, no Tiam1 staining was observed in intestines from Tiam1Δ/Δ mice (Fig. 1A). We did not find abnormalities in the intestines of adult Tiam1Δ/Δ mice when compared with wild type mice. Analysis of markers of differentiation (such as lysozyme staining of Paneth cell granules, periodic acid Schiff staining of mucin production in goblet cells, and alkaline phosphatase staining of enterocytes) and proliferation (Ki-67 expression) revealed no differences between wild type and Tiam1Δ/Δ mice (Fig. 1, B–D, and data not shown). This indicates that Tiam1 deficiency does not visibly affect intestinal development or subsequent homeostasis.

In contrast to the restricted expression of Tiam1 at the base of small intestine crypts of mice, the protein was expressed uniformly in adenomatous polyps arisen in Min/+ mice (Fig. 2A). These mice carry a nonsense mutation in the APC gene and produce intestinal tumors as a result of increased Wnt signaling (11). In addition, immunohistochemical staining of human colon adenomas from both familial adenomatous...
polypsis patients (n = 38) and sporadic cases (n = 13) revealed strong and uniform Tiam1 protein expression in all cases examined. The protein levels exceeded the levels in adjacent normal mucosa as found in mice (for example see Fig. 2B). Additionally, in normal human colonic mucosa, we observed a gradient of Tiam1 protein decreasing from cells at the base of the crypt to the luminal surface (blue arrowheads) and decreases toward the luminal surface (blue arrowheads). Note strongly stained plasma cells in the stroma. (The boxed regions in A–C are magnified and shown in adjacent right panels).

FIGURE 2. Tiam1 expression in mouse neoplastic intestinal mucosa and in human normal and neoplastic colonic mucosa. A, Tiam1 is uniformly expressed in adenoma cells of Tiam1+/Min+/ mice. Note the absence of Tiam1 expression in normal tissue (arrowheads in right panel). B, sections from human colon adenomas were immunohistochemically stained for Tiam1. In all of the cases studied and as shown here for a representative familial adenomatous polyposis polyp, Tiam1 expression was decreased in dysplastic crypts compared with adjacent normal colonic mucosa. The dashed line depicts the boundary between normal (N) and tumor (T) tissues. C, in normal human colonic mucosa, the level of Tiam1 protein is highest within cells at the base of the crypt (black arrowheads) and decreases toward the luminal surface (blue arrowheads). Note strongly stained plasma cells in the stroma. (The boxed regions in A–C are magnified and shown in adjacent right panels).

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The specific expression of Tiam1 at sites of endogenous canonical Wnt signaling and its apparent up-regulation in neoplastic lesions resulting from excessive Wnt signaling prompted us to evaluate further whether Tiam1 is a Wnt-responsive gene. For this we used a human CRC cell line, DLD1, engineered to express inducible forms of TCF transcription factors upon the addition of doxycycline (2). Abrogation of Wnt signaling by the induced expression of the inhibitory forms of TCF resulted in a complete loss of Tiam1 protein mRNA by 48 h (Fig. 3A). Protein expression was also significantly reduced in this interval (Fig. 3B). Down-regulation of Tiam1 was temporally correlated with the appearance of the inhibitory forms of TCF and occurred with similar kinetics to c-Myc and cyclin D1 (Fig. 3B), both considered direct TCF targets (21, 22). Together these findings indicate that Tiam1 is a Wnt-responsive gene and potentially a direct TCF target. To further substantiate these findings, we overexpressed Wnt1 and an oncogenic form of β-catenin (lacking the Ser33 regulatory phosphorylation site) in RIE cells in which the Wnt pathway is not activated. Exogenous expression of both Wnt1 and β-catenin in RIE cells resulted in a significant up-regulation of Tiam1 protein levels (Fig. 3C), consistent with the canonical Wnt pathway controlling levels of expression of Tiam1.

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To further study the consequences of changes in the levels of Tiam1 in colon tumor cells, we engineered the human DLD1 cells to express siRNA designed to knock down Tiam1 upon the addition of doxycycline. In earlier studies, we have successfully used the Tiam1-specific siRNA sequence to down-regulate Tiam1 protein levels in other cell types (15). We generated several independent DLD1 cell clones in which doxycycline addition induced a selective reduction in Tiam1 expression. The induced expression of siRNA of Tiam1 significantly reduced Tiam1 levels in the presence of doxycycline for either 24 or 48 h. RNA was isolated, and Northern blot analysis was performed for Tiam1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels. B, TR7, DN15, and P4 cells were incubated with (+) or without (-) doxycycline for the indicated times before protein lysates were isolated and immunoblotting performed for Tiam1, c-Myc, cyclin D1, TCF, and Rac. C, RIE cells were infected with either empty vector (EV), a retroviral vector encoding for Wnt1 or a retroviral vector encoding for a dominant active form of β-catenin. Protein was extracted 48 h later, and immunoblotting for Tiam1 and β-actin (loading control) was performed. wt, wild type.
FIGURE 4. Tiam1 modifies intestinal tumorigenesis. A, Tiam1 protein levels in a representative clone of DLD1 cells inducibly expressing Tiam1 siRNA (clone C2) as compared with control TR7 cells. Protein was harvested 48 h after plating in the presence (+) or absence (−) of doxycycline (dox) and immunoblotted for Tiam1 or β-actin (loading control). B, C2 cells induced with doxycycline grow slower than untreated C2 cells or control TR7 cells in the presence or absence of doxycycline. C, phase contrast images of control TR7 cells or C2 cells 48 h after plating in the presence (+) or absence (−) of doxycycline. Control cells grow in tightly packed colonies even in the presence of doxycycline. C2 cells display reduced colony formation specifically in the presence of doxycycline. D, confocal images of colonies of either control TR7 cells or C2 cells fixed and stained for β-catenin 48 h after plating in the presence (+) or absence (−) of doxycycline. Tiam1 siRNA induction leads to reduced β-catenin at sites of cell-cell contact.

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Tiam1 influences human CRC cell growth and cell-cell adhesion. A, Tiam1 protein levels in a representative clone of DLD1 cells inducibly expressing Tiam1 siRNA (clone C2) as compared with control TR7 cells. Protein was harvested 48 h after plating in the presence (+) or absence (−) of doxycycline (dox) and immunoblotted for Tiam1 or β-actin (loading control). B, C2 cells induced with doxycycline grow slower than untreated C2 cells or control TR7 cells in the presence or absence of doxycycline. C, phase contrast images of control TR7 cells or C2 cells 48 h after plating in the presence (+) or absence (−) of doxycycline. Control cells grow in tightly packed colonies even in the presence of doxycycline. C2 cells display reduced colony formation specifically in the presence of doxycycline. D, confocal images of colonies of either control TR7 cells or C2 cells fixed and stained for β-catenin 48 h after plating in the presence (+) or absence (−) of doxycycline. Tiam1 siRNA induction leads to reduced β-catenin at sites of cell-cell contact.

nies containing scattered, fibroblast-like cells (Fig. 4C) and release of β-catenin from the plasma membrane (Fig. 4D). These findings are consistent with the earlier reported requirement of Tiam1 in the formation and maintenance of cadherin-based cell adhesions (15, 23). From these in vitro studies we conclude that Tiam1 is a Wnt-responsive gene that influences the growth rate of colorectal cancer cells as well as the strength of cadherin-based adhesions between these cells. Reduced cell-cell adhesion is frequently associated with the progression of epithelial tumors.

To address whether Tiam1 expression contributes to the formation and progression of Wnt-induced intestinal tumors in vivo, we examined tumor formation in Min/+ mice in different Tiam1 backgrounds. In comparison with Tiam1+/+//Min/+ mice, Tiam1−/−//Min/+ mice demonstrated a 50% reduction (p < 0.005, t test) in intestinal tumor numbers (Fig. 5A). A similar proportional reduction in numbers was observed across the spectrum of tumor types including hyperplasias, adenomas, and adenocarcinomas (see Fig. 5B). Further analysis of the various tumors revealed that their growth was also significantly reduced in heterozygous Tiam1−/−//Min/+ mice and was reduced further in Tiam1−/−//Min/+ mice (Fig. 5C). The intermediate phenotype of the heterozygous mice indicates that the observed differences depend on the level of Tiam1 expression. From these studies we conclude that Tiam1 is a Wnt-responsive gene that is implicated in the formation and growth of Wnt-induced intestinal tumors.

We also attempted to determine the rate of malignant progression of intestinal tumors in Tiam1+/+ or Tiam1−/− backgrounds. However, malignant progression is infrequently observed in Min/+ mice, because they are typically sacrificed at a young age because of morbidity arising from bowel obstruction and anemia. Malignant progression in the small intestine is defined as tumor invasion into the lamina propria or beyond. Upon sacrificing mice at 14 weeks, we observed a similar fraction of adenocarcinomas (~8% of all lesions) independently of Tiam1 status, suggesting that Tiam1 deficiency does not affect the progression from adenomas to adenocarcinomas. However, when examining the extent of tumor invasion, we found a significantly larger fraction (p < 0.05; chi-squared test) of malignant tumors to have invaded into the submucosa and muscularis propria in Tiam1−/−//Min/+ mice compared with Tiam1+/+//Min/+ mice (Fig. 6A). Representative examples of invasion of malignant tumors into the submucosa and muscularis propria are shown in Fig. 6 (B and C, respectively). These data suggest that adenocarcinomas arisen in Tiam1-deficient mice are more aggressive than those arisen in Tiam1 wild type.

Female Min/+ mice also develop mammary tumors at low incidence that are likewise attributed to aberrant Wnt signaling. Interestingly, in addition to the effect of Tiam1 depletion on intestinal tumorigenicity, we also observed a dramatic reduction in the incidence of mammary tumors in Tiam1−/−//Min/+ mice that was even more apparent in Tiam1+/+//Min/+ mice (Fig. 7A). Mammary tumors in Tiam1+/+// Min/+ mice were invariably squamous cell carcinomas and, when immunohistochemically stained for Tiam1, displayed Tiam1 up-regulation compared with normal mammary tissue (Fig. 7B). These findings further support our conclusions that Tiam1 is a Wnt-responsive gene that is implicated in the formation of Wnt-induced tumors including intestinal and mammary tumors.

DISCUSSION

Because of its central role in intestinal tumorigenesis, several groups have concentrated on the identification of genes that are regulated by the canonical Wnt signaling pathway and especially those up-regulated in CRC cells (2, 24–27). Certain of these targets have been demonstrated to regulate intestinal homeostasis, including notably c-Myb and EphB family members. However, few Wnt target genes have as yet been demonstrated to influence intestinal tumorigenesis in vivo (28–31). Our data presented here indicate that Tiam1 is a Wnt target gene.
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Absence of Tiam1 impairs intestinal tumor formation in Min/+ mice. A, average number of total intestinal lesions per mouse found in Tiam1+/+/Min/+; Tiam1+/−/Min/+; and Tiam1−/−/Min/+ mice. The error bars show standard error of the mean. *, 50% reduction in lesions in Tiam1−/−/Min/+ mice compared with Tiam1+/+/Min/+ mice is statistically highly significant (p < 0.005; Student’s t test). B, average number of intestinal hyperplasias, adenomas, and adenocarcinomas per mouse is depicted for each genotype. The error bars show standard error of the mean. *, reduction in hyperplasias and adenomas in Tiam1−/−/Min/+ mice compared with Tiam1+/−/Min/+ mice is statistically highly significant (p < 0.005; Student’s t test). C, percentage of tumors with a largest dimension bigger than 1 mm. *, reduction in tumor size in both Tiam1−/−/Min/+ and Tiam1−/−/Min/+ mice is statistically highly significant (p < 0.005; analysis of variance).

Knock-down of Tiam1 in DLD1 cells by siRNA reduced their ability to form cadherin-mediated cell-cell adhesions, consistent with earlier in vitro experiments using a human CRC-derived cell line in which Tiam1 was down-regulated confirmed the requirement for Tiam1 for optimal growth. Moreover, we observed increased Tiam1 expression in human colorectal tumors, suggesting that this gene is implicated in both mouse and human intestinal tumorigenesis.

We previously observed that susceptibility to develop Ras-induced skin tumors, following application of a two-step chemical carcinogenesis protocol, was significantly reduced in Tiam1−/− mice (18). Tiam1 physically associates with the activated Ras-GTPase, and this association stimulates Rac activity (32). The Tiam1/Rac signaling module thus appears to be selectively recruited by at least two independent oncogenic signaling pathways of major clinical significance, albeit by distinct mechanisms. This suggests that Tiam1-mediated Rac activation is a significant modifier of tumor development and therefore a potentially interesting therapeutic target. The requirement for Tiam1 for optimal tumor cell growth is a consistent finding in both skin and intestinal tumor models and was also recently demonstrated for Madin-Darby canine kidney cells, an immortalized kidney epithelial cell line (15). Our data are also consistent with a previously published study showing that targeted expression of a dominant active mutant of Rac1 to the mouse intestine resulted in enlarged crypts and increased proliferation (15, 33).

We observed the increased susceptibility to apoptosis of initiated intestinal epithelial cells, although this is very difficult to address in this model. However, analysis of apoptotic cells by TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling) did not reveal increased apoptosis in polyps arising in Tiam1−/−/Min/+ mice (data not shown).
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FIGURE 7. Absence of Tiam1 impairs mammary tumor formation in Min/+ mice. A, percentage of female mice with one or more mammary tumors. The reduction in incidence in both Tiam1+/−/Min/+ (n = 34) and Tiam1−/−/Min/+ (n = 12) mice is statistically significant when compared with Tiam1+/+/Min/+ (n = 23) mice (p < 0.05; chi-squared test). B, Tiam1 immunohistochemical staining in a representative mammary tumor found in a Tiam1+/−/Min/+ mouse. The arrowhead points to a normal mammary gland that is negative for Tiam1.

vitro studies showing that Tiam1 is critical for the formation and maintenance of cell-cell adhesions and for suppressing epithelial cell motility and invasion (15, 23). In vivo, and particularly with Ras-induced skin tumors, it appears that the loss of Tiam1 expression facilitates malignant progression, presumably by its effect on cadherin-based cell adhesions. The observed increased invasive growth in the intestinal tumors could be due to the same phenomena, because we found reduced cadherin-based adhesions in DLD1 cells in which Tiam1 was down-regulated by siRNA. In a recent study overexpression of Tiam1 was found to increase the in vitro migration of SW480 colorectal carcinoma cells as well as their ability to metastasize in an orthotopic nude mouse model (34). The apparent discrepancy between our present data and the above study could potentially be attributable to the specific nature of the selected SW480 cells, in which Tiam1 overexpression does not promote cadherin-based cell-cell adhesions. Earlier studies have indicated that the effect of Tiam1 on invasion is cell type-specific and dependent on its capacity to influence E-cadherin-based adhesions (35). Intriguingly, another Rac-specific exchange factor, Asef, expressed in CRC-derived cells, is able to antagonize cadherin-mediated adhesion and to promote cell motility in vitro (36). Asef is hyperactivated by truncated, mutant APC, found in the majority of CRC cells. Knock-down of Asef impaired CRC cell motility. Thus despite both molecules activating Rac, Asef and Tiam1 promote different biological outcomes, consistent with the concept that guanine nucleotide exchange factors not only activate Rho-like molecules but also direct that activity to particular downstream signaling and biological ends (37). Taken together, our data indicate cross-talk between the canonical Wnt and Tiam1/Rac signaling pathways that influences the initiation, growth, and progression of intestinal tumors.

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REFERENCES
1. Nelson, W. J., and Nusse, R. (2004) Science 303, 1483–1487
2. van de Wetering, M., Sancho, E., Verweij, C., de Lau, W., Oving, I., Hurlstone, A., van der Horn, K., Batlle, E., Coudreuse, D., Haramis, A. P., Tjon-Pon-Fong, M., Moerer, P., van den Born, M., Soete, G., Pals, S., Eilers, M., Medema, R., and Clevers, H. (2002) Cell 111, 241–250
3. Batlle, E., Henderson, J. T., Begthel, H., van den Born, M., Sancho, E., Huls, G., Meedijdik, J., Robertson, J., van de Wetering, M., Pawson, T., and Clevers, H. (2002) Cell 111, 251–263
4. van Es, J. H., Jay, P., Gregorieff, A., van Gijn, M. E., Jonkheer, S., Hatzis, P., Thiele, A., van den Born, M., Begthel, H., Brabletz, T., Taketo, M. M., and Clevers, H. (2005) Nat. Cell Biol. 7, 381–386
5. Korinek, V., Barker, N., Moerer, P., van Donselaar, E., Huls, G., Peters, P. J., and Clevers, H. (1997) Nat. Genet. 19, 379–383
6. Pinto, D., Gregorieff, A., Begthel, H., and Clevers, H. (2003) Genes Dev. 17, 1709–1713
7. Kuhner, F., Davis, C. R., Wang, H. T., Chu, P., Lee, M., Yuan, J., Nusse, R., and Kuo, C. J. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 266–271
8. Bierzu, M., and Clevers, H. (2000) Cell 103, 311–320
9. Kinzler, K. W., Nilbert, M. C., Su, L. K., Vogelstein, B., Bryan, T. M., Levy, D. B., Smith, K. J., Preisninger, A. C., Hedge, P., McKechnie, D., Finnear, R., Markham, A., Groffen, J., Boguski, M. S., Atsels, S. F., Hori, A., Ando, H., Miyoshi, Y., Miki, Y., Nishibashi, I., and Nakamura, Y. (1991) Science 253, 661–665
10. Morin, P. J., Sparks, A. B., Korinek, V., Barker, N., Clevers, H., Vogelstein, B., and Kinzler, K. W. (1997) Science 275, 1787–1790
11. Su, L. K., Kinzler, K. W., Vogelstein, B., Preisninger, A. C., Moser, A. R., Luongo, C., Gould, K. A., and Dove, W. F. (1992) Science 256, 668–670
12. Michiels, F., Habets, G. G., Stam, J. C., van der Kammen, R. A., and Collard, J. G. (1995) Nature 375, 338–340
13. Burridge, K., and Weinberg, R. (2004) Cell 116, 167–179
14. Coleman, M. L., Marshall, C. J., and Olson, M. F. (2004) Nat. Rev. Mol. Cell Biol. 5, 355–366
15. Gallinger, S., and Preisinger, A. C. (2001) J. Cell Biol. 151, 609–615
16. He, T. C., Sparks, A. B., Rago, C., Hermeking, H., Zawel, L., da Costa, I. T., Morin, P. J., Vogelstein, B., and Kinzler, K. W. (1998) Science 281, 1509–1512
17. Tsutsui, O., and McCormick, F. (1999) Nature 398, 422–426
18. Nordi, L. P., ten Klooster, J. P., van der Kammen, R. A., Michiels, F., Oomen, L. C., and Collard, J. G. (1997) Science 278, 1464–1466
19. Fujita, M., Fukurawa, Y., Tsumoda, T., Tanaka, T., Ogawa, M., and Nakamura, Y. (2001) Cancer Res. 61, 7722–7726
20. He, T. C., Chan, T. A., Vogelstein, B., and Kinzler, K. W. (1999) Cell 99, 335–345
21. Mann, B., Gelos, M., Siedow, A., Hanks, M. L., Gratchev, A., Ilyas, M., Bodmer, W. F., Moyer, M. P., Riecken, E. O., Buhr, H. J., and Hanks, S. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1603–1608
22. Sansom, O. J., Reed, K. R., Hayes, A. J., Ireland, H., Brinkmann, H., Newton, I. P., Batlle, E., Simon-Assmann, P., Clevers, H., Nathke, I. S., Clarke, A. R., and Winton, D. J. (2004) Genes Dev. 18, 1385–1390
23. Park, B. H., Vogelstein, B., and Kinzler, K. W. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 2598–2603
24. Wilson, C. L., Hepper, K. J., Labowsky, P. A., Hogan, B. L., and Matrisian, L. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1402–1407
25. Zhu, P., Martin, E., Mengawasser, J., Schlag, P., Janssen, K. P., and Gottlicher, M. (2004) Cancer Cell 10, 455–463
26. Batlle, E., Baconi, J., Begthel, H., Jonker, S., Gregorieff, A., van den Born, M., Malats, N., Sancho, E., Boon, E., Dawson, S., and Collard, J. G. (2002) Nat. Cell Biol. 4, 621–625
27. Stappenbeck, T. S., and Gordon, J. I. (2001) Development 128, 2603–2614
28. Minard, M. E., Herynk, M. H., Collard, J. G., and Gallick, G. E. (2005) Oncogene 24, 2568–2573
29. Sander, E. H., van Delft, S., ten Klooster, J. P., Reid, T., van der Kammen, R. A., Michiels, F., and Collard, J. G. (1998) J. Cell Biol. 143, 1385–1398
30. Kawasum, T., Sato, R., and Akiyama, T. (2003) Nat. Cell Biol. 5, 211–215
31. Malliri, A., and Collard, J. G. (2003) Curr. Opin. Cell Biol. 15, 583–589

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