

Regulation of Vascular Endothelial Growth Factor by the Wnt and K-ras Pathways in Colonic Neoplasia

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Abstract

Angiogenesis is not restricted to advanced stages of tumor development but is also observed in benign precursor lesions. Vascular endothelial growth factor (VEGF) is a key regulator of tumor angiogenesis, but the genetic mechanisms controlling its expression in premalignant lesions are poorly described. The Wnt signaling pathway, which is commonly mutated in benign colonic adenomas, was found to strongly up-regulate VEGF. A T-cell factor-4-binding element at –805 bp in the VEGF promoter is an important mediator of this effect. Signaling through the K-ras oncogene, also frequently mutated in benign colonic polyps, up-regulated VEGF in a phosphatidylinositol 3-kinase-dependent manner. Furthermore, K-ras activation appeared to enhance Wnt signaling, which suggests a unique interaction between these two pathways. These studies thus identify VEGF as a novel target of the Wnt pathway in early colonic neoplasia and serve to underscore the importance of angiogenesis in premalignant disease.

Introduction

VEGF² is one of the key pro-angiogenic stimuli in physiological and pathological states. In colon cancer, VEGF levels are elevated and correlated with a poor clinical outcome (1). However, angiogenesis is not restricted to the advanced stages of cancer but can also be observed early in premalignant stages of tumor development. In benign colorectal adenomas, VEGF protein and RNA levels exceed those of normal colonic mucosa (2, 3). Interestingly, VEGF protein levels and blood vessel counts are similar in adenomas and nonmetastatic malignancies (1), whereas levels in metastatic colorectal tumors are significantly higher.

The mechanisms that up-regulate VEGF in cancerous states are complex. HIF-1, a heterodimeric transcription factor comprised of the bHLH proteins HIF-1 α and HIF-1 β , is one key regulator (4). Although mutant H-Ras regulates VEGF in epithelial, fibroblast, and endothelial cell lines (5), the relevance to gastrointestinal malignancies is unclear, because mutations in K-ras are almost exclusively identified in colon polyps and cancer. Inhibition of the PI3K effector pathway with wortmannin can reduce this stimulatory effect in endothelial cells (6), but independent studies have demonstrated that the ERK-1,-2 (p42/p44 MAP kinase) effector pathway is more relevant in fibroblasts (7). Thus, the pathways that regulate VEGF expression are cell-specific. In colon cancer, activation of ERK pathways plays a role in the up-regulation of VEGF in conditions of serum starvation (8). Otherwise, the precise transcriptional mechanisms that regulate VEGF in colonic epithelial cells are not well described.

Two key genetic events underlying the pathogenesis of benign colonic adenomas are activation of the Wnt and K-ras signaling pathways. The current studies identify VEGF as a novel target of the Wnt signaling pathway. In addition, K-ras appears to enhance signaling through the Wnt pathway in a PI3K-dependent manner. By defining a physiologically relevant target of these signaling pathways, these findings provide a molecular basis for the angiogenesis observed early in premalignant disease.

Materials and Methods

Cell Lines. Lovo, ColoHSR, Colo205, Hct116, 293, and HeLa cell lines (all from American Type Culture Collection), and Caco2 cells (gift of Dr. Loyal Tillotson) were maintained in recommended growth media with 10% fetal bovine serum supplemented with 2% penicillin/streptomycin (Bio-Whittaker).

Plasmids and Constructs. Upstream regulatory sequence (2.8 kb) of the human VEGF promoter (–1.8 kb to +1.0 kb from the transcriptional start site; gift of Dr. Ramnik Xavier) was subcloned into the pGL2-basic luciferase vector (Promega). Deletion constructs were prepared by digesting the 2.8-kb VEGF insert at the *Sfa*NI site at –850 bp (to yield the 1.9-kb insert), *Bgl*II site at –420 bp (to yield the 1.4-kb insert), and *Sma*I site at –90 bp (to yield the 1.1-kb insert). The 0.4-kb fragment was prepared by digesting the 1.0-kb insert at the 3' end with *Sac*I. Site-directed mutants were generated using the Quik-Change protocol (Stratagene) and the mutagenic primers 5'-CTGCCGCTCACTTTACTGTCTGCAGGCCAG and 5'-CTGGCCTGCAGACAGTAAAGTGAGCGGCAG. The introduced mutations were confirmed by DNA sequencing. A TCF4 reporter construct was designed by subcloning the consensus TCF-4 binding element into the heterologous promoter construct PT81 (9).

The previously described β -catenin^{S37A} (10), dominant negative Δ 35-TCF4 (11), APC (12), kinase mutant ERK-1,-2 (13), pZipKras^{12Val} (14), pVL Raf301 (15), p85 and p110 subunits of PI3K (16), Myr-Akt (17), and dominant-negative Akt-K179A (17) expression plasmids were transfected in varying amounts, as described in the "Results" section. Typically, 1 μ g of reporter vector was transfected with 0.25–0.5 μ g of expression vector. An empty pcDNA3.1 or pZipNeo vector was used so that equal amounts of expression plasmid were transfected within a given experiment.

Transfections. Transient transfections were performed using the cationic lipid Lipofectamine-PLUS (Life Technologies, Inc.) according to the manufacturer's specifications. All of the experiments were performed in 6-well tissue culture dishes with cells plated to reach 70% confluence on the day of transfection. Experiments were performed in duplicate wells a minimum of two times. Luciferase activity was measured 48 h after transfection.

Immunoblotting. Protein lysates were harvested from ColoHSR cells transiently transfected with pZipKras^{12Val} or empty vector (pZipNeo) after 48 h. Total cellular protein (75 μ g) was separated on a denaturing acrylamide gel and electrotransferred onto nitrocellulose membranes (Schleicher and Schuell). Blots were hybridized with a phospho-Akt-specific antibody, stripped, and rehybridized with a total-Akt antibody (both Cell Signaling and both used at 1:1000). Visualization was performed with chemiluminescence.

Reverse Transcription-PCR. Total RNA was harvested using Trizol (Life Technologies, Inc.) and treated with DNase I. After reverse transcription using an oligo-dT primer, PCR was performed with specific primers for VEGF and G3-PDH.

Received 5/30/01; accepted 6/21/01.

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² The abbreviations used are: VEGF, vascular endothelial growth factor; PI3K, phosphatidylinositol 3-kinase; HIF, hypoxia-inducible factor; ERK, extracellular signal-regulated kinase; APC, adenomatous polyposis coli; GSK-3 β , glycogen synthase kinase-3 β ; TCF, T-cell factor; MAP, mitogen-activated protein; MEK, MAP/ERK kinase.

Results

VEGF Expression Is Regulated by the Wnt Pathway in Colon Cancer Cells. A 2.8-kb fragment of the regulatory sequence of the human *VEGF* gene (−1.8 kb from the transcriptional start site to +1.0 kb) was subcloned into the pGL2-basic luciferase vector. This promoter fragment has previously been demonstrated to be highly up-regulated in murine tumor models (18). The 2.8-kb VEGF-pGL2 construct was active in colon cancer cell lines with endogenously activated Wnt signaling, a consequence of mutations of the *APC* tumor suppressor gene (Lovo), the β -catenin oncogene (Hct116), or both (Colo205). To inhibit Wnt signaling, a dominant negative TCF4 (DN Δ 35-TCF4) construct was expressed. In Lovo, Hct116, and Colo205 cells, cotransfection of DN Δ 35-TCF4 suppressed VEGF promoter activity dramatically (Fig. 1A). In contrast, expression of DN Δ 35-TCF4 in 293 kidney cells that also vigorously express VEGF failed to inhibit promoter activity. APC, another negative regulator of Wnt signaling, was expressed in Lovo cells that endogenously harbor a mutant *APC* gene. The VEGF promoter was suppressed 45% in Lovo cells transfected with wild-type APC, but no such inhibition was observed in 293 kidney cells (Fig. 1B). These studies suggest that the Wnt pathway up-regulates VEGF expression. Moreover, this appears to be a distinctive feature of colonic epithelial cells.

The effects of Wnt signaling on the VEGF promoter were confirmed through measurements of endogenous VEGF mRNA levels. Wild-type APC or DN Δ 35-TCF4 was expressed in Lovo cells, and each reduced endogenous VEGF mRNA levels 45 and 55%, respectively (Fig. 1C). These results thus confirm the previous studies using a VEGF promoter construct.

To determine whether activation of the Wnt pathway directly up-regulates the VEGF promoter, an oncogenic β -catenin^{S37A} was transfected with the 2.8-kb VEGF-pGL2 reporter into HeLa cells. A critical serine phosphorylation site at amino acid 37 that ordinarily targets β -catenin for degradation has been mutated (10). Fig. 1D illustrates a linear dose response to the effects of β -catenin^{S37A}. The

peak response was observed when 1.25 μ g of expression vector was cotransfected with 0.75 μ g of reporter vector, resulting in a >30-fold stimulation of VEGF promoter activity. In independent studies, HeLa cells were treated with 25 mM LiCl, an inhibitor of GSK-3 β kinase activity. GSK-3 β is a negative regulator of Wnt signaling, and inhibiting GSK-3 β with LiCl resulted in a 2.2-fold increase in VEGF promoter activity. Collectively, these findings indicate that activation of Wnt signaling up-regulates VEGF expression in colonic neoplasia.

Mutational Analysis Defines Key *cis*-Regulatory Regions of the VEGF Promoter. To delineate the *cis*-regulatory elements that mediate the effects of β -catenin on the VEGF promoter, a series of promoter deletion constructs were designed. These constructs (1.9-kb VEGF-pGL2, 1.4-kb VEGF-pGL2, 1.1-kb VEGF-pGL2, 0.4-kb VEGF-pGL2) were tested in Lovo, Colo205, Caco2, and HCT 116 colon cancer cells, all of which have an endogenously activated Wnt signaling pathway. The 2.8-kb and 1.9-kb constructs were consistently the most active among the four cell lines. Promoter activity in all of the colon cell lines was reduced ~50% when sequences at the 5' end of the 1.9-kb construct were deleted (Fig. 2A). There was a second point between the 1.1-kb and 0.4-kb promoter fragments at which the activity fell only in Lovo cells. These findings argue for key regulatory elements that lie within the 1.9-kb VEGF fragment but not the 1.4-kb fragment. Of note, the HIF-response element located −935 bp upstream of the transcriptional start site did not appear to play an important regulatory role, because the activity of the 1.9-kb VEGF construct that lacks the HIF-response element, was similar to the 2.8-kb VEGF construct that does contain the element (4).

The sequence deleted between the 1.9-kb and the 1.4-kb VEGF promoter fragments contains a potential TCF-binding element (5'-CTTTGATG) located −805 bp upstream of the transcriptional start site. This site was selectively mutated to 5'-CTTTACTG (mut1.9 kb VEGF-pGL2). Fig. 2B illustrates a significant decline in VEGF promoter activity in Hct116 and Lovo cells when this TCF site was selectively mutated. However, no change was observed in Caco2

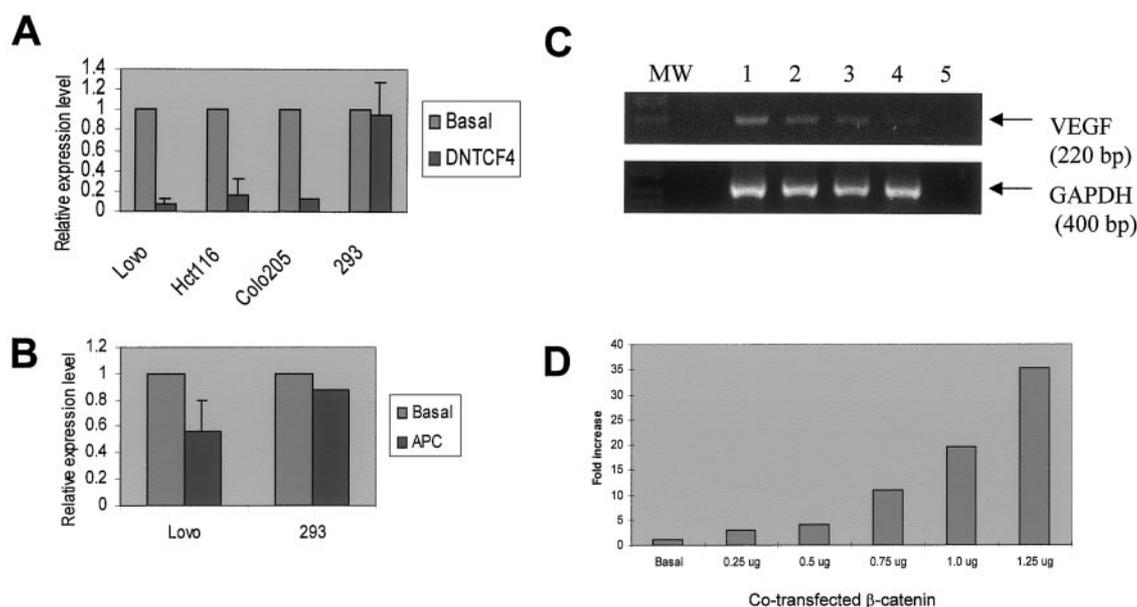


Fig. 1. VEGF is regulated by the Wnt pathway. Expression levels of the 2.8-kb VEGF-pGL2 construct were determined in multiple cell lines cotransfected with DN-TCF4 (A), or wild-type APC (B). The levels were compared with basal conditions (set at 1.0) when empty vector was cotransfected. The results represent the mean \pm SE of experiments performed in duplicate and repeated at least twice. C, endogenous VEGF mRNA levels in Lovo cells transfected with empty vector (Lane 1), wild-type APC (Lane 2), DN-TCF4 (Lane 3), or incubated with 1 μ M wortmannin (Lane 4). One μ g of total RNA was DNase treated, reverse transcribed, PCR-amplified with specific primers for VEGF (25 cycles) or GAPDH (20 cycles), and then separated on an agarose gel. Lane 5, negative control (no reverse transcriptase). The relative expression levels were determined by densitometry. With Lane 1 set to 1.0, the relative levels of expression were 0.55 (Lane 2), 0.45 (Lane 3), and 0.15 (Lane 4). D, dose response of the 2.8-kb VEGF-pGL2 promoter construct (0.75 μ g) to varying amounts of cotransfected β -catenin^{S37A} (0.25–1.25 μ g). Total of 2 μ g of plasmid was transfected per well.

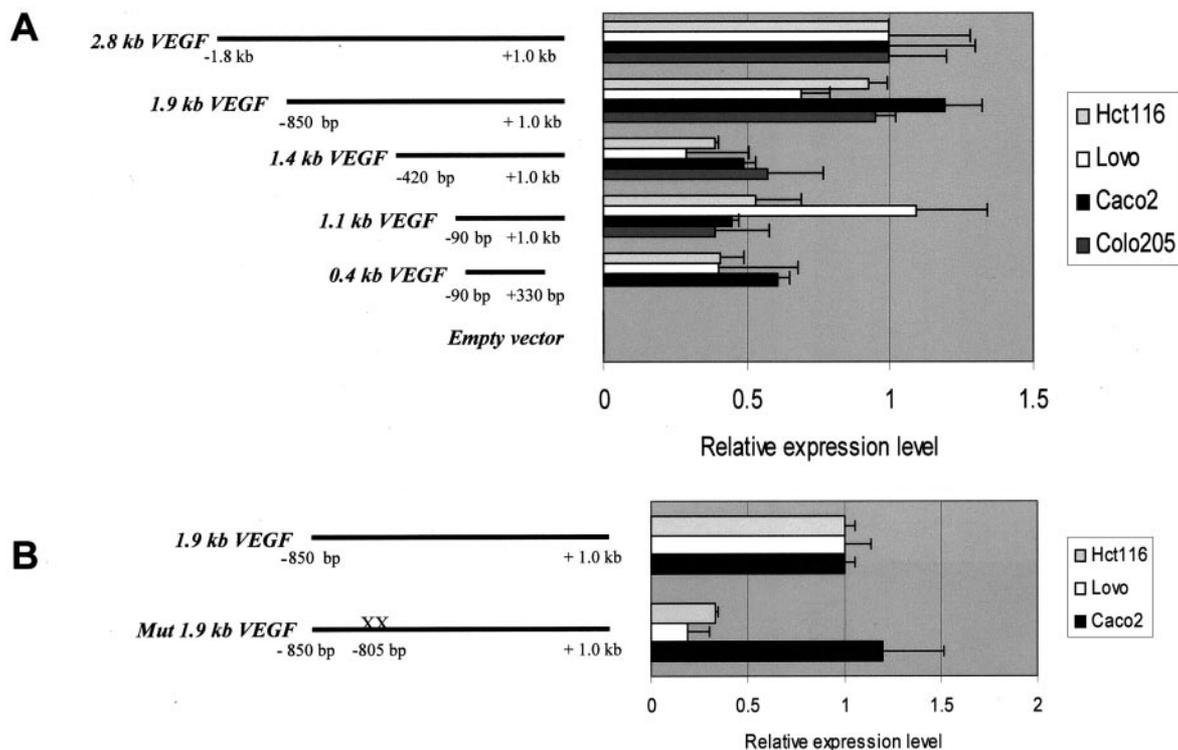


Fig. 2. Identification of key regulatory elements in the VEGF promoter. *A*, 5' deletion constructs of the VEGF promoter were transfected into four colon cancer cell lines. The results are expressed relative to the activity of the full-length 2.8-kb VEGF-pGL2 construct (set to 1.0) and represent the mean \pm SD of at least two separate transfections performed in duplicate. See "Materials and Methods" for details on the design of each promoter deletion. *B*, transcriptional activity of the wild-type 1.9-kb VEGF promoter construct and a mutant 1.9-kb VEGF promoter construct harboring a site-specific alteration of a putative TCF4 binding site at -805 bp. The expression of the wild-type 1.9-kb construct is set to 1.0.

cells. In contrast to Hct116 and Lovo cells, which both harbor an oncogenic *K-ras*^{13A_{sp}}, the *K-ras* proto-oncogene is wild-type in the Caco2 cell line (14). This suggests that regulation of VEGF by the Wnt pathway in colon cancer may depend on interactions with oncogenic *K-ras* signaling. Consistent with this hypothesis, expression of mutant *K-ras*^{12Val} in Caco2 cells led to a 33% decrease in the activity of the mut1.9 kb VEGF promoter construct.

K-ras Up-Regulates VEGF through PI3K-dependent Pathways in Colon Cancer Cells. Previous studies in fibroblast and endothelial cell lines have demonstrated that VEGF can be induced by activation of the *H-Ras* oncogene (5, 6). The relevance to gastrointestinal malignancies is not clear, because mutations in *K-ras* are primarily identified in colon polyps and cancer. To determine whether *K-ras* also up-regulates VEGF, HeLa cells were transfected with the oncogenic *K-ras*^{12Val} isoform frequently identified in colon cancer. The 2.8-kb VEGF promoter was up-regulated 2.9-fold (± 0.4) compared with cotransfection of empty vector, pZipNeo.

K-ras activates multiple effector pathways, including the Raf/MEK/ERK, PI3K, and jun NH₂-terminal kinase pathways. To characterize the effectors through which *K-ras* up-regulates VEGF in colon cancer, specific inhibitors were tested in cell lines that endogenously carry a mutant *K-ras* oncogene. In Lovo cells, cotransfection of dominant-negative Raf301 or dominant-negative kinase-deficient ERK1 and ERK2 failed to inhibit VEGF promoter activity (Fig. 3A), which indicates that the Raf/MEK/ERK pathway is unlikely to mediate the effects of *K-ras* signaling on the VEGF promoter. Similar results were obtained in the Hct116 cell line, which also contains the *K-ras*^{13A_{sp}} mutation. However, inhibition of ERK activity with the dominant-negative ERK mutants reduced VEGF promoter activity 42% in Hct116 cells, which suggests that ERK-mediated signaling may play a role in some colon tumors. Additional confirmation was obtained using the MEK-specific inhibitor PD98059. Incubation of Lovo and

Hct116 cells with 20 μ M PD98059 failed to inhibit VEGF expression and was associated with a slight up-regulation of VEGF promoter activity (1.3- to 1.4-fold above basal). This suggests that ERK may not be activated by MEK in Hct116 cells.

Another key *K-ras* effector pathway activates PI3K. Incubation of Lovo and Hct116 cell lines with 1 μ M wortmannin, a specific inhibitor of PI3K, was associated with a significant decline in VEGF promoter activity, which suggests that PI3K-dependent pathways are critical in colon tumor cells with a mutant *K-ras* oncogene (Fig. 3B). Endogenous VEGF mRNA levels were also reduced 85% after treatment with 1 μ M wortmannin in Lovo cells (Fig. 1C). A key target of phosphorylation by PI3K is Akt/protein kinase B. Transfection of the dominant-negative Akt-K179A vector led to a 60% reduction of basal VEGF promoter activity in Lovo cells and a 75% reduction in Hct116 cells, which indicates that PI3K may act through Akt to up-regulate VEGF (Fig. 3B).

To determine whether activation of the PI3K pathway directly up-regulates VEGF, the p85 and p110 subunits of PI3K or a constitutively active Akt (Myr-Akt) were expressed. All stimulated the full-length VEGF promoter 3- to 4-fold in HeLa cells, similar to the level of induction by mutant *K-ras*^{12Val} (Fig. 3C). The induction of VEGF by mutant *K-ras*^{12Val} in Caco2 cells was completely blocked by Akt-K179A (data not shown). Finally, ColoHSR cells that are *K-ras*^{wild-type} were transiently transfected with the mutant *K-ras*^{12Val} vector. Immunoblotting studies demonstrated that Akt is phosphorylated in response to mutant *K-ras* signaling (Fig. 3D). These studies demonstrate that PI3K-mediated activation of Akt may be the key *K-ras* effector pathway that regulates VEGF in colon cancer cells.

K-ras and Wnt Pathways Interact to Up-Regulate VEGF. To determine whether signals from the Wnt and *K-ras* pathways interact to regulate VEGF, HeLa cells were transfected with *K-ras*^{12Val} and/or constitutively active β -catenin^{S37A}. As illustrated in Fig. 4A, *K-ras*

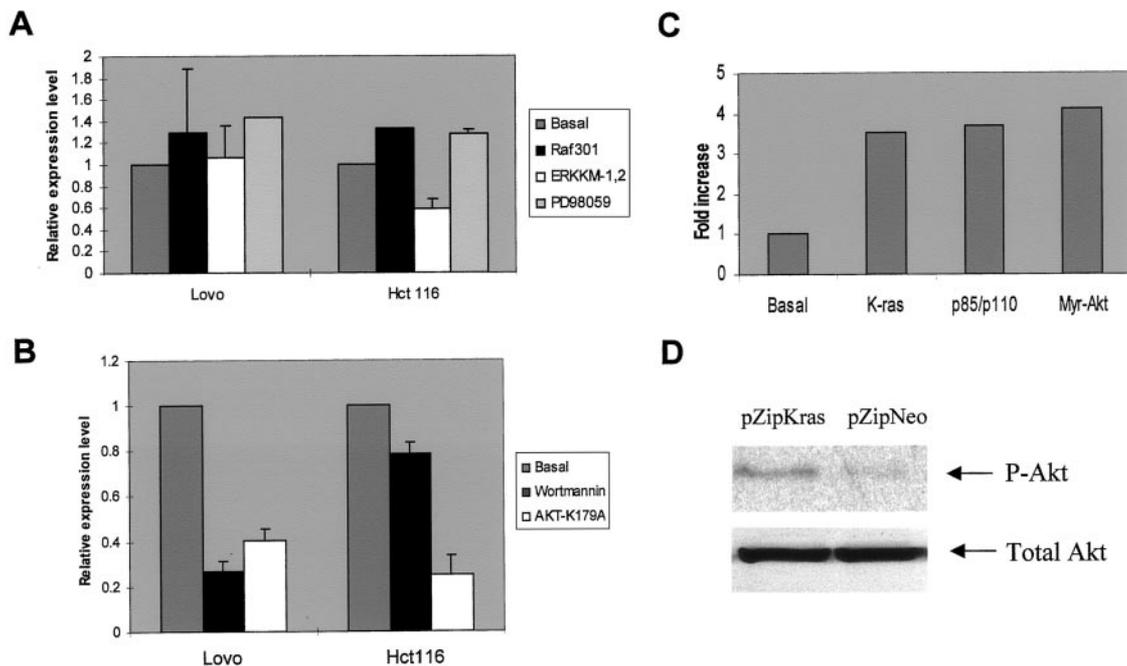


Fig. 3. Definition of K-ras effector pathways that regulate VEGF expression. **A**, effect of K-ras pathway inhibitors on the activity of the 2.8-kb VEGF promoter in Lovo and HCT 116 cells. The expression levels (\pm SE) in the presence of dominant-negative Raf301, kinase-inactive ERK-1 and -2, or the MEK inhibitor PD98059, are illustrated relative to the basal activity of the VEGF promoter alone (set to 1.0). **B**, effect of PI3K pathway inhibitors on the activity of the 2.8-kb VEGF promoter. The expression levels in the presence of wortmannin ($1 \mu\text{M}$) or dominant-negative Akt-K179A are illustrated relative to basal expression (set to 1.0). **C**, K-ras and PI3K pathway activators up-regulate the 2.8-kb VEGF promoter construct in HeLa cells. The results are illustrated relative to basal transcriptional activity of reporter plasmid alone (set to 1.0). **D**, K-ras induces phosphorylation of Akt. Serum-starved ColoHRSR cells were transfected with either the mutant pZipKras^{Val12} vector or the empty vector (pZipNeo). Immunoblots were performed on harvested protein after 48 h using a phospho-Akt-specific antibody. The blot was stripped and reprobbed with an antibody to total Akt.

up-regulates VEGF promoter activity 2.9-fold, and β -catenin increases it 4.5-fold. However, the combination yields a 6.2-fold increase in VEGF promoter activity, which indicates a strong interaction between the two signaling pathways. To determine whether these

effects are mediated through TCF4, a reporter construct bearing one copy of a consensus TCF4 element (5'-CCTTTGATCTTAC), upstream of the heterologous TK-promoter in the PT-81 luciferase vector, was used (9). β -catenin^{S37A} up-regulated activity of this TCF reporter 1.8-fold, and K-ras up-regulated activity 1.4-fold. However, the combination led to a 3.3-fold increase in reporter activity, which suggests that K-ras can up-regulate VEGF by enhancing Wnt signaling through TCF4. Control studies with an empty PT-81 vector did not demonstrate such synergy.

HeLa cells, expressing β -catenin, were then treated with the PI3K inhibitor wortmannin to determine whether the Wnt pathway may reciprocally regulate PI3K. In the absence of β -catenin expression, wortmannin inhibited VEGF promoter activity as shown previously (Fig. 4B). However, when Wnt signaling was activated by overexpressing β -catenin, the addition of wortmannin failed to down-regulate VEGF. This suggests that signaling through the Wnt pathway does not lead to the downstream activation of PI3K in the regulation of VEGF.

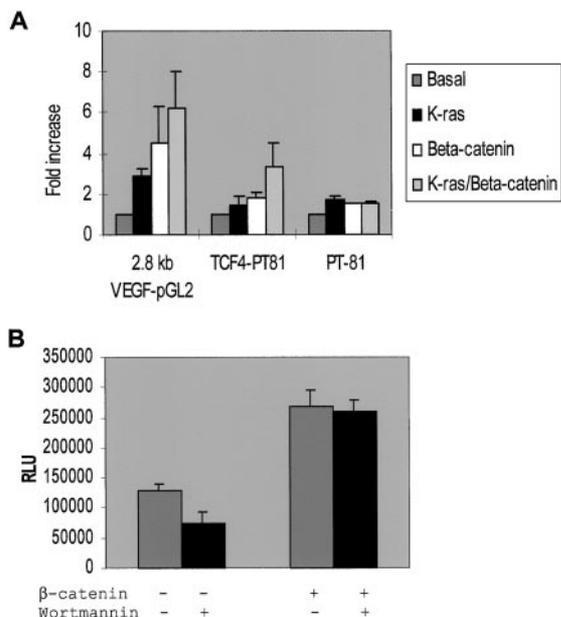


Fig. 4. Interaction between the Wnt and K-ras signaling pathways. **A**, the 2.8-kb VEGF promoter construct, a heterologous promoter construct with a consensus TCF4 site, or an empty PT81 vector were cotransfected into HeLa cells with K-ras, β -catenin^{S37A}, or both expression vectors. The changes in expression levels are displayed relative to the baseline activity of the reporter construct alone (set to 1.0 for each reporter construct). The results are the mean of at least two separate experiments \pm SE. **B**, transcriptional activity of the 2.8-kb VEGF reporter construct in HeLa cells expressing β -catenin^{S37A} that are incubated in the presence or absence of $1 \mu\text{M}$ wortmannin. The mean relative light units (RLU) \pm SE are illustrated.

Discussion

The Wnt signaling cascade regulates not only key patterning events in development but also the process of tumorigenesis. This is particularly relevant to colorectal malignancies, because the pathway is activated in the majority of colorectal adenomas and cancers. Although several gene targets of the Wnt pathway, including *c-MYC* (19), *cyclin D1* (20), and *PPAR δ* (21), have been identified, it is likely that this list is incomplete. These studies demonstrate that VEGF is a novel target of the Wnt pathway in colon cancer.

The regulation of *VEGF* gene expression is complex, and part of this complexity stems from cell-specific variations in its regulation. In colonic epithelial cells, Wnt signaling plays an important role, and this effect appears to be mediated in part through a consensus TCF4 element -805 bp upstream of the transcriptional start site. However,

there are likely to be additional TCF elements that may also regulate VEGF expression. Furthermore, K-ras activates VEGF in colon cancer cells through a PI3K/Akt-dependent pathway. Although there are likely to be multiple effects of PI3K activation on the VEGF promoter, we have identified a unique cooperative interaction with the Wnt pathway.

There are many potential mechanisms that may link these two pathways. PI3K activates Akt/protein kinase B, which subsequently phosphorylates GSK-3 β . Although phosphorylation of GSK-3 β at Ser9 by Akt inhibits GSK-3 β activity in response to insulin signaling (22), no such inhibition has previously been demonstrated in colon cancer cell lines. However, there may be alternative mechanisms through which GSK-3 β could link the PI3K and Wnt pathways. Other possibilities include activation of the MAP-kinase-like enzyme Nemo-like kinase that regulates phosphorylation of TCF4 (23), or induction of the coactivator p300 that binds to β -catenin (24).

The observation that K-ras induction of VEGF may be linked to Wnt signaling in colon cancer implies an interaction between two critical pathways that regulate cellular proliferation and transformation. Such a coordinate pattern of gene regulation is likely to have broad implications for additional genes that underlie the pathogenesis of colon cancer. One such candidate is gastrin, previously demonstrated in independent studies to be regulated by both the Wnt and K-ras pathways (9, 14).

The observation that VEGF is up-regulated by the Wnt and K-ras pathways thus provides a molecular basis for the angiogenesis observed in benign colonic adenomas. This also serves to highlight the significance of angiogenesis in benign premalignant disease and reinforce the principle that angiogenesis is not confined to advanced stages of cancer. Finally, these findings imply the importance of antiangiogenic approaches in the design of chemopreventive strategies for colon polyps and cancer.

Acknowledgments

We thank Drs. Daniel Podolsky and Timothy Wang for helpful discussions and Drs. Timothy Wang (University of Massachusetts Medical Center, Worcester, MA), Ramnik Xavier (Massachusetts General Hospital, Boston, MA), Loyal Tillotson (University of North Carolina, Chapel Hill, NC), Michael Quon (NIH, Bethesda, MD), and Christian Reinecker (Massachusetts General Hospital, Boston, MA) for generously sharing plasmid vectors and PCR primers. Oligonucleotides were provided by the Center for the Study of Inflammatory Bone Disease at the Massachusetts General Hospital.

References

1. Takahashi, Y., Kitadai, Y., Bucana, C. D., Cleary, K. R., and Ellis, L. M. Expression of vascular endothelial growth factor and its receptor, KDF, correlates with vascularity, metastasis, and proliferation of human colon cancer. *Cancer Res.*, *55*: 3964–3968, 1995.
2. Ono, T., and Miki, C. Factors influencing tissue concentration of vascular endothelial growth factor in colorectal carcinoma. *Am. J. Gastroenterol.*, *95*: 1062–1067, 2000.
3. Wong, M., Cheung, N., Yuen, S., Leung, S., and Chung, L. Vascular endothelial growth factor is up-regulated in the early pre-malignant stage of colorectal tumor progression. *Int. J. Cancer*, *81*: 845–850, 1999.
4. Forsythe, J., Jiang, B-H., Iyer, N. V., Agani, F., Leung, S. W., Koos, R. D., and Semenza, G. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol. Cell. Biol.*, *16*: 4604–4613, 1996.
5. Rak, J., Mitsuhashi, Y., Bayko, L., Filmus, J., Shirasawa, S., Sasazuki, T., and Kerbel, R. Mutant *ras* oncogenes upregulate VEGF/VPF expression: implications for induction and inhibition of tumor angiogenesis. *Cancer Res.*, *55*: 4575–4580, 1995.
6. Arbiser, J., Moses, M., Fernandez, C. A., Ghiso, N., Cao, Y., Klauber, N., Frank, D., Brownlee, M., Flynn, E., Parangi, S., Byers, H., and Folkman, J. Oncogenic *H-ras* stimulates tumor angiogenesis by two distinct pathways. *Proc. Natl. Acad. Sci. USA*, *94*: 861–866, 1997.
7. Milanini, J., Vinals, F., Pouyssegur, P., and Pages, G. p42/p44 MAP kinase module plays a key role in the transcriptional regulation of the vascular endothelial growth factor gene in fibroblasts. *J. Biol. Chem.*, *273*: 18165–18172, 1998.
8. Jung, Y. D., Nakano, K., Liu, W., Gallick, G., and Ellis, L. Extracellular signal-regulated kinase activation is required for up-regulation of vascular endothelial growth factor by serum starvation in human colon carcinoma cells. *Cancer Res.*, *59*: 4804–4807, 1999.
9. Koh, T., Bulitta, C., Fleming, J., Dockray, G., Varro, A., and Wang, T. Gastrin is a target of the β -catenin/TCF-4 growth-signaling pathway in a model of intestinal polyposis. *J. Clin. Invest.*, *106*: 533–539, 2000.
10. Orford, K., Crockett, C., Jensen, J., Weissman, A. M., and Byers, S. Serine phosphorylation-regulated ubiquitination and degradation of β -catenin. *J. Biol. Chem.*, *272*: 24735–24738, 1997.
11. Korinek, V., Barker, N., Morin, P. J., van Wichen, D., de Weger, R., Kinzler, K. W., Vogelstein, B., and Clevers, H. Constitutive transcriptional activation by a β -catenin-TCF complex in APC^{-/-} colon carcinoma. *Science (Wash. DC)*, *275*: 1784–1787, 1997.
12. Easwaran, V., Song, V., Polakis, P., and Byers, S. The ubiquitin-proteasome pathway and serine kinase activity modulate adenomatous polyposis coli protein-mediated regulation of β -catenin-lymphocyte enhancer-binding factor signaling. *J. Biol. Chem.*, *274*: 16641–16645, 1999.
13. Robbins, D. J., Zhen, E., Owaki, H., Vanderbilt, C. A., Ebert, D., Geppert, T. D., and Cobb, M. H. Regulation and properties of extracellular signal-regulated protein kinases 1 and 2 *in vitro*. *J. Biol. Chem.*, *268*: 5097–5106, 1993.
14. Nakata, H., Wang, S-L., Chung, D. C., Westwick, J. K., and Tillotson, L. G. Oncogenic *ras* induces gastrin gene expression in colon cancer. *Gastroenterology*, *115*: 1144–1153, 1998.
15. Williams, N. G., Roberts, T. M., and Li, P. Both p21ras and pp60v-src are required, but neither alone is sufficient to activate the Raf-1 kinase. *Proc. Natl. Acad. Sci. USA*, *89*: 2922–2926, 1992.
16. Weng, Q. P., Andrabi, K., Klippel, A., Kozlowski, M. T., Williams, L. T., and Avruch, J. Phosphatidylinositol 3-kinase signals activation of p70 S6 kinase *in situ* through site-specific p70 phosphorylation. *Proc. Natl. Acad. Sci. USA*, *92*: 5744–5748, 1995.
17. Cong, L. N., Chen, H., Li, Y., Zhou, L., McGibbon, M. A., Taylor, S. I., and Quon, M. J. Physiological role of Akt in insulin-stimulated translocation of GLUT4 in transfected rat adipose cells. *Mol. Endocrinol.*, *11*: 1881–1890, 1997.
18. Fukumura, D., Xavier, R., Sugiura, T., Chen, Y., Park, E-C., Lu, N., Selig, M., Nielsen, G., Taksir, T., Jain, R., and Seed, B. Tumor induction of VEGF promoter activity in stromal cells. *Cell*, *94*: 715–725, 1998.
19. He, T-C., Sparks, A. B., Rago, C., Hermeking, H., Zawel, L., da Costa, L. T., Morin, P. J., Vogelstein, B., and Kinzler, K. W. Identification of c-MYC as a target of the APC pathway. *Science (Wash. DC)*, *281*: 1509–1512, 1998.
20. Tetsu, O., and McCormick, F. β -catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature (Lond.)*, *398*: 422–426, 1999.
21. He, T-C., Chan, T. A., Vogelstein, B., and Kinzler, K. W. PPAR δ is an APC-regulated target of nonsteroidal anti-inflammatory drugs. *Cell*, *99*: 335–345, 1999.
22. Cross, D., Alessi, D., Cohen, P., Andjelkovich, M., and Hemmings, B. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature (Lond.)*, *378*: 785–789, 1995.
23. Ishitani, T., Ninomiya-Tsuji, J., Nagai, S., Nishita, M., Meneghini, M., Barker, N., Waterman, M., Bowerman, B., Clevers, H., Shibuya, H., and Matsumoto, K. The TAK1-NLK-MAPK-related pathway antagonizes signaling between β -catenin and transcription factor TCF. *Nature (Lond.)*, *399*: 798–802, 1999.
24. Hecht, A., Vlemminckx, K., Stemmler, M. P., van Roy, F., and Kemler, R. The p300/CBP acetyltransferases function as transcriptional coactivators of β -catenin in vertebrates. *EMBO J.*, *15*: 1839–1850, 2000.

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Cancer Res 2001;61:6050-6054.

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