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Post-transcriptional regulation of vascular endothelial growth factor mRNA by the product of the *VHL* tumor suppressor gene

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ABSTRACT **The *VHL* tumor suppressor gene is inactivated in patients with von Hippel–Lindau disease and in most sporadic clear cell renal carcinomas. Although VHL protein function remains unclear, VHL does interact with the elongin BC subunits *in vivo* and regulates RNA polymerase II elongation activity *in vitro* by inhibiting formation of the elongin ABC complex. Expression of wild-type VHL in renal carcinoma cells with inactivated endogenous VHL resulted in unaltered *in vitro* cell growth and decreased vascular endothelial growth factor (VEGF) mRNA expression and responsiveness to serum deprivation. VEGF is highly expressed in many tumors, including VHL-associated and sporadic renal carcinomas, and it stimulates neoangiogenesis in growing solid tumors. Despite 5-fold differences in VEGF mRNA levels, VHL overexpression did not affect VEGF transcription initiation or elongation as would have been suggested by VHL–elongin association. These results suggest that VHL regulates VEGF expression at a post-transcriptional level and that VHL inactivation in target cells causes a loss of VEGF suppression, leading to formation of a vascular stroma.**

The hereditary cancer syndrome, von Hippel–Lindau (VHL) disease, is associated with constitutional mutations of the *VHL* gene (1, 2). VHL disease has an autosomal dominant inheritance pattern with variable penetrance, and affected individuals are predisposed to develop a number of tumors, including renal carcinomas and vascular tumors of the central nervous system and retina (2). Tumorigenesis in VHL disease is associated with somatic loss or inactivation of the wild-type *VHL* allele (3, 4) and, therefore, supports Knudson's "two-hit" hypothesis for tumor formation (5). In addition, as would be predicted from the Knudson tumor suppressor gene model, *VHL* is somatically inactivated by either mutation (6–9) or hypermethylation, leading to transcriptional silencing (10) in the majority of nonhereditary clear-cell renal carcinomas. However, VHL is not inactivated in renal tumors of the papillary histologic type (6).

The human *VHL* gene encodes a protein of 213 amino acids that is highly conserved across species, and it has no significant sequence similarity to other cloned genes (1). Immunofluorescence studies of the VHL protein indicate a complex regulation of subcellular localization, including nuclear staining when cultured cells are grown under sparse conditions but cytosolic staining when cells grow to confluency (11). A clue for VHL protein function was obtained through biochemical studies, which demonstrated that VHL associates with the elongin B and C subunits of the elongin/SIII complex (12, 13). The elongin complex, made up of the catalytic A subunit and the regulatory B and C subunits, potentiates RNA polymerase II transcription activity by increasing elongation rates and decreasing polymerase pausing *in vitro* (14). VHL binding to the elongin BC complex *in vitro* inhibits elongin activity, resulting in decreased RNA polymerase II activity (12). Loss of elongin BC binding is a consequence of a number of naturally occurring missense mutations of *VHL*.

The functional role of the VHL/elongin BC (VBC) association *in vivo* and its relationship with suppression of solid tumor growth is under investigation. Solid tumors are composed of a mixture of tumor cells and supporting stromal cells that are recruited by the tumor cells. Tumor growth requires neoangiogenesis, and the migration, proliferation, and differentiation of vascular endothelial cells is stimulated by the release of polypeptide growth factors by the tumor cells (15–17). These factors include acidic fibroblast growth factor, basic fibroblast growth factor (bFGF), epidermal growth factor, transforming growth factors α and β, tumor necrosis factor α, and vascular endothelial growth factor (VEGF). VEGF is secreted by a number of tumors and is normally expressed in the kidney, brain, and other tissues (18–22). VEGF is also markedly elevated in both VHL-associated and sporadic central nervous system hemangioblastomas (23, 24) and renal carcinomas (25, 26). In addition to having mitogenic activity on endothelial cells, VEGF also induces vascular permeability, which may lead to extravasation of plasma proteins and deposition of fibrin, providing an extracellular support for tumor cell and endothelial cell growth.

In the present study the effects of retroviral transduction of the VHL cDNA into renal carcinoma cell lines was evaluated. We show that expression of wild-type VHL in cells containing a mutated endogenous VHL does not affect *in vitro* growth characteristics, but does lead to decreased expression of VEGF in the cells tested. These results indicate that *VHL* inactivation, an early event in VHL-associated tumorigenesis as well as in sporadic renal tumorigenesis, may lead to loss of regulation of polypeptide growth factors that mediate cell–cell interactions and promote inappropriate cell growth.

MATERIALS AND METHODS

***VHL* Subcloning into a Retroviral Vector and Establishment of VHL Transductants.** The *VHL* coding sequences were excised from the g7 VHL cDNA (1) as either *Eco*RI/*Xba*I or *Bam*HI/*Xba*I restriction fragments and subcloned into the Moloney murine leukemia virus retroviral vector pLXSN. The *VHL* frameshift construct was generated as a *Bam*HI/*Bgl*II restriction fragment and subcloned into pLXSN, where it encoded a 215-amino acid protein with a frameshift at amino

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Abbreviations: VHL, von Hippel–Lindau (protein); VBC, VHL/elongin BC; BCE, bovine capillary endothelial; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; FBS, fetal bovine serum.

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acid 187. Clones were characterized for orientation by restriction enzyme digestions. Plasmids were transfected by calcium phosphate precipitation (BRL/Life Technologies) into a mixed culture of the retroviral packaging cells GP+E86 and PA317 to obtain amphitrophic virus. All cell lines used were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and gentamicin, and transduced cells were selected and maintained in 600 μg/ml G418 (BRL/Life Technologies). Viral titers were determined with NIH 3T3 cells and ranged from 1 to 5 × 10^6 colony-forming units/ml. Transductions were performed in 35-mm dishes or six-well tissue culture plates by plating various renal carcinoma cell lines and allowing them to grow to about 50% confluence. The medium was removed and replaced by virus-containing supernatants from the appropriate packaging cell containing 5 μg/ml DEAE-dextran. Supernatants were removed after 18 hr and replaced with fresh medium. G418 selection was initiated after an additional 24 hr. Transduction efficiencies were high and all of the cell lines used in this study were bulk transductants. The renal carcinoma cell lines transduced were UOK 101, 111, 117, 121, 123, 127, and 130, and KN41, which were derived from clear cell tumors, and UOK 112, 124, and 132, which were derived from papillary tumors.

Cell Proliferation and Anchorage-Independent Growth Assays. *In vitro* cell growth was assessed by plating 1 × 10^4 cells per well in six-well tissue culture dishes in triplicate. Cells were harvested at 24-hr intervals by trypsinization and counted using a hemocytometer.

Bovine capillary endothelial (BCE) cells (obtained from J. Folkman, Harvard Medical School, Boston) were used to evaluate mitogenic activity in culture supernatants of renal carcinoma cells. Conditioned media were collected from 3-day cultures (with comparable growth) of parent renal carcinoma cells or transductants (grown in the absence of G418). BCE cells were plated at a density of 1 × 10^4 cells per well in DMEM containing 10% calf serum and gentamicin (BCE medium) in 24-well tissue culture dishes. After 18 hr, the medium was removed and replaced in triplicate wells with 200 μl of conditioned medium plus 200 μl of BCE medium. Cells were harvested after 72 hr by trypsinization and counted using a hemocytometer. Recombinant bFGF was obtained from Promega, BRL/Life Technologies, or R&D Systems, and recombinant VEGF was obtained from R&D Systems. Neutralizing monoclonal antibodies (R&D Systems) were used at a final concentration of 1 μg/ml.

Measurement of anchorage-independent growth was performed by plating 1 × 10^5 cells on duplicate 60-mm gridded tissue culture dishes (Nunc) in DMEM plus 10% fetal bovine serum (FBS)and antibiotics and containing 0.7% agarose. Cells were grown for 2 weeks with the overlaying medium changed at 3- to 5-day intervals. Growth was evaluated by counting colonies in six 1-cm squares and calculating the means.

Tumorigenicity Analysis and Anti-VEGF Staining. Cells were harvested by trypsinization and washed three times in phosphate-buffered saline (PBS). Final washed cell pellets were resuspended at a density of 5 × 10^6 cells per 0.1 ml of PBS, and 0.1 ml was injected subcutaneously into either nude (*nu/nu*) or BNX (*bg/nu/xid*) mice. Growth was observed from 4 weeks to 20 weeks after tumor cell injection. The mice were observed for a minimum of 12 weeks, and where tumor growth was not evident they were sacrificed and the site of injection was analyzed, using a dissecting microscope to determine presence or absence of tumor cell growth. Subcutaneous tumors were excised from the mice and frozen in O.C.T. compound (Miles). Sections (8 mm) were prepared and stained with an anti-VEGF peptide antiserum or a control anti-carrier serum as described previously (27).

Growth Under Serum-Free Conditions and RNA Analysis. Cells were grown in 150-mm diameter tissue culture dishes to a density of 50% to 80% confluence, at which point the medium was removed and replaced with either fresh DMEM containing 10% FBS and antibiotics, or the cells were washed two times with DMEM with antibiotics but without FBS (serum-free medium). Thirty milliliters of serum-free medium was then added to each dish and the cells were grown for an additional 12–24 hr. During this time, the cultures grown in serum-free medium appeared to proliferate relatively little but did not show evidence of cell death. Total RNA was prepared by using Trizol reagent (BRL/Life Technologies). Total RNA was loaded at 10 μg per lane, electrophoresed on 1% agarose gels containing 2.2 M formaldehyde, and capillary blotted to Nytran plus membranes (Schleicher and Schuell). VEGF, VHL, and β-actin DNA probes were radiolabeled by using a random primer labeling kit (Ambion), and hybridizations were done in Hybrisol (Oncor) at 42°C at a probe concentration of 1 × 10^6 dpm/ml of hybridization solution.

Nuclear Run-on Assays. Preparation of nuclei and nuclear run-on assays were performed as described previously (28). Probes were generated by using the polymerase chain reaction (PCR) and the appropriate plasmid clones as templates. Probes were purified, and 2 μg of DNA was applied to GeneScreen*Plus* membranes (DuPont/NEN) using a slot-blot apparatus. The VEGF-specific probes included a 1040-bp PCR fragment from the promoter and, upstream of the transcription initiation site, a 1112-bp PCR fragment from the 5′ untranslated region and including the translation initiation codon and the peptide leader sequences, a 363-bp PCR fragment representing mature $VEGF_{121}$ sequence, and a 124-bp PCR fragment corresponding to exon 7 of the VEGF gene. A 600-bp PCR fragment from the β-actin gene, a 350-bp PCR fragment from the neomycin phosphotransferase gene, and pBluescript plasmid DNA (5 μg applied per slot) were used as controls.

RESULTS

We subcloned into a retroviral vector either the wild-type human VHL cDNA or a VHL restriction fragment that would encode a VHL protein with a frameshift mutation at amino acid 187, a region where several naturally occurring mutations (frameshift and amino acid substitutions) have been identified. Eleven renal carcinoma cell lines were transduced, including eight cell lines derived from clear cell tumors with defined VHL mutations (6, 10) and three cell lines derived from papillary renal tumors without VHL mutations (6). We were unable to obtain G418-resistant cells from three of the clear cell tumor-derived cell lines tested, suggesting that VHL overexpression may have suppressed growth of those cells. This characteristic of VHL overexpression in renal carcinoma cells was recently described (29). All of the cell lines that were successfully transduced with either VHL-expressing retrovirus were analyzed for exogenous VHL expression on the basis of the unique transcript size from the retroviral long terminal repeat (LTR) promoter. In addition, transductants were evaluated for changes in growth rates in culture, effects of VHL on anchorage-independent growth, and effects on xenograft formation in immunocompromised mice. While the VHL wild-type and frameshift constructs were equally expressed in transduced cells, the level of each was found to be 5- to 10-fold greater than the level of the endogenous transcript (data not shown; also see Fig. 4 below). Although VHL was overexpressed in these cells, their *in vitro* growth characteristics remained unchanged. In this report we focus on the cell lines UOK101, which has an exon 2 splice acceptor mutation, resulting in a two-exon form of VHL, encoding a 172-amino acid protein (6), and UOK 121, which is hypermethylated at the VHL locus and expresses no detectable VHL transcript (10). The UOK 101 cell line expressing either wild-type VHL (101 wt) or the VHL frameshift mutation (101 fs) showed

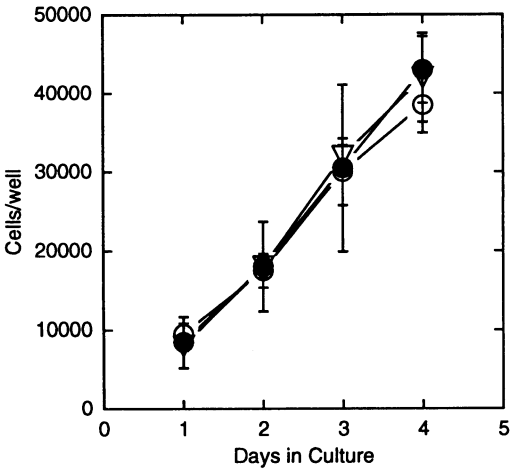

FIG. 1. *In vitro* proliferation rates are unaffected by expression of wild type VHL in renal carcinoma cells. UOK 101 cells expressing wild-type VHL (○) or a frameshift VHL mutation (●) show proliferation similar to that of the parent 101 cell line (▽). Cells were plated at a density of 1×10^4 cells per well in 24-well tissue culture dishes and trypsinized and counted using a hemocytometer at the indicated intervals after plating.

proliferation rates *in vitro* that were similar to those of the parental 101 cells (Fig. 1). Furthermore, UOK 101 and its transductants were equally capable of colony formation under anchorage-independent growth conditions (Table 1). Similar *in vitro* growth characteristics in cells expressing either wild-type VHL or the frameshift mutation were seen in all eight transduced cell lines examined.

VHL expression did not affect tumorigenicity in any of the three papillary tumor-derived cell lines tested or in KN41, a clear cell tumor-derived line. When UOK101 and its transductants were tested for tumorigenesis, the parent cell line did not form tumors in nude mice, UOK101 wt cells infrequently formed tumors, and UOK101 fs efficiently formed tumors (Table 1). Interestingly, expression of the VHL frameshift mutation appeared to have a dominant phenotype in UOK101 cells in the tumorigenesis assay. This observation is under further investigation.

Table 1. Soft agar colony formation and tumorigenicity of UOK 101 renal carcinoma and transductants expressing wild-type VHL cDNA or a frameshift mutation

Cell line	Colonies/cm²*	Tumor formation†	Tumor size,‡ cm³
101	14 ± 4.2	0/10	
101 wt	15.7 ± 4.3	2/15	0.108, 0.32
101 fs	17.2 ± 3.9	14/15	1.12, 2.02§

*Cells $(1 \times 10)^5$ were plated in 0.7% agarose on 60-mm tissue culture dishes with 2-mm grids. Six 1-cm² fields from two dishes were counted.

†Xenograft formation was evaluated after subcutaneous injection of 5×10^6 tumor cells into nude or BNX mice.

‡Tumor sizes were calculated by measuring the length, width, and height of the xenografts with calipers.

§The two tumors indicated resulted from 101 fs cells that were injected at the same time as the 101 wt cells that formed tumors. These tumors were evaluated 12 weeks after injection. Other mice with 101 fs xenografts were typically sacrificed before the tumors exceeded 0.5 cm³.

Because 101 fs cells clearly had an *in vivo* but not *in vitro* growth advantage relative to the 101 wt cells, we hypothesized that part of this difference might be due to differing expression of angiogenic factors by the two cell lines. Immunohistochemical staining for VEGF protein in the 101 fs and 101 wt xenografts showed that VEGF was highly expressed in the 101 fs tumor but expressed at much lower levels in the 101 wt tumor (Fig. 2). Therefore, the capacity of 101 fs cells to efficiently form tumors in nude mice may be related at least in part to VEGF expression.

To examine expression of angiogenic growth factors, conditioned medium was prepared from parental and transduced renal carcinoma cells and tested for mitogenic activity on BCE cells, which are dependent on exogenous growth factors for proliferation *in vitro*. Conditioned media from UOK 101, 101 wt, and 101 fs cells all stimulated BCE cell proliferation 5- to 8-fold over medium alone (Fig. 3). Neutralizing anti-VEGF monoclonal antibody inhibited the mitogenic activity of con-

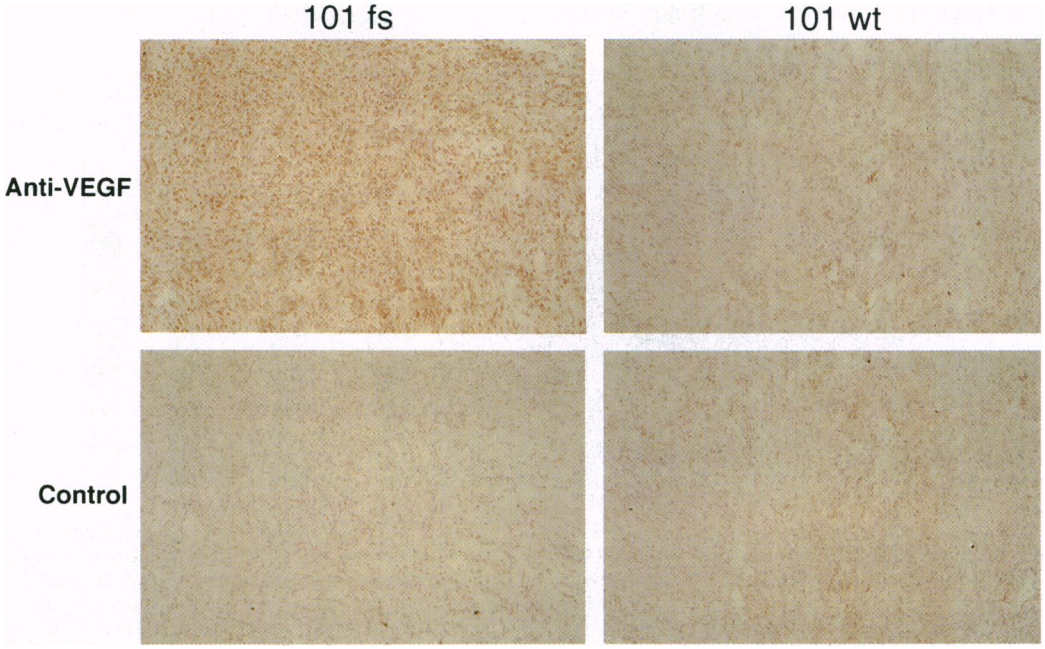

FIG. 2. VEGF is highly expressed in UOK 101 fs xenografts (*Left*) but not in 101 wt xenografts (*Right*). Cells (5×10^6) were injected subcutaneously into nude mice, and the mice were sacrificed after 12 weeks. Immunohistochemistry of the excised tumors with anti-VEGF serum or a control preimmune serum was performed as described (27). (×100.)

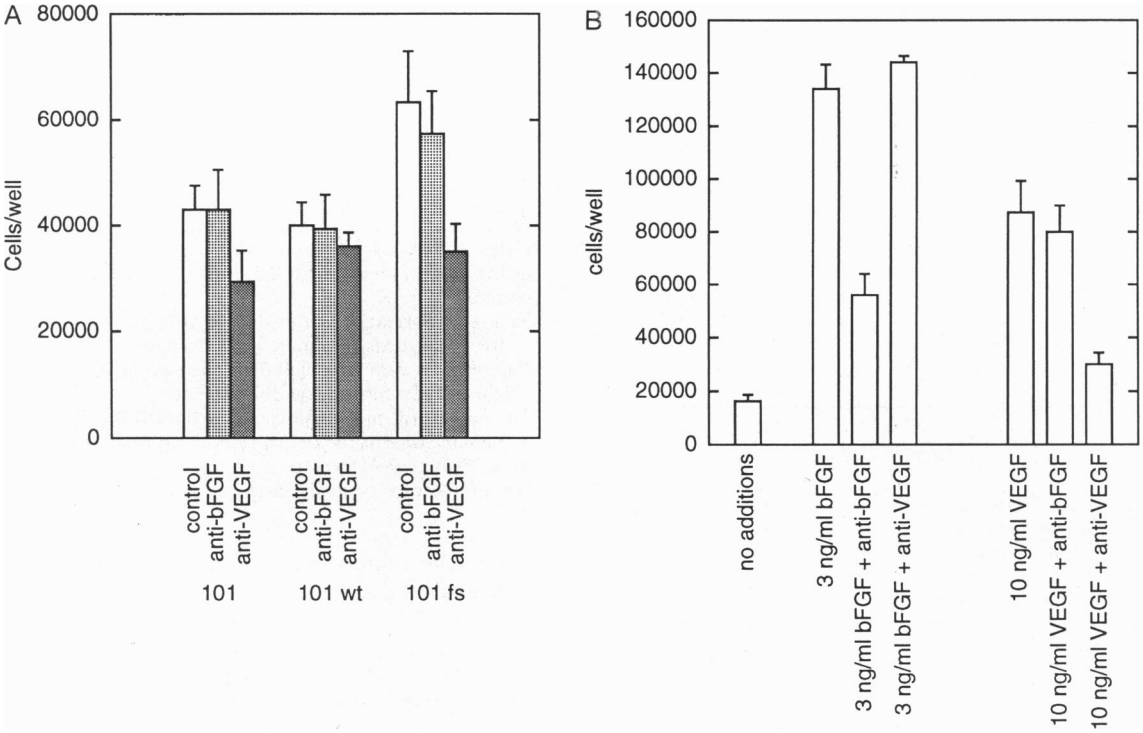

FIG. 3. Proliferation of BCE cells in response to conditioned medium from renal carcinoma cells (*A*) or purified recombinant bFGF or VEGF (*B*). BCE proliferation was tested as described in the text. Anti-bFGF or anti-VEGF monoclonal antibodies were used at a concentration of 1 μg/ml. Recombinant bFGF or VEGF were tested at the indicated concentrations with or without addition of anti-bFGF or anti-VEGF antibody at 1 μg/ml.

ditioned media from 101 cells or 101 fs cells by 32% and 45%, respectively, while anti-bFGF monoclonal antibody had no effect (Fig. 3). The mitogenic activity seen in 101 wt cell conditioned medium was not affected by either antibody tested (Fig. 3). Therefore, while VEGF is a component of the conditioned medium from 101 and 101 fs cells, VEGF may not be significantly expressed by the VHL-expressing 101 wt cells. Elevated mitogenic activity of 101 fs cells was reproducibly detected and could be due to increased expression of a common growth factor, induced expression of a novel growth factor, or diminished expression of an inhibitory factor. Similar BCE cell mitogenic activity and anti-VEGF inhibition were detected in conditioned media from UOK 121, 121 wt, and 121 fs cells (data not shown).

Steady-state VEGF mRNA expression was analyzed by Northern blotting. UOK 101 cells and 101 fs cells expressed similarly high levels of VEGF mRNA (Fig. 4). In contrast, cells expressing wild-type VHL had 5- to 10-fold lower VEGF mRNA levels (Fig. 4). VEGF mRNA expression patterns were similar for the UOK 121 cell line and its transductants. Thus, overexpression of wild-type VHL in renal carcinoma cells is

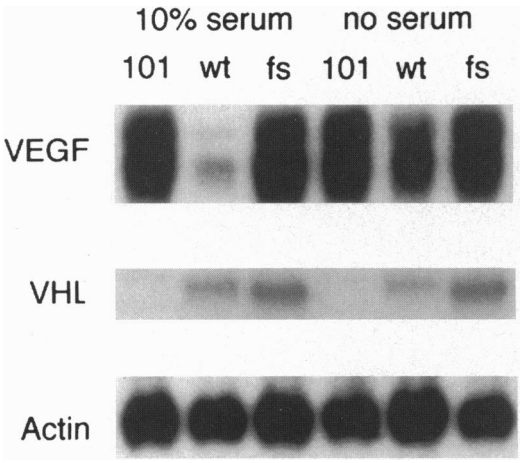

FIG. 4. Northern blot analysis of VEGF mRNA expression. RNA was isolated from cells grown in medium containing 10% fetal bovine serum or in serum-free medium for 18 hr and 10 μg of total RNA was loaded per lane. Northern blots were hybridized with the indicated radiolabled probes. The VEGF probe detects 4.0- and 3.7-kb transcripts; the VHL probe detects the 5.5-kb endogenous transcript and transcripts of 5.0 and 4.7 kb corresponding to the wild-type and frameshift VHL retroviral constructs, respectively.

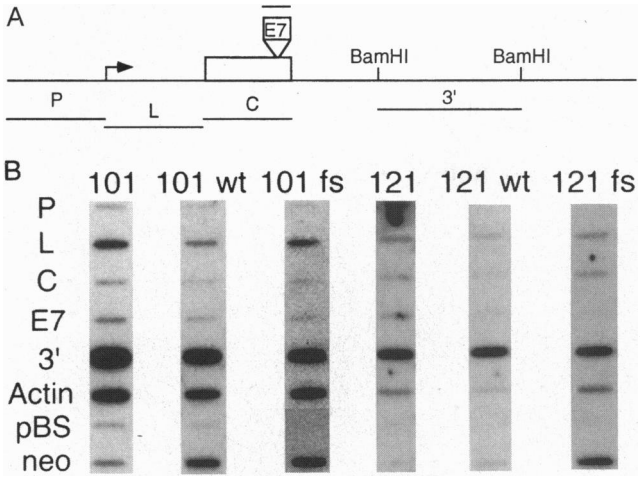

FIG. 5. VEGF expression is not regulated at the transcriptional level in renal carcinoma cells expressing wild-type VHL. (*A*) Diagram of the VEGF gene indicating the probes used for nuclear run-on assays, not drawn to scale. (*B*) Nuclear run-on assay using nuclei isolated from renal carcinoma cell lines UOK 101, 121, or their transductants. P, VEGF promoter probe; L, VEGF leader probe; C, VEGF coding sequences probe; E7, VEGF exon 7; 3′, VEGF 3′ untranslated region probe; actin, β-actin gene probe; pBS, pBluescript plasmid DNA; neo, neomycin phosphotransferase gene probe.

associated with a repression of VEGF mRNA expression. We tested whether VEGF mRNA levels could be induced in the wild-type VHL-expressing cells by altering growth conditions. When cells were cultured in the absence of serum, VEGF mRNA expression increased 4-fold in wild-type VHL-expressing cells, to levels similar to those seen in the parent cells and frameshift VHL-expressing cells which were not significantly affected by serum deprivation (Fig. 4). Exogenous VHL mRNA expression directed by the retroviral LTR promoter was unchanged when cells were grown in serum-free medium (Fig. 4), indicating that increased VEGF expression was not due to modulation of VHL expression.

VEGF mRNA and protein levels increase in cells subjected to hypoxia (30, 31). Hypoxia-induced VEGF expression is controlled at multiple levels, including initiation of transcription and stabilization of mRNA (32–35). The demonstration that VHL regulates elongin activity *in vitro* (12) suggests that wild-type VHL expression in renal carcinoma cells may regulate target gene expression through repression of transcription elongation. Nuclear run-on assays were performed to assess VEGF transcription in renal carcinoma cells. If expression of wild-type VHL led to increased pausing of RNA polymerase at defined sites along the VEGF template (as might be predicted by VHL regulation of the elongin complex), this would be detected with probes directed against the 5′ *versus* 3′ regions of the VEGF transcript (Fig. 5*A*). Nuclear run-on reactions from UOK 101 or 121 showed 3- to 4-fold greater hybridization to the VEGF leader probe than to the coding sequence probes (Fig. 5*B*), consistent with either an RNA polymerase pause site(s) in the VEGF leader or differential hybridization of the probes used. However, the nuclear run-on reactions showed that expression of wild-type VHL or the VHL frameshift mutation in these cells did not affect the level of VEGF expression or the pattern of hybridization across the length of the VEGF gene (Fig. 5*B*). Therefore, while VHL regulates VEGF mRNA levels, that regulation appears not to be at the level of either transcription initiation or elongation. In addition, the c-*myc* gene with its well-characterized RNA polymerase II pause sites at the 5′ end (36) was examined, and while pausing was detected at the appropriate sites, there was no influence of wild-type VHL on c-*myc* transcription (data not shown).

DISCUSSION

VHL was introduced into renal carcinoma cell lines by using a retroviral vector, and VHL overexpression did not affect *in vitro* proliferation rates or anchorage-independent growth of the cells tested. We could not introduce the VHL-expressing retrovirus into 3 of the 4 cell lines that were derived from clear cell renal carcinomas and that were known to form xenografts in immunocompromised mice, and therefore, the effects of VHL overexpression on tumorigenicity in those lines could not be directly assessed. One line, KN41, did overexpress VHL, but its growth in nude mice was unaffected (data not shown). Iliopoulos *et al.* (37) did show that VHL overexpression in the 786-O cell line resulted in decreased tumorigenic potential. None of the three papillary tumor-derived cell lines that were transduced with VHL showed altered tumorigenicity. The VHL frameshift construct showed no affect on cell growth *in vitro* but appeared to have a dominant tumorigenic function in UOK 101 cells, since 101 cells did not form tumors but 101 fs cells did form tumors very efficiently. UOK 101 cells have a single endogenous copy of *VHL* containing a deletion of the exon 2 splice acceptor site, resulting in loss of expression of exon 2 (6). Alternative splicing of exon 2 is normally seen in human VHL (6), and the resultant 172 amino acid VHL (VHL_{172}) protein would be co-expressed with the full-length 213-amino acid protein. Only VHL_{172} would be expressed in 101 cells. The elongin BC binding site is contained within VHL exon 3, and therefore is retained in VHL_{172} (12, 13), and VHL_{172} has been shown to interact with elongin BC *in vitro* and *in vivo* (D. Chen, W.M.L., and R.D.K., unpublished observations). If the 215-amino acid protein encoded by the VHL fs construct, which contains VHL exon 2 sequences, has a dominant function in UOK 101 cells, we would predict that the function would be as a result of expression of VHL exon 2 (amino acids 115–155) and potentially independent of VHL-elongin binding activity.

We have found that the product of the *VHL* tumor suppressor gene regulates VEGF mRNA expression at a post-transcriptional level. The disparity between transcription rates and steady-state mRNA levels leads us to conclude that mRNA half-life is different in cells expressing versus those not expressing wild-type VHL. Since VEGF mRNA expression is regulated post-transcriptionally through mRNA stability, we predict that the low VEGF mRNA steady-state levels seen in wild-type VHL-expressing cells may be due to reduced message stability. We measured VEGF mRNA expression after treatment with the transcription inhibitor actinomycin D, but we could not demonstrate that expression of wild-type VHL in UOK 101 or 121 cells resulted in a reduced VEGF mRNA half-life (data not shown). In a separate study Iliopoulos *et al.* (38) did show a 4-fold decrease in VEGF mRNA stability in VHL-expressing 786-O cells. Our results are not necessarily contradictory to those of Iliopoulos *et al.*, since there is evidence that in some cell types and for some transcripts, mRNA degradation requires ongoing RNA and protein synthesis (39–42). Therefore, in the present study actinomycin D treatment could itself confer stabilization of VEGF mRNA in cells expressing VHL due to the inhibition of synthesis of a specific factor(s) that regulates VEGF mRNA degradation. Alternatively, VHL may affect the nuclear fate of the VEGF transcript, which may not be observed by measuring cytoplasmic mRNA turnover. These data indicate that the VBC complex may have a cellular function that is independent of the elongin ABC complex and distinct from the RNA polymerase II elongation regulation activity conferred by elongin A. VHL in the VBC complex may regulate VEGF gene expression at the level of the fate of the transcript. Perhaps since elongin C has homology to the prokaryotic rho protein (43), the elongin C-containing complex may interact directly with RNA, functioning in elongation in the ABC complex, while in the VBC complex it helps target proteins to specific transcripts for the control of half-life. Interestingly, VHL appears to regulate the expression of a number of genes that are controlled by hypoxia (38). In our studies, the effect of hypoxia is mimicked by serum deprivation. This suggests that VHL may function to integrate signals, particularly those involved in tissue modeling, that control the normal ability to precisely regulate the determinants of tissue architecture such as angiogenesis.

The findings presented in this report suggest a mechanism for tumorigenesis in VHL patients. Cells with an inherited inactivating *VHL* mutation and *VHL* loss of heterozygosity might express high levels of VEGF which would recruit endothelial cells and promote tumor formation. The results presented also suggest novel therapeutic targets for cancer treatment. VHL is ubiquitously expressed in human tissues, yet the only common cancer in which VHL is inactivated is renal carcinoma. Many other types of tumors overexpress VEGF, but VHL activity, or the biochemical pathway of which it is a part in other cell types, remains uncharacterized. Chemotherapeutic modulators of VHL activity may complement inhibitors of angiogenesis which are currently being tested for use against some forms of cancer and other diseases and act through inhibition of proliferation and migration of endothelial cells and not on the tumors themselves.

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