

Expression of Hypoxia-inducible Factors in Human Renal Cancer: Relationship to Angiogenesis and to the von Hippel-Lindau Gene Mutation¹

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ABSTRACT

The von Hippel-Lindau tumor suppressor protein acts as the substrate recognition component of a ubiquitin E3 ligase that targets hypoxia-inducible factor (HIF)- α subunits for proteolysis. Stabilization of HIF- α subunits has been described in VHL-defective cell lines, leading to HIF activation and up-regulation of hypoxia-inducible mRNAs. Mutations of the von Hippel-Lindau tumor suppressor protein are found in most clear cell renal cell carcinomas (CC-RCCs) but not other renal tumors, raising a question about the importance of activation of the HIF pathway in CC-RCC development. To address this question, we have examined the expression of HIF- α subunits in 45 primary renal tumors and related this to tumor subtype, the presence of VHL mutations, and measures of angiogenesis. We show that HIF- α is up-regulated in the majority of CC-RCCs, and that the pattern of expression is biased toward the HIF-2 α isoform. Expression of HIF- α proteins was associated significantly with up-regulation of VEGF mRNA and protein and increased microvessel density. Up-regulation of HIF- α in CC-RCC was found to involve increased mRNA as well as protein expression, suggesting that both VHL-dependent and VHL-independent mechanisms are involved. These results suggest that activation of the HIF pathway is functionally important in CC-RCC development and might provide a new therapeutic target.

INTRODUCTION

Exposure to hypoxia results in the up-regulation of many genes (1). Well-characterized examples include genes involved in glucose metabolism (*GLUT-1* and *LDHA*) and proangiogenic factors such as VEGF³ (2, 3). The transcription factor HIF-1 is a key regulator of these target genes. HIF-1 is a heterodimer of two basic helix-loop-helix Per-ARNT-Sim proteins designated HIF-1 α and HIF-1 β /ARNT (4). In the presence of oxygen, HIF-1 α protein is destroyed rapidly by the proteasome so that steady-state levels are low (5). In hypoxia, this process is suppressed, allowing activation of the transcriptional response. HIF-2 α also dimerises with HIF-1 β /ARNT and is functionally and structurally similar to HIF-1 α (6, 7).

In cancer, the HIF system can be activated by microenvironmental hypoxia; this response appears to be amplified by a variety of oncogenic mechanisms (8). Clear insight into these processes has been provided by studies of the pVHL. pVHL functions as the recognition component of a multicomponent ubiquitin ligase that targets specific sequences in HIF- α subunits (9–12).

Individuals with VHL syndrome have a high lifetime risk of de-

veloping CC-RCC. In sporadic CC-RCCs (which account for 85% of renal cancers), the *VHL* gene is mutated in 50–60% of cases (13) and silenced by hypermethylation in an additional 15% (14). VHL mutations are not found in association with other subtypes of renal cancer such as papillary, chromophobe, and collecting duct carcinomas and the essentially benign oncocytomas (15).

These observations raise questions about the frequency of HIF- α up-regulation in CC-RCC versus other tumor types and its relation to the oncogenic process. We have investigated the expression of HIF- α in a series of human renal cancers and related the expression of HIF- α to parameters of angiogenesis. We show that up-regulation of HIF- α , particularly HIF-2 α , is a common and striking feature of CC-RCC, and that HIF- α up-regulation in CC-RCC is correlated with enhanced VEGF expression and increased MVD.

MATERIALS AND METHODS

Renal Sample Collection. Samples were collected from renal tumors immediately after radical nephrectomy and from macroscopically normal tissue in the same kidney distant from the tumor. Samples were snap frozen in liquid nitrogen. Sections from frozen tumor were stained to confirm the presence and subtype of renal cancer, and the frozen samples were representative of the tumor as a whole. Patient details and histological subtypes are shown in Table 1.

RNAse Protection. Total RNA was extracted from ~200 mg of tissue using TRI reagent (Sigma-Aldrich, Gillingham, Dorset, United Kingdom). RNAse protection assays were performed in duplicate on 10–30 μ g of total RNA as described (16). Labeled riboprobes for VEGF, HIF-1 α (accession no. U224311), and HIF-2 α (U81984) were prepared using [³²P]CTP. To attenuate the signal strength of the highly abundant loading control U6 small nuclear RNA (accession no. X01366), a riboprobe of significantly lower specific activity was prepared by addition of unlabeled CTP to the labeling reaction.

The protected fragment size for VEGF₁₂₁ was 517 nucleotides and for VEGF₁₆₅ and VEGF₁₈₉ was 439 nucleotides. Those for HIF-1 α , HIF-2 α , and the loading control were 253, 210, and 106 nucleotides, respectively. In each assay, a positive and negative (tRNA only) control and undigested riboprobes were analyzed. Intensity of signal, quantified on a PhosphorImager (Molecular Dynamics, Chesham, Buckinghamshire, United Kingdom) was calculated as the signal of interest:U6 signal ratio to account for minor loading differences. To allow comparison between samples, signal intensity for tissue samples was standardized against the positive control. The fold induction was determined by calculating the signal in the tumor:signal in paired normal tissue ratio, having previously adjusted for loading differences by comparison with the U6 control.

Protein Extraction and Immunoblot Analysis. Frozen tumor samples were homogenized in extraction buffer [8 M urea, 10% glycerol, 10 mM Tris-HCl (pH 6.8), 1% SDS, 5 mM DTT, 0.5 mM phenylmethylsulfonyl, 1 μ g/ml aprotinin, 10 μ g/ml pepstatin, and 10 μ g/ml leupeptin] using an IKA Ultra-Turrax T8 homogenizer (Janke & Kunkel and Co.) for 15 s at full speed. Extracts were quantified using the BCA protein assay (Pierce). Immunoblotting was performed as described (17).

Measurement of VEGF Protein in Tumor Samples. Tumor protein extracts were prepared and analyzed using the Quantikine ELISA kit (R&D Systems Ltd., Abingdon, Oxfordshire, United Kingdom) as described (18).

Detection of VHL Mutations. VHL genomic DNA sequence GenBank reference AF010238 was used as a reference sequence throughout.

Received 8/30/01; accepted 3/18/02.

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¹ Supported in part by a Royal College of Surgeons of England Research Fellowship (to K. J. T.) and by the Imperial Cancer Research Fund.

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³ The abbreviations used are: VEGF, vascular endothelial growth factor; HIF, hypoxia-inducible factor; VHL, von Hippel-Lindau; pVHL, VHL tumor suppressor protein; CC-RCC, clear cell renal cell carcinoma; MVD, microvessel density; K-W, Kruskal-Wallis; M-W, Mann-Whitney.

Table 1 Patient details and results summary for HIF-1 α /2 α protein expression, MVD, and expression of VEGF (protein and RNA)^a

Protein and RNA were extracted from snap-frozen samples of 45 human renal tumors of various histological types and from paired normal renal tissue. The expression of HIF- α proteins was determined by Western blotting, VEGF protein by ELISA, and VEGF RNA by RNase protection. MVD was assessed in histological tumor sections by staining vessels with antibody to CD34 and Chalkley vessel counting.

Tumor type (no. of cases)	Patient characteristics	HIF protein expression by Western blotting (All cases examined)		MVD Chalkley count Mean (SD)	VEGF ELISA (pg/ml/mg protein) Mean (SD)	VEGF mRNA Mean fold induction (SD)
		No. of cases expressing HIF-1 α	No. of cases expressing HIF-2 α			
Clear cell (34)	Female, 17 Male, 17 Mean age, 63 Stage 1-4	26	27	11.5 (3.3)	1139.8 (1395.4)	18.4 (25.4)
Papillary (5)	Female, 0 Male, 5 Mean age, 60 Stage 1-3A	1	0	4.4 (1.1)	33.2 (74.2)	0.8 (0.2)
Chromophobe (4)	Female, 2 Male, 2 Mean age, 72 Stage 1-2	2	0	8.3 (3)	130.3 (100.4)	2.6 (2.0)
Collecting duct (1)	Female Age, 48 Stage 3A	0	0	5 (n/a)	756 (n/a)	0.7 (n/a)
Oncocytoma (1)	Female Age, 75 Stage 1	0	0	10 (n/a)	0 (n/a)	Not done

^a MVD and VEGF ELISA were performed on 31 of 34 clear cell tumors and on all other cases. RNase protection was performed on 17 of 34 clear cell tumors, 4 of 5 papillary, 3 of 4 chromophobe, and on the single collecting duct tumor. The units for RNase protection are arbitrary. Fold induction describes the tumor:normal ratio.

PCR. PCR was carried out using Amplitaq Gold (PE Biosystems, Warrington, Cheshire, United Kingdom). Four sense/antisense primer pairs were used (exon 1a, exon 1b, exon 2, and exon 3). Details of primers and reaction conditions are available on request. For fluorescent single-strand conformation polymorphism, primers were labeled with HEX and 6-carboxyfluorescein; unlabelled primers were used for denaturing high-performance liquid chromatography.

Mutation Detection. Denaturing high-performance liquid chromatography and fluorescent single-strand conformation polymorphism analyses were as described (19), the latter using 49:1 acrylamide gels at 18°C. Mutations were confirmed by DNA sequencing using BigDye terminator chemistry (PE Biosystems, Warrington, Cheshire, United Kingdom; manufacturer's protocol), with the addition of halfBD sequencing reagent (Genpak Ltd., New Milton, Hampshire, United Kingdom).

Immunohistochemistry. Sections (4 μ m) were floated on to X-Tra slides (Surgipath Europe Ltd.). Slides were dewaxed and rehydrated before antigen retrieval by pressure cooking for 3 min in Tris/EDTA buffer (pH 9.0). After incubation with 1:100 monoclonal antibody QBEnd10 (Dako) in TBS for 1 h, slides were incubated with goat antimouse IgG (Dako) for 30 min and then with alkaline phosphatase anti-alkaline phosphatase (Dako) for 30 min. The last two steps were repeated twice with 10-min incubations. New Fuchsin Red substrate (Dako) was applied for 15 min, and slides were counterstained with hematoxylin before mounting (Aquamount). MVD was determined by Chalkley vessel counting as described (20). The mean of the three Chalkley counts was then calculated to give the MVD score.

Statistics. All tests were performed using the Stata package release 5 (Stata Corporation). Tests used were the K-W and M-W nonparametric tests, the *t* test, and ANOVA. In the analysis of the MVD data, scores were grouped into <7 and \geq 7.

RESULTS

Patient and Tumor Characteristics. Forty-five patients were studied, representing the expected frequencies of the main types of human renal cancer (Table 1). Tumor staging was performed in accordance with the 1997 joint International Union Against Cancer (UICC) and American Joint Commission on Cancer staging system (21). Tumors were classified according to the Heidelberg classification (15).

VHL Mutational Analysis. To characterize further our series of CC-RCCs, we analyzed a subset for VHL mutations. Mutations were

detected in 15 of 27 (56%) clear cell cases analyzed for mutation (data not shown). One tumor was from a patient with clinically verified VHL syndrome. No point mutations were found in this tumor, but our analysis would not have detected large deletions of the *VHL* gene, which account for a substantial proportion of VHL kindreds (22). For the same reason, or through hypermethylation, cases in which we sought but did not identify VHL mutations might have had functional silencing of VHL.

Expression of HIF-1 α and HIF-2 α Protein. HIF-1 α and HIF-2 α protein expression was determined by immunoblotting in every case (Fig. 1; Table 1). HIF-1 α was detected in the majority of CC-RCCs (26 of 34; 77%). HIF-2 α expression was detected in CC-RCCs alone (27 of 34 cases; 79%). In all 35 cases where paired normal tissue was available, expression of HIF-1 α /HIF-2 α in normal tissue was either absent or negligible in comparison to the corresponding tumor tissue. Of the CC-RCC cases, 22 of 34 (65%) expressed both factors, 4 of 34 (12%) expressed HIF-1 α alone, 5 of 34 (15%) expressed HIF-2 α alone, and 3 of 34 (9%) expressed neither factor. Of the cases known to be VHL defective, 11 of 16 (69%) expressed HIF-1 α , and 15 of 16 (94%) expressed HIF-2 α . In the cases where mutations were sought but not found (12 of 27), the frequency of HIF-1 α expression was similar (9 of 12; 75%), but fewer cases expressed HIF-2 α (6 of 12; 50%).

Expression of HIF-1 α and HIF-2 α mRNA. Expression of HIF-1 α and HIF-2 α mRNA was assessed by RNase protection in 25 cases (Table 2; Fig. 1). Expression of HIF-1 α mRNA in the tumor was greater than in paired normal tissue in 12 of 17 CC-RCCs and in 4 of 4 papillary tumors, 2 of 3 chromophobe tumors, and 1 of 1 collecting duct tumors. For HIF-1 α mRNA, the difference between tumor and paired normal tissue was not statistically significant [$P = 0.08$, paired *t* test; mean (SD) RNA expression in tumors = 0.26 (0.17); mean (SD) RNA expression in paired normal tissue = 0.16 (0.18)]. Expression of HIF-2 α mRNA was greater in tumor than in paired normal tissue in 17 of 17 CC-RCCs and in 1 of 3 chromophobe tumors but in no other tumors. For HIF-2 α mRNA, there was a significant difference between tumor and paired normal tissue [$P < 0.001$, paired *t* test; mean (SD) RNA expression in tumors = 0.58 (0.44); mean (SD) RNA expression in paired normal tissue = 0.23 (0.13)]. The up-regulation

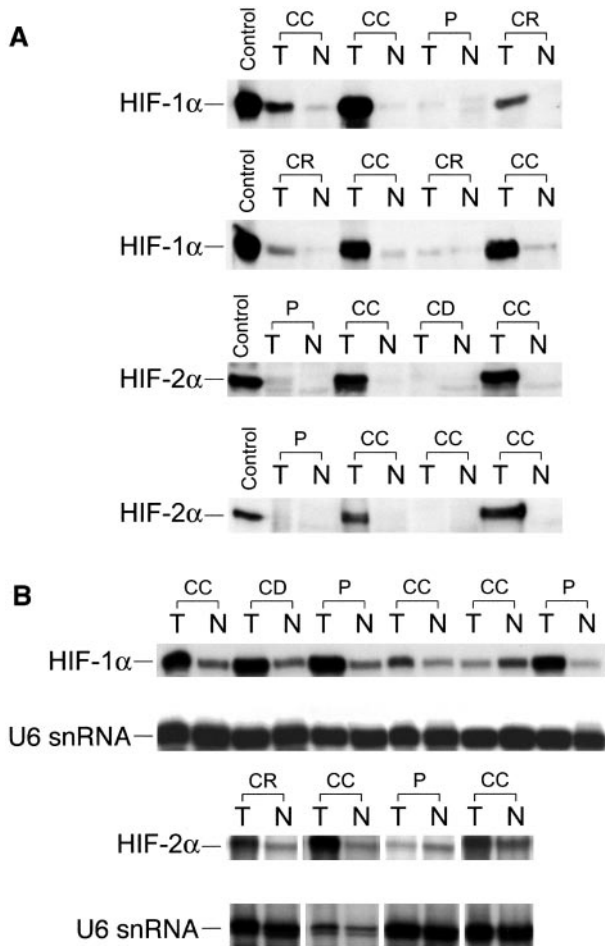


Fig. 1. A, expression of HIF-1 α /HIF-2 α protein in paired tumor/normal biopsies from human renal cancer. CC, clear cell; P, papillary; CR, chromophobe; CD, collecting duct; T, tumor; N, normal. B, RNase protection showing HIF-1 α /HIF-2 α mRNA expression in paired tumor and normal human renal cancers. U6 snRNA was used as a loading control. T, tumor; N, normal.

of HIF-1 α mRNA was not significantly greater in cases that expressed HIF-1 α protein compared with those that did not [$P = 0.7$, M-W; cases that expressed HIF-1 α protein, $n = 14$, mean RNA expression (SD) = 10.5 (14.8); cases that did not express HIF-1 α protein, $n = 11$, mean RNA expression (SD) = 5.5 (6.2)]. However, the relationship was significant for HIF-2 α with 5-fold greater up-regulation in tumors positive for HIF-2 α protein expression [$P < 0.001$, M-W; cases that expressed HIF-2 α protein, $n = 15$, mean RNA expression (SD) = 5.6 (5.9); cases that did not express HIF-2 α protein, $n = 10$, mean RNA expression (SD) = 1.0 (0.7)].

Relationship of Expression of HIF-1 α /2 α to Angiogenesis. All tumors were categorized into those that expressed neither HIF-1 α nor HIF-2 α (“neither” group), those that expressed one factor but not the

other (“either” group), and those that expressed both factors (“both” group). These three categories were used to assess the relationship between the expression of HIF- α proteins and parameters of angiogenesis (Fig. 2). We examined the relationship between expression of HIF-1 α proteins and tumor MVD. This relationship was examined in 31 of 34 CC-RCCs and in all non-clear cell tumors (Table 1; Fig. 2). CC-RCCs had a significantly higher MVD than non-clear cell tumors ($P < 0.001$, t test). In the series as a whole, there was a significant association between high MVD and expression of HIF- α proteins [$P < 0.001$, ANOVA; “neither” group, $n = 11$, mean (SD) = 6.4 (2.9); “either” group, $n = 12$, mean (SD) = 10.8 (3.6); “both” group, $n = 19$, mean (SD) = 11.9 (3.1)].

A role for mediators of angiogenesis in regulating this effect on MVD was assessed by determining the expression of the key angiogenic factor VEGF in the cases in which we had determined MVD (Table 1; Fig. 2). VEGF protein was significantly higher in CC-RCCs than in non-clear cell tumors ($P = 0.002$, M-W), and in the series as a whole there was a significant relationship between VEGF expression and MVD ($P = 0.01$, M-W). Furthermore, in the series as a whole, there was a significant relationship between expression of HIF- α proteins and increased VEGF [$P = 0.002$, K-W; “neither” group, $n = 11$, mean (SD) = 97 (226); “either” group, $n = 12$, mean (SD) = 910 (1040); “both” group, $n = 19$, mean (SD) = 1304 (1573)]. This relationship was also significant for CC-RCCs alone [$P = 0.05$, K-W; “neither” group, $n = 3$, mean (SD) = 8.3 (14); “either” group, $n = 9$, mean (SD) = 1170 (1087); “both” group, $n = 19$, mean (SD) = 1304 (1573)].

Because any effect of HIF- α protein expression on VEGF is likely to be mediated, at least in part, at the transcriptional level, VEGF mRNA expression was determined in 25 cases, representing the same subset in which we had assayed HIF- α mRNA (Table 1; Fig. 2). Expression of VEGF mRNA was significantly higher in CC-RCCs than in tumors of other types ($P = <0.001$, t test), although expression in the paired normal tissue did not differ between the two groups ($P = 0.81$, t test). Furthermore, in these 25 cases, fold up-regulation of VEGF mRNA in the tumor was significantly related to expression of HIF- α protein [$P = 0.001$ K-W; “neither” group, $n = 7$, mean (SD) = 1.5 (1.7); “either” group, $n = 7$, mean (SD) = 7 (5); “both” group, $n = 11$, mean (SD) = 24 (30)].

DISCUSSION

In this work, we have examined the expression of HIF- α proteins in human renal tumors. We show that HIF-1 α expression is up-regulated strongly in the majority of CC-RCCs in comparison with normal kidney, where expression was negligible or absent. This result contrasted with the findings in renal tumors of other types where strong HIF-1 α up-regulation was much less frequent. For HIF-2 α , the contrast between tumor types was even greater. HIF-2 α expression was commonly and strikingly up-regulated in CC-RCCs but was below the threshold of detection in renal tumors of other types.

Table 2 RNA was extracted from 25 human renal tumours of various histological types and from paired normal renal tissue. Expression of HIF-1 α and HIF-2 α mRNA was determined by RNase protection.

Tumor type (no. of cases assessed/total no. of cases)	HIF-1 α		HIF-2 α	
	No. of cases showing induction of RNA (i.e., tumor >normal)	Fold induction (tumor vs. normal) Mean (SD)	No. of cases showing induction of RNA (i.e., tumor >normal)	Fold induction (tumor vs. normal) Mean (SD)
Clear cell (17/34)	12/17	2.3 (2.5)	17/17	5.2 (5.7)
Papillary (4/5)	4/4	8.7 (10.0)	0/4	0.8 (0.3)
Chromophobe (3/4)	2/3	2.5 (2.2)	0/3	0.9 (0.3)
Collecting duct (1/1)	1/1	4.3 (n/a)	0/1	0.5 (n/a)
Oncocytoma (0/1)	Not done	Not done	Not done	Not done

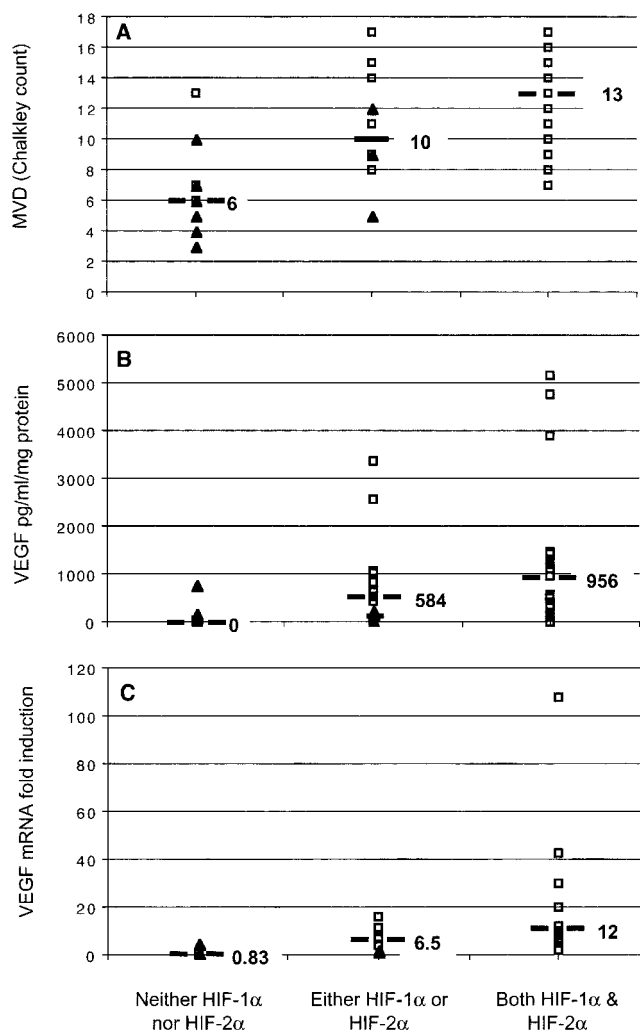


Fig. 2. Relationship of expression of HIF-1 α /HIF-2 α to expression of MVD (Chalkley count; A), VEGF protein expression (ELISA; B), and VEGF mRNA (fold induction, tumor versus normal; C). Clear cell tumors (\square) and non-clear cell tumors (\blacktriangle) are shown separately. Horizontal bars, median values.

Recent studies have established that pVHL acts as the substrate recognition component of a ubiquitin ligase that targets HIF- α proteins for ubiquitin-mediated proteolysis (9, 10, 12). Furthermore, HIF- α subunits are stabilized in VHL-defective cell lines, resulting in constitutive activation of the HIF transcriptional response (23). The *VHL* gene is mutated or silenced by hypermethylation in the majority of CC-RCCs (13, 14). This raises a question about the importance of HIF activation in CC-RCC development. We and others have previously assayed a range of tumor-associated pVHL mutations for their ability to restore normal regulation of HIF-1 α to pVHL-defective tissue culture cell lines (9, 12). Thus far, all pVHL mutations associated with CC-RCC have been found to be defective in these assays. To pursue this, we have now assayed HIF- α expression in primary renal tumors. Our finding that most CC-RCCs manifest striking up-regulation of HIF- α subunits extends the findings in tissue culture cell lines (24) and is consistent with a major role for HIF activation in CC-RCC oncogenesis.

Nevertheless, up-regulation of both HIF- α subunits was not always observed across the series of tumors. Because some CC-RCC lines (for instance CAKI-1) are wild type for VHL and show normal regulation of HIF- α (23), we considered whether tumors that did not express high levels of HIF- α proteins might form a subgroup of CC-RCCs that were wild type for VHL. However, even when the analysis was confined to cases in which a VHL mutation was proven,

up-regulation of both HIF- α subunits was not universal. Feasibly some pVHL mutations could differentially affect regulation of one or other HIF- α subunit, but we have not observed this with different mutated pVHLs in transfection assays (9). Most probably, HIF- α subunit expression is subject to other controls that determine the extent to which HIF-1 α or HIF-2 α accumulation occurs when pVHL-dependent proteolysis is disabled.

This possibility is supported strongly by the analysis of HIF-1 α and HIF-2 α mRNA levels, which showed striking up-regulation in many tumors when compared with normal kidney. This observation is supported by the results of another study that used reverse transcription-PCR (25). In VHL-defective cell lines, we found previously that restoration of wild-type pVHL did not self-regulate the mRNA of either HIF- α subunit, suggesting that up-regulation of HIF- α mRNAs in CC-RCC is not a direct consequence of the VHL defect itself (23). In contrast, others authors found that reintroduction of a wild-type *VHL* gene into a VHL-deficient RCC cell line resulted in up-regulation of HIF-1 α mRNA but self-regulation of HIF-2 α mRNA (24). Overall, the mechanisms regulating HIF- α mRNA levels are still largely unclear.

Also of interest was the pattern of HIF- α subunit expression. In CC-RCCs, expression of HIF- α subunits was biased toward HIF-2 α . When the analysis was confined to CC-RCCs with a proven VHL mutation, HIF-2 α up-regulation was particularly frequent, being observed in 94% of cases versus none of the non-CC-RCC tumors. This bias was seen both at the protein and mRNA level. A similar bias has been observed in analyses of VHL-defective cell lines (23, 24). Moreover, the same shift toward HIF-2 α mRNA expression has been observed in CNS hemangioblastomas, another VHL-associated tumor where VHL inactivation is common not only in familial but also in sporadic cases (26). In these tumors, HIF-2 α (but not HIF-1 α) mRNA was strikingly elevated in comparison with normal tissue and correlated with up-regulation of VEGF mRNA. Taken together, these findings suggest that the shift toward HIF-2 α subunit expression is somehow important in promoting VHL-associated oncogenesis. Conceivably, this may be attributable to differences in the range of target genes of HIF-2 α relative to HIF-1 α . Studies of HIF target genes have defined examples with putative antitumorigenic properties, suggesting that it is the overall balance of target gene activation that determines the outcome of HIF activation (27). Although the determinants of target gene specificity have not been identified, forced expression studies have indicated that HIF-2 α is relatively more active on the promoters of VEGF and the angiopoietin receptor, Tie-2, suggesting a particular role in the regulation of angiogenesis (6, 7).

Tumor growth and the development of metastases are dependent upon angiogenesis. Furthermore, measures of angiogenesis are prognostic in many tumor types. The role of HIF in the regulation of VEGF therefore provides a potential link between HIF activation and the promotion of tumor growth. In support of this, tumor xenografts from HIF-1 β -deficient hepatomas were found to be less well vascularized than their wild-type counterparts (28). Surprisingly, experimental studies of HIF- α -deficient cells have revealed conflicting data with one study of HIF-1 α $-/-$ embryonic stem cells demonstrating reduced vascularization and a different study of HIF-1 α $-/-$ fibroblasts reporting no effect on vascularization (29, 30). The link between HIF and tumor vascularization may depend on the cell background or on the mode and extent of HIF inactivation. Here we observed that up-regulation of HIF- α is associated with significantly higher vascular density in human renal tumors. The significant associations between the expression of HIF- α subunits and that of VEGF mRNA/protein suggest a functional role for HIF in enhancing expression of VEGF and ultimately angiogenesis. Two studies published during the course of this work support this possibility. Semiquantitative immunohistochemistry was used to show an association between

vascularization and HIF-1 α expression in human gliomas (31), and *in situ* hybridization was used to show concordance in the distribution of HIF-2 α and VEGF mRNA in a study limited to 13 RCCs (25).

HIF-1 α protein expression in renal tumors was investigated in another recent study (32). HIF-1 α protein was expressed in 24 of 32 (75%) of CC-RCCs, which is virtually identical to our findings. That study also indicated associations between VHL loss of function and induction of HIF-1 α and between induction of HIF-1 α and expression of target genes. However, Wiesener *et al.* (32) did not evaluate expression of HIF-2 α . The differences observed here between the two factors suggest that the HIF-2 α pathway is particularly significant in RCC. Furthermore, unlike Wiesener *et al.* (32), we found up-regulation of HIF- α mRNA in the majority of tumors. Although this up-regulation was not significant for HIF-1 α , it was highly significant for HIF-2 α .

Our findings demonstrate that up-regulation of the HIF system is common in CC-RCC, and they support the hypothesis that mutations of pVHL promote CC-RCC development by disabling regulation of HIF- α subunits. Concurrent up-regulation of HIF- α subunit mRNAs in CC-RCC suggests additional mechanisms contributing to HIF activation and is consistent with a functional role for HIF in CC-RCC development. Growth of experimental tumors derived from breast and colon cancer cell lines is suppressed by antagonism of HIF activation (33). Our work suggests that CC-RCC may be a particularly good target for this new approach to therapy.

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