Abstract

Renal cell carcinoma (RCC) is the most common primary cancer arising from the kidney in adults, with clear cell renal cell carcinoma (ccRCC) representing approximately 75% of all RCCs. Increased expression of the hypoxia-inducible factors-1α (HIF1α) and HIF2α has been suggested as a pivotal step in ccRCC carcinogenesis, but this has not been thoroughly tested. Here, we report that expression of a constitutively activated form of HIF2α (P405A, P530A, and N851A, named as HIF2αM3) in the proximal tubules of mice is not sufficient to promote ccRCC by itself, nor does it enhance HIF1αM3 oncogenesis when coexpressed with constitutively active HIF1αM3. Neoplastic transformation in kidneys was not detected at up to 33 months of age, nor was increased expression of Ki67 (MKI67), γH2AX (H2AFX), or CD70 observed. Furthermore, the genome-wide transcriptome of the transgenic kidneys does not resemble human ccRCC. We conclude that a constitutively active HIF2α is not sufficient to cause neoplastic transformation of proximal tubules, arguing against the idea that HIF2α activation is critical for ccRCC tumorigenesis. Cancer Res; 73(9): 1–10. ©2013 AACR.

Introduction

Renal cell carcinoma (RCC) is the most common primary cancer arising from the kidney in adults, with clear cell renal cell carcinoma (ccRCC) representing approximately 75% of all RCCs (1, 2). Loss of expression or mutation of the von Hippel-Lindau (VHL) tumor suppressor gene is found in hereditary and most sporadic ccRCCs (1, 3). This suggests an etiologic role for VHL gene loss in renal carcinogenesis. However, the exact pathway by which loss of VHL leads to ccRCC has not been elucidated. The best studied and likely the most important effect of VHL loss is the increased expression of the hypoxia-inducible factor-1α (HIF1α) and hypoxia-inducible factor-2α (HIF2α; ref. 4–6). Increased expression of these 2 transcription factors has been proposed as a key step in ccRCC carcinogenesis (4). HIF2α shares approximately 48% amino acid homology with HIF1α (7). Both HIF1α and HIF2α are regulated by prolyl hydroxylation at proline sites and by asparaginyl hydroxylation at an asparagine site under normoxic conditions (7), and each pair with HIF1β and binds to hypoxia responsive elements (HREs, 5′-RCGTG-3′, ref. 7). HIF2α protein levels are increased and HIF2α is transcriptionally activated in VHL−/− renal carcinomas (8). Furthermore, elimination of HIF2α in RCC cell lines is sufficient to suppress VHL−/− tumor growth in xenograft models (9, 10). Tumor suppression by pVHL can be overridden by HIF2α but not HIF1α in tumor xenografts of cultured 786-0 cells (9, 11, 12). These data collectively suggest that HIF2α may be more oncogenic than HIF1α for tumor xenograft growth, although other data indicate that HIF1α is more oncogenic (13–18).

At present, few murine models that exhibit the pertinent features of human ccRCC exist. Human tumorgraft models of ccRCC have significant limitations, including the immunodeficient state of the animals (19). We recently reported a transgenic model of cancer of the kidney (TRACK) model, which mimics early-stage human ccRCC through expression of a triple mutant (P402A, P564A, and N803A) human HIF1α construct (13). We have now used the same kidney proximal tubule specific type 1 γ-glutamyl transpeptidase (GGT or γGT) promoter (20, 21) to drive expression of a triple mutant (P405A, P530A, and N851A), constitutively active HIF2α protein specifically in the proximal tubule cells (PTC) to determine if this results in carcinogenesis. We show here that this triple mutant, HIF2α protein (HIF2αM3) is active, even under normoxic conditions. Transgenic mice that express this triple mutant, constitutively active HIF2α construct specifically in the kidney exhibit glycogen accumulation and hydropic degeneration, but no lipid deposition. We also do not observe renal cysts or disorganized proximal tubules resembling in situ carcinoma. We do not observe overexpression of molecular markers of cancer, for example, Ki67, γH2AX, and CD70, in the kidneys of HIF2αM3 transgenic positive (TG+) mice. Furthermore, we analyzed entire transcriptomes of cells from the HIF2αM3 TG+ kidney cortex by Next Generation Sequencing/RNA-seq. The kidney cortex transcriptome of HIF2αM3 TG+ mice does...
not closely resemble that of human ccRCC, consistent with the lack of tumorigenesis in these mice.

Materials and Methods

**Plasmid construction and generation of transgenic mice**

Mutated, constitutively active mouse HIF2α cDNA was created by site-directed mutagenesis (Invitrogen) of conserved proline residues (proline 405 and 530) and a conserved asparagine (asparagine 851) into alanine residues. The rat GGT promoter (−1930 to −246) was amplified by PCR from a plasmid (21). The GGT promoter, mutated HIF2α, and β-globin poly-A were cloned into pBlueScript and named γGT-HIF2α triple mutant (γ-HIF2αM3).

A linearized XhoI-Xbal fragment (vector sequence removed) was injected into pronuclei of one-cell embryos (C57BL/6 x C57BL/6) at the Well Cornell Medical College (WCAC) Mouse Genetics Core. Southern Blot analysis was then conducted (13). The γ-HIF2αM3 transgene was carried in the heterozygous state. The γ-HIF2αM3-1 and the γ-HIF2αM3-17 lines were mated with the TRACK mice to obtain γ-HIF1αM3; γ-HIF2αM3 double TG+ mice. Both γ-HIF1αM3 and γ-HIF2αM3 transgenes are carried in the heterozygous state in the double TG+ mice. All animal procedures were conducted following guidelines of the Well Cornell Research Animal Resource Center.

**Tissue dissection, processing, pathological review, and histology/staining**

Tissues were fixed, processed, sectioned, and hematoxylin and eosin stained (13). Slides were reviewed in a blinded manner by Dr. Shevchuk, an experienced clinical pathologist specializing in human kidney cancer, and independently by a veterinary pathologist, Dr. Linda Johnson, from the Laboratory of Comparative Pathology, WCAC. Immunohistochemistry was carried out as described (13). Antibodies used: HIF2α (100–122, Novus Biologicals); CA-IX (sc-25600, Santa Cruz); Glut-1 (ab14683, Abcam); Ki67 (M7249, Dako); and γH2AX (9718S, Cell Signaling). Periodic acid-Schiff (PAS) stain was conducted on paraffin-embedded and cryopreserved sections (13). Oil red O (ORO) staining was performed as described (13).

**Reverse transcriptase PCR, whole genome RNA sequencing, and data analysis**

Total RNA was extracted using mini-RNAeasy columns (Qiagen). Semiquantitative reverse transcriptase (RT)-PCR was then carried out (13). Total RNA from thin, outer slices of kidney cortex was used for whole genome sequencing. The complete transcriptomes of kidney cortex from 3 γ-HIF2αM3 18-month-old TG+ male mice and 3 age-matched wild-type (WT) C57BL/6 male mice were sequenced on an Illumina HiSeq2000 Sequencer. The reads were aligned to the mouse genome (NCBI37.55/MM9) using the Burrows-Wheeler Aligner (BWA; ref. 22) in GobyWeb software (23). Comparisons of gene expression changes between γ-HIF2αM3 TG+ and WT male mice were carried out using differential expression analysis with Goby in the GobyWeb. Benjamini and Hochberg FDR adjustment (q-value) for Student t test (Student t test-BH-FDR-q value) and Benjamini and Hochberg FDR adjustment (q value) for Fisher exact test (Fisher exact test-BH-FDR-q value) were used to determine statistical significance. The data have been in the GEO database (accession no. GSE45254).

**Statistical analysis**

Results are expressed as the mean ± SEM. Student t test was used to determine the statistical significance of the γH2AX+ and Ki67+ cell number differences between TG+ and WT kidneys.

**Results**

**Generation of transgenic mice that express mutated, constitutively active HIF2α**

To examine the role of HIF2α in ccRCC carcinogenesis, we constructed a GGT-HIF2α triple-mutant plasmid (γ-HIF2αM3, Fig. 1A). After confirmation of activity in a cultured normal kidney proximal tubule cell line, a linearized γ-HIF2αM3 Xbal-Xhol fragment was used to generate γ-HIF2αM3 transgenic mice. A total of 10 of 50 founder mice harbored the integrated target gene by Southern analysis (Fig. 1B, founders #17 to #21). Three lines (#21, #34, and #38) did not show germ line transmission. The other 7 lines were evaluated by RT-PCR, using a transgene-specific primer pair (primers 1 and 2, Fig. 1A) for transgene mRNA levels in the kidney, spleen, liver, heart, lung, intestine, skeletal muscle, and testis/ovary. The triple-mutant HIF2α (γ-HIF2αM3) was expressed only in the kidneys of TG+ lines #1, #17, #27, #31, and #48 (Fig. 1C, TG+, #1 as an example). The transgene was not expressed in the other organs analyzed, except for low expression in the testis (Fig. 1D). VHL, endogenous HIF1α, and endogenous HIF2α mRNA levels were not changed in the kidneys of TG+ mice compared with the kidneys of transgenic negative (TG−) mice (Fig. 1C, TG−). All 5 TG+ founder lines, γ-HIF2αM3-1, γ-HIF2αM3-17, γ-HIF2αM3-27, γ-HIF2αM3-31, and γ-HIF2αM3-48, developed normally and could pass the transgene to offspring following a Mendelian pattern of inheritance. Because the GGT promoter is not active until about 3 weeks after birth (20, 21), we did not expect and did not observe any gross developmental abnormalities in the kidneys.

**HIF2α and its target genes are upregulated in TG+ kidneys**

As described above, the HIF2α transgene mRNA is expressed in TG+ kidneys (Fig. 1C). We next examined the expression by semiquantitative RT-PCR of CA-IX (NP_647466) and Glut-1 (NP_035530), which are HIF target genes, and CD70 (TNFSF7), which is a marker of human ccRCC (24–27) but not a known HIF2α target gene. We detected increased mRNA levels of CA-IX and Glut-1, but not CD70 (Fig. 2A).

By immunohistochemistry, we confirmed increased HIF2α, CA-IX, and Glut1 protein staining in the abnormal proximal tubules (Fig. 2B, C, D, TG+), but not in the morphologically normal proximal tubules of the same γ-HIF2αM3 mice. As expected, weak or no detectable HIF2α, CA-IX, or Glut1 signals are observed in the proximal tubules of TG− and WT mice (Fig. 2B, C, D, TG−).
**γ-HIF2α M3-1 and γ-HIF2α M3-17 TG+ mice exhibit mild vacuolation**

Clear vacuoles were detected circumferentially around some, but not all, proximal tubule cell nuclei in the γ-HIF2α M3 mice. The γ-HIF2α M3-1 and γ-HIF2α M3-17 lines exhibited the strongest phenotype (Fig. 3A); the other lines had similar phenotypes, but displayed fewer proximal tubules containing clear vacuoles (data not shown). We identified vacuoles with a pale, eosinophilic to clear feathery cytoplasm without displacement of the nucleus, consistent with glycogen accumulation and hydropic degeneration (Fig. 3A, arrows) in one-year-old γ-HIF2α M3-1 and γ-HIF2α M3-17 mice. Increased numbers of vacuoles in some proximal tubules were observed in 2-year-old γ-HIF2α M3-1 and γ-HIF2α M3-17 mice (Fig. 3B, arrows). The histologically abnormal proximal tubule cells in TG+ mice were large, simple cuboidal epithelial cells (Fig. 3A and B), and these cells were surrounded by a tubular basement membrane, suggesting that these cells are under proper growth control. In ccRCC, the clear cytoplasm is caused by deposition of glycogen, phospholipids, and neutral lipids, particularly cholesterol esters (28). The cytoplasm of the abnormal cells in the HIF2α TG+ mice contained increased amounts of glycogen, as shown by PAS staining (Fig. 3C, arrows), but no increase in lipid as determined by ORO staining (Fig. 3D).

We examined 21 mice of the γ-HIF2α M3-1 line and 16 γ-HIF2α M3-17 TG+ mice and compared them with WT mice. The oldest examined was a 33-month-old TG+ male mouse. Six TG+ mice older than 24 months, 13 between the ages of 18 and 24 months, 9 between the ages of 12 and 18 months, and 9 younger than 12 months were analyzed. No renal cysts or disorganized proximal tubules resembling *in situ* carcinoma were identified in any of the 37 TG+ animals examined.

**Expression of markers of proliferation and DNA double strand breaks in the γ-HIF2α M3 TG+ mice**

Uncontrolled cell division/proliferation is one of the most prominent features of tumor cells. Genomic instability is another universal feature of tumor cells (29). An increased DNA mutation rate/genomic instability may cause neoplastic transformation (30). Ki67 is a marker for proliferation (31). The

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**Figure 1.** Generation of γ-HIF2α M3 transgenic mice. A, construction of the γ-HIF2α M3 plasmid. The fragment used to create the TG+ mice is shown. Three mutations (P405A, P530A, and N851A), dashed arrows, a new exon (shaded square) was included in the β-globin poly A. Primers 1 and 2 were used to amplify the transgene by RT-PCR. B, Southern blot of the TG+ and TG− founders. Only founder #17 to #21 mice are shown. Founders #17 and #21 are TG+, others shown are TG−. C, HIF2α transgene, endogenous VHL, HIF1α, and HIF2α RT-PCR in kidneys of the γ-HIF2α M3-1 line. The HIF2α transgene was only detected in TG+ mice. Endogenous VHL, HIF1α, and HIF2α mRNAs were expressed at similar levels in TG+ and TG− mice, β-Actin, loading control. D, HIF2α transgene RT-PCR in multiple organs of the γ-HIF2α M3-1 line mice. The HIF2α transgene was detected specifically in the kidneys and testes of TG+ mice from the γ-HIF2α M3-1 line. No expression of the γ-HIF2α M3 transgene was detected in any organ of the TG− mouse. β-Actin, loading control.
serine 139 phosphorylated form of H2A histone family, member X (γH2AX) is a widely used marker that indicates double strand breaks (DSB). We examined Ki67 and γH2AX protein levels in TG+ versus TG− kidneys (Fig. 4). We detected few Ki67+ and γH2AX+ cells in WT proximal tubules (1.5 or 1.9 TG− cells/field, respectively). In the γ-HIF2αM3 TG+ mice, we observed statistically significantly decreased numbers of Ki67+ and γH2AX+ cells in the morphologically abnormal proximal tubules. There were 0.1 (P = 0.01, compared with TG−) and 0.3 (P = 0.02, compared with TG−) Ki67+ cells/field in the γ-HIF2αM3-1 and γ-HIF2αM3-17 TG+ kidneys, respectively; there were 0.9 (P = 0.03, compared with TG−) and 0.8 (P = 0.005, compared with TG−) γH2AX+ cells/field in the γ-HIF2αM3-1 and γ-HIF2αM3-17 TG+ kidneys, respectively. Thus, the kidneys from TG+ mice exhibited a reduction in Ki67 and γH2AX staining relative to WT.

The transcriptome of γ-HIF2αM3 TG+ kidneys does not closely resemble the transcriptome of human ccRCC

We next examined gene expression using next generation sequencing of the whole transcriptome (RNA-seq). We cut a thin slice of kidney cortex, which contains the preponderance of proximal tubules, and extracted total RNA for whole transcriptome analysis. High levels of proximal tubule marker mRNAs, for example, GGT, indicate kidney cortex identity.

We identified upregulation of 1,253 and downregulation of 749 transcripts in γ-HIF2αM3 TG+ versus WT kidney cortexes from 18-month-old mice using less stringent conditions (fold change > 1.2, Student t test-BH-FDR-q value < 0.05, Fisher exact test-BH-FDR-q value < 0.05). We identified upregulation of 206 and downregulation of 86 transcripts in γ-HIF2αM3 TG+ versus WT mice when data were filtered with a more stringent condition (fold change > 1.5, Student t test-BH-FDR-q value < 0.01, Fisher exact-test-BH-FDR-q value < 0.01). Certain HIF2α target genes, such as CA-IX, show significant overexpression in the HIF2αM3 TG+ versus WT samples. However, other putative HIF2α target genes, such as Oct4, cyclin D1, and TGFα (32, 33), did not exhibit increased expression in the γ-HIF2αM3 TG+ versus WT kidneys. Cyclin D1 shows a fold change of 1.1 (Student t test-BH-FDR-q value = 0.49) and TGFα transcripts show a fold change of 1.24, (Student t test-BH-FDR-q value = 0.19).

To compare the transcriptome of the γ-HIF2αM3 TG+ kidneys to human ccRCC, we identified the 20 genes most highly overexpressed at the RNA level in human ccRCC from Oncomine (Compendia Bioscience), combining 5 different datasets of human ccRCC patient samples (34–37). We identified a total of 5 datasets of cancer versus normal analysis of the ccRCC cancer type (Table 1). The 5 datasets we used are in Table 1. One of the 20 genes highly expressed in human ccRCC, EGLN3, shows significant upregulation in our γ-HIF2αM3 TG+ kidneys (> 3 fold; ref. Table 1). However, the remaining 19 genes that are highly overexpressed in human ccRCC are not represented in the datasets we used.
ccRCC are either not changed (P < 0.05) or are not significantly upregulated (< 3 fold) in the γ-HIF2αM3 TG+ compared with WT kidneys (Table 1).

We also identified the top genes overexpressed at the RNA level in the γ-HIF2αM3 TG+ compared with WT kidneys, and compared the transcript levels of these genes with those in the combined Oncomine datasets used in Table 1. One of these genes, CD163 molecule-like 1, shows overexpression (fold change > 3) in the combined Oncomine datasets (Table 2). The other 19 genes that are highly overexpressed in the γ-HIF2αM3 TG+ relative to WT kidneys do not show over-expression in the combined Oncomine human ccRCC versus normal datasets (Table 2). We conclude from analysis of these data that expression of a mutant, constitutively active HIF2α protein in kidney proximal tubules does not result in a transcriptome that closely resembles that of human ccRCC.

Discussion

The γ-HIF2αM3 TG+ kidneys do not resemble human ccRCC histologically

Several researchers have suggested that overexpression of HIF2α is a key step in the development of ccRCC (4). However, we detected no obvious ccRCC phenotype in the kidneys of γ-HIF2αM3 TG+ mice. In contrast, in our γ-HIF1α-M3 (TRACK) transgenic mice, we identified 2 types of vacuolation: large round, discrete vacuoles, displacing the nucleus, that contained lipid accumulation (13); and vacuoles with a pale, eosinophilic to clear feathery cytoplasm without displacement of the nuclei, consistent with glycogen accumulation and hydropic degeneration (13). Although some PTCs of these γ-HIF2αM3 transgenic mice contain clear spaces around the nuclei, the vacuoles do not resemble the large, almost completely clear vacuoles found in the TRACK kidneys (13) and in human ccRCC (38). Furthermore, no renal cysts or disorganized proximal tubules resembling in situ carcinoma were identified in any of the 37 TG+ animals examined, including 6 between 24- and 33-months-old. These data suggest that overexpression of HIF2α alone in PTCs does not result in PTCs that resemble ccRCC histologically.

The γ-HIF2αM3 TG+ kidneys do not show neoplastic transformation and do not express cancer markers

That there is no ccRCC phenotype in these γ-HIF2αM3 TG+ kidneys is unexpected, given previous data in the literature using cultured human ccRCC cells (9–12, 32, 33, 39). HIF2α has previously been reported to be more oncogenic than HIF1α and to play an important role in ccRCC carcinogenesis (4, 40, 41). However, we detected no renal cysts or neoplasia in the kidneys of γ-HIF2αM3 TG+ mice. In contrast, we observed multiple renal cysts and disorganized proximal tubules resembling in situ carcinoma in the γ-HIF1α-M3 TG+ TRACK mice (13). Uncontrolled cell division/proliferation and genomic instability are two of the most prominent features of tumor cells. By staining, using Ki67 or H2AX antibodies, we previously showed elevated expression of both Ki67 and γH2AX in γ-HIF1α-M3 TG+ mice (13). However, we did not detect higher levels of Ki67 or γH2AX in the γ-HIF2αM3 TG+ kidneys (Fig. 4). In fact, the levels were lower than in WT, suggesting that constitutively active HIF2α reduces cell proliferation and genomic instability. The CD70 protein level is higher in the majority of human ccRCC and CD70 is a marker and therapeutic target in human ccRCC (24). CD70 mRNA levels are several fold higher in older TRACK versus WT mice (13). However, we did not observe any CD70 mRNA expression in 18-month-old γ-HIF2αM3 TG+ kidneys by RT-PCR (Fig. 2), again indicating a lack of neoplastic transformation.

Using the methods described in the supplementary data, we calculated and compared the relative levels of the HIF1αM3 and HIF2αM3 transcripts in the HIF1αM3 TG+ (TRACK) mice.

Figure 4. Decreased expression of γH2AX and Ki67 in the γ-HIF2αM3 kidneys. The Ki67+ and γH2AX+ cells in 10 random fields of sections of 3 different TG+ and 3 WT kidneys were counted. All TG+ mice show a statistically significant decrease in Ki67+ and γH2AX+ cells. *, P < 0.05; **, P < 0.01.
Table 1. Twenty genes showing the highest mRNA expression in human ccRCC compared with normal human kidney, from the Oncomine database. These transcripts are compared to the transcripts in the γ-HIF2αM3 transgenic kidney cortex.

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<th>Oncomine median rank</th>
<th>Oncomine median-fold change</th>
<th>Gene</th>
<th>Gene description</th>
<th>Fold-change γ-HIF2αM3/WT</th>
<th>Student t-test BH-FDR q-value</th>
<th>Fisher exact-R γ-HIF2αM3/WT-BH-FDR q-value</th>
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NOTE: The list of the top 20 genes overexpressed at the RNA level in human ccRCC was compiled by Oncomine by combining 5 different datasets of human ccRCC patient samples, totaling 175 patients. These 5 datasets are (i) hereditary ccRCC versus normal (32), (ii) nonhereditary ccRCC versus normal (32), (iii) ccRCC versus normal (33), (iv) ccRCC versus normal (34), and (v) ccRCC versus normal (35). The fold changes in mRNA levels of these genes in γ-HIF2αM3 TG+ mice versus WT kidneys are listed.

aOne of these genes (EGLN3) shows statistically significant (P < 0.05) overexpression (fold change > 3) in γ-HIF2αM3 TG+ versus WT mice.
### Table 2. Top 20 genes overexpressed at the mRNA level in the γ-HIF2α-M3 TG+ versus WT kidneys by RNAseq

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NOTE: The list of the top 20 genes overexpressed at the mRNA level in the γ-HIF2α-M3 TG+ versus WT kidneys was compiled from the RNAseq results. The median fold change in mRNA levels of these genes in human ccRCC was compiled by Oncomine by combining 5 different sets of human ccRCC patient samples, totaling 175 patients (see Table 1). Genes that have no measurements from all 5 datasets were excluded from this list.

<sup>a</sup>One of these genes (Cd163l1) shows overexpression (fold change > 3) in the Oncomine datasets.
and HIF2αM3 TG+ mice, respectively. The HIF2αM3 mRNA levels in the HIF2αM3 TG+ mice are about 26% of the HIF1αM3 mRNA levels in the highest expressing TRACK line, and about the same as the HIF1αM3 mRNA levels in other TRACK transgenic lines (Supplementary Table S2). The HIF2αM3 protein is also overexpressed in the HIF2αM3 kidneys (Fig. 2B). Thus, the difference we observe in tumorigenic phenotype is likely a result of the specific HIF protein expressed and not the level of transgene expression.

The transcriptome of γ-HIF2αM3 TG+ kidneys does not closely resemble the transcriptome of human ccRCC

Furthermore, we compared the changes in gene expression in the γ-HIF2αM3 TG+ kidneys with human ccRCC. Only one of the top 20 genes overexpressed in 5 different sets of human ccRCC patient samples showed statistically significant overexpression in the γ-HIF2αM3 TG+ kidneys (Table 1). In fact, some of these genes showed a statistically significant reduction in expression, for example, caveolin 1 showed a fold change of 0.79 (Student t test-BH-FDR q-value = 0.04) when we compared the γ-HIF2αM3 TG+ with WT kidneys. Conversely, only one of the top 20 genes overexpressed at the RNA level in the γ-HIF2αM3 TG+ kidneys showed overexpression in the combined human ccRCC Oncomine datasets (Table 2). These data further indicate that expression of mutated, constitutively active HIF2α in kidney PTCs does not lead to a phenotype resembling human ccRCC, though some HIF2α target genes, such as CA-IX, show increased expression in the PTCs of kidneys of γ-HIF2αM3 TG+ mice (Fig. 2). We did not detect high levels of the putative HIF2α targets TGFα, cyclin D1, and Oct4 (42, 43) in the HIF2αM3 TG+ mice. This might be because different types of cells were used in those assays. For example, Oct4 was identified as a HIF2α target in embryonic stem cells (42), whereas TGFα and cyclin D1 were identified as HIF2α targets in cultured ccRCC cancer cells (43).

Expression of constitutively active HIF2α does not cause neoplastic transformation of proximal tubule cells

Our data suggest that expression of a mutated, constitutively active HIF2α in proximal tubule cells, the cells from which ccRCC originates (44, 45), does not cause neoplastic transformation. This conclusion is also supported by a recent publication reporting that overexpression of a similarly mutated (P405A, P530G, and N851A), constitutively active HIF2α in murine distal tubule cells is insufficient for renal carcinogenesis (16). However, it is possible that HIF2α may cooperate with HIF1α to drive renal cell carcinogenesis, because an increase in HIF2α expression is seen after an increase in HIF1α expression in early kidney lesions in patients with VHL disease (46). We crossed our TRACK and γ-HIF2αM3 TG+ mice to determine whether constitutive activation of both HIF1α and HIF2α results in more rapid neoplastic transformation of normal PTCs. From pathologic examination of 8 γ-HIF2αM3; γ-HIF2αM3 TG+ mice up to 11 months old, we conclude that constitutively active HIF2α does not result in more rapid neoplastic transformation in the presence of constitutively active HIF1α in the kidney. However, we can not rule out the possibility that this lack of tumorigenicity of HIF2αM3 is caused by potential biologic differences between human and mouse kidney PTCs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: L. Fu, M.M. Shevchuk, D.M. Nanus, L.J. Gudas
Development of methodology: L. Fu, L.J. Gudas
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): L. Fu, G. Wang, M.M. Shevchuk, L.J. Gudas
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): L. Fu, G. Wang, M.M. Shevchuk, D.M. Nanus, L.J. Gudas
Writing, review, and/or revision of the manuscript: L. Fu, M.M. Shevchuk, D.M. Nanus, L.J. Gudas
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): L.J. Gudas
Study supervision: L.J. Gudas

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References


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