The Ets Transcription Factor *ELF5* Functions as a Tumor Suppressor in the Kidney

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Renal cell carcinoma is an important clinical disease with poorly understood etiology. *ELF5* is an epithelial-specific member of the Ets family of transcription factors, characterized by the 80 amino acid Ets domain that binds the purine-rich GGAA/T Ets motif found in the promoter regions of a variety of genes. Since ELF5 is highly expressed in kidney and has been postulated to function as a tumor suppressor, at least in the context of the breast, we investigated its role in kidney cancer. In renal cell carcinoma *ELF5* expression was consistently decreased in tumor samples versus normal. *ELF5* mRNA was decreased in 94% of lesions tested and *ELF5* protein was undetectable in 40/40 kidney-derived carcinomas. Re-expression of the *ELF5* gene in 786-O renal carcinoma cells suppressed their tumorigenic capacity *in vitro* and *in vivo*. This work is the first to suggest that *ELF5* has tumor suppressor activity in the kidney.

∎ **Keywords:** *ELF5*, Ets transcription factor, renal carcinoma

The Ets transcription factors are a large family of proteins implicated in the control of cellular proliferation and tumorigenesis (reviewed in Seth & Watson, 2005). The Ets genes are characterized by a region of conserved sequence, called the Ets domain. This 80 amino acid region forms the winged helix-turn-helix (wHTH) DNA binding domain that recognises a core GGAA/T sequence in the promoters of target genes (Donaldson et al., 1994). Most of the Ets genes are expressed in hemopoietic cells and are important in subtypes of leukemia and lymphoma. The isolation of an epithelial-specific subset of Ets transcription factors, comprising *ELF3*, *ELF5*, *ESE-3* and the prostate-specific *PSE/PDEF*, opened a new avenue in Ets biology and considering the prevalence of epithelial-derived cancer in the human population, these genes may be of clinical significance.

The epithelial-specific Ets transcription factor *ELF5* was first described by this laboratory (Zhou et al., 1998). We postulate that *ELF5* may function as tumor suppressor based on its lack of expression in a large variety of carcinoma cell lines (Oettgen et al., 1999; Zhou et al., 1998) and primary breast cancers (Ma et al., 2003), and on its

chromosomal localisation to 11p13, a region that frequently undergoes LOH and hypermethylation in carcinomas of the breast, prostate, bladder and lung (Gudmundsson et al., 1995; Karnik et al., 1998; Ludwig et al., 1991; Shipman et al., 1993; Winqvist et al., 1995). *ELF5* is expressed strongly in mammary epithelium and in other epithelium-rich organs such as the kidney (Lapinskas et al., 2004; Oettgen et al., 1999; Zhou et al., 1998). We reported that the loss of one functional allele of the *ELF5* gene in the mouse results in developmental arrest of the mammary gland during pregnancy and established that *ELF5* is required for differentiation of mammary epithelial cells into milk-producing units (Zhou et al., 2005).

Relatively little is known about the signal transduction pathways in which Elf5 participates. In the mammary

gland *ELF5* has been placed downstream in the prolactin/Jak/Stat signaling pathway (Harris et al., 2006; Zhou et al., 2005) and in embryonic mouse lung is downstream of fibroblast growth factor (FGF) signaling, acting via FGFR2b and the PI3-Kinase/Akt pathway (Metzger et al., 2007). *ELF5* is also induced during keratinocyte differentiation (Oettgen et al., 1999) and may act in a signal transduction pathway involving keratinocyte-restricted factor (KRF) (Tummala & Sinha, 2006). Finally, *ELF5* was shown to induce expression of the *parotid gland PSP* promoter and the *prostate-specific PSA* promoter (Oettgen et al., 1999) and our studies have shown that Elf5 is also required for expression of a panel of genes encoding milk proteins (Rogers et al., 2010; Zhou et al., 2005).

Renal cell carcinoma is an increasingly important clinical disease. Apparent incidence has increased due to improvements in detection, but treatment has not made equivalent progress and the etiology of the disease is not well understood (Rathmell & Godley, 2010). Metastatic renal cell carcinoma is refractory to chemotherapy, hormonal therapy and radiation therapy (reviewed in Liou et al., 2004). Increased risk of kidney cancer is associated with specific diseases such as kidney infection and diabetes type I and II (Schlehofer et al., 1996) and there is an unidentified genetic component, with an increased relative risk of the cancer if a first-degree relative has also been diagnosed with kidney cancer (Schlehofer et al., 1996) but despite this knowledge, the molecular mechanisms involved are poorly understood.

The majority of renal cell carcinomas are sporadic in origin but a small percentage of cases arise due to a predisposition associated with a heritable syndrome. Seven hereditary renal cell cancer syndromes have been recognized and the genes for five of these identified: von Hippel–Lindau (*VHL*); the *MET* proto-oncogene; fumarate hydratase (*FH*); Birt–Hogg–Dubé (*BHD*); and hyperparathyroidism 2 (*HRPT2*) (reviewed in Pavlovich and Schmidt, 2004). The *VHL* gene, the first gene identified for hereditary renal cell carcinoma and defective in 75% of sporadic renal cancers, was also discovered along with two other tumor suppressor genes, a Ras effector known as RAS-association domain family 1 (*RASSF1A*) and the DNA repair gene, *OGG1* in studies examining allele loss at chromosome 3p, the most frequent genetic alteration in renal cell carcinoma (Audebert et al., 2000; Clifford et al., 1998; Gnarra et al., 1995; Morrissey et al., 2001). The diverse nature and functions of the genes so far identified in renal cell carcinoma implies that various biological pathways and mechanisms are involved. Other genes such as *TRC8* (translocation in renal cancer from chromosome 8), *JAB1* (Jun activation domain binding protein 1) and *HIF-1a* (Hypoxia-inducible factor-1alpha) have been implicated due to their participation in a cellular pathway involving *VHL* (Gemmill et al., 2002; Tanimoto et al., 2000).

In this study we tested the hypothesis that *ELF5* behaves as a tumor suppressor in cancer of the kidney. We clearly demonstrate that in renal cell carcinoma, *ELF5* expression was consistently decreased and that re-expression of the *ELF5* gene in a renal carcinoma cell line suppressed their tumorigenic capacity. Thus, *ELF5* may function as a tumor suppressor in the kidney.

Method

Quantitative RT-PCR Analysis of Renal Carcinomas

Eighteen renal cell carcinoma cDNAs were obtained from the Ludwig Institute for Cancer Research Oncology Unit (Melbourne). All samples were obtained according to appropriate human ethical guidelines. Normal reference kidney RNA was obtained from Stratagene. QRT-PCR was performed on the LightCycler (Roche) using FastStart DNA Master SYBR Green I (Roche). *ELF5* transcript was detected using oligonucleotides ELF5-18 (5′- TGC-CTTTGAGCATCAGACAG-3′) and ELF5-19 (5′-AGTATCATCTTGTTCGG-AGG-3′), in 3 mM MgCl$_2$, with 10 sec denaturation at 95ºC, 5 sec annealing at 55ºC and 11 sec extension at 72ºC. *Cytokeratin 8* was used as a marker for simple epithelium. Oligonucleotide sequences were CK8F (5′- CTGGAGTCTGCCTGG-AAGG-3′) and CK8R (5′-CCTCGTACTGTGCCTTGACC-3′). Amplification was performed as for *ELF5* but with 5 sec annealing at 59ºC and 8 sec extension at 72ºC.

Protein Expression of ELF5 in Cancer Sections

Renal cancer biopsy slides containing punch biopsies 2 mm in diameter and sectioned at 4 µm were obtained from SuperBioChips Laboratories (Seoul, South Korea). Anti-ELF5 immunohistochemistry was performed on this formalin-fixed, paraffin-embedded tissue using a polyclonal anti-ELF5 antibody along with the TSA Indirect kit (NEN Life Sciences) essentially as described (Lapinskas et al., 2004).

Production of 786-O Renal Carcinoma Cell Lines Expressing ELF5

Full length *ELF5* cDNA was cloned in the *pEF-BOS* expression vector (Mizushima and Nagata, 1990). 786-O cells were maintained in RPMI 1640 medium with 10 % (v/v) FCS. 786-O cells were co-transfected (FuGene 6) with *ELF5-pEF-BOS* and a puromycin-resistance plasmid (Ramirez-Solis et al., 1995). Clonal lines were selected with 3 µg/ml puromycin (ICN Biochemicals). After selection, puromycin was removed for all subsequent procedures. Expression of *ELF5* cDNA was confirmed by RT-PCR using primer pair *ELF5*-18 and *ELF5*-19.

Growth of Transfected Cells Under Anchorage-Independent Conditions

Two ml of 0.7 % (w/v) agarose in culture medium was plated in 6 well plates (BD Labware). 1×10^4 cells were resuspended in 1 ml of 0.35 % (w/v) agarose in culture

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medium and layered upon the 0.7 % (w/v) bottom agarose. Wells were overlaid with culture medium and incubated at 37°C/5% CO_2 for 21 days. Each of 3 clones was plated in triplicate and the assay repeated three times independently. For scoring, three fields of each well were counted as small colonies (cells that had not divided more than twice) or large colonies (cells that had divided more than twice).

Tumorigenic Capacity of Transfected Cells *in vivo*

The tumor-forming capacity of *ELF5*-transfected renal carcinoma cell clones compared with mock-transfected cells was tested by subcutaneous cell inoculation into immuno-compromised SCID mice. 1×10^6 786-O derived cells were resuspended in 100 μl Matrigel (BD BioSciences) and injected subcutaneously under the hind leg of 5- to 6-week-old female SCID mice (Animal Research Council, WA, Australia). The tumor size (mm^3) was evaluated by measurement of three perpendicular diameters with microcalipers and using the formula $4/3\ L \times W \times H$, where L is the longest diameter, W is the diameter perpendicular to L and H is the height of the tumor. When tumors reached an L diameter of 10 mm, or at 21 days post-inoculation, the mice were culled and regions of the tumor excised and examined by H&E staining.

Results

ELF5 is Down-Regulated in Renal Cell Carcinoma

Relatively high levels of *ELF5* mRNA (Zhou et al., 1998) and protein (Lapinskas et al., 2004) are observed in the kidney so we reasoned that if *ELF5* was a tumor suppressor, its loss may be involved in renal cell carcinoma. Also, loss of *ELF5* transcript had been observed in kidney-derived carcinoma cell lines (Zhou et al., 1998). Since the expression of *ELF5* mRNA in renal cell cancer had not been previously studied we quantified *ELF5* expression levels in 18 renal cell cancers compared to normal kidney reference RNA by QRT-PCR (Figure 1). All but one sample showed a reduction in *ELF5* expression.

To determine if the loss of *ELF5* mRNA correlated with a loss of *ELF5* protein, we examined protein expression of *ELF5* in kidney carcinoma samples by immunohistochemistry. We used a polyclonal anti-ELF5 antibody that we had previously characterized for specificity (Lapinskas et al., 2004). The slides contained 9 normal samples matched to 9 of the cancer samples on the same slide (i.e., tumor and normal kidney tissue from the same person). In all cases, the normal tissue stained with the anti-*ELF5* antibody and the tumor samples did not. *ELF5* protein was not detectable in 40 kidney-derived carcinomas whereas it was detected in the 9 normal kidney tissue samples
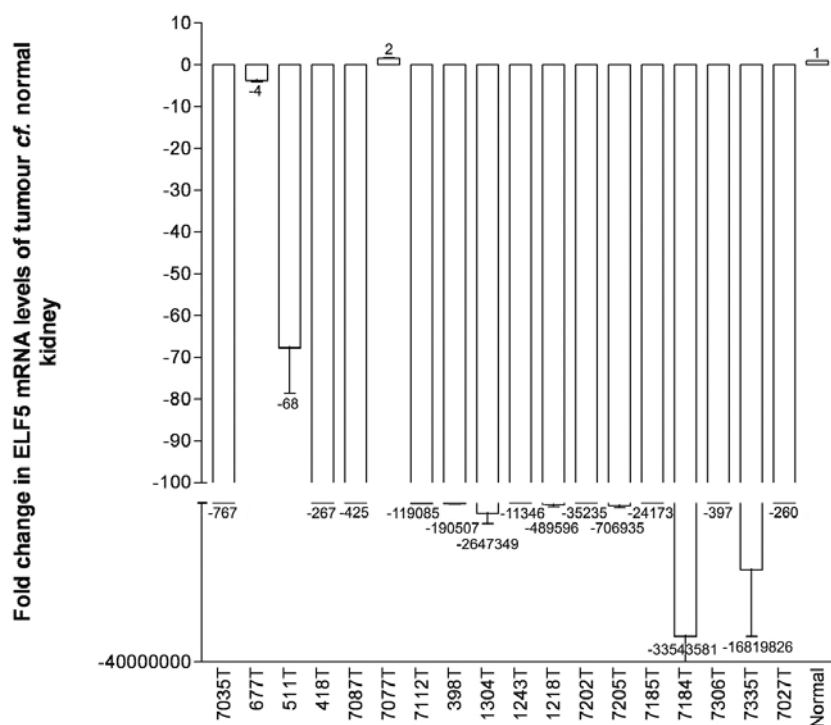

FIGURE 1

ELF5 transcript levels are reduced in renal cell carcinoma.

Note: In the kidney all samples were compared with a reference normal sample as matched normal cDNA was not available. The amount of *ELF5* in each sample was normalized to the amount of *cytokeratin 8*, which represents the quantity of epithelium present (*n* = 3). Data is presented as fold-change +/- SEM.
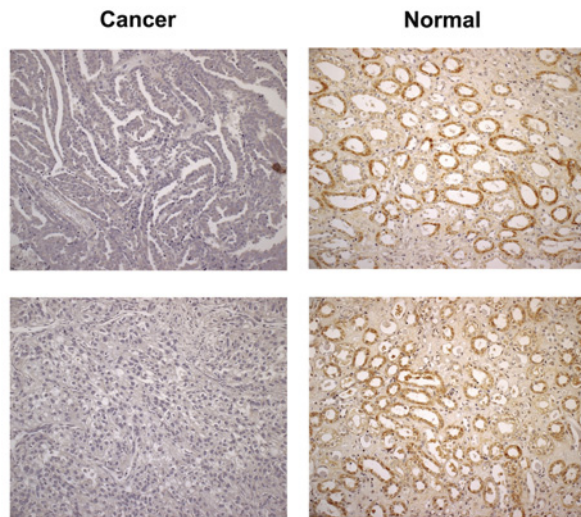
FIGURE 2

Immunohistochemical analysis of ELF5 in kidney carcinoma.

Note: Two carcinomas that lack epithelial staining for ELF5 in abnormal kidney epithelium are representative of the cancer sample population. Two normal kidneys showing staining for ELF5 in normal kidney epithelium are representative of the normal sample population. Magnification: 200X.

(Figure 2). These results concur with the loss of *ELF5* mRNA expression in kidney-derived carcinomas and indicate that the loss of protein is due to a decrease in transcription rather than translation.

ELF5 Functions as a Tumor Suppressor in Renal Carcinoma Cells

Since the loss of ELF5 was associated with tumorigenesis in kidney carcinomas we investigated whether ELF5 was capable of functioning as a tumor suppressor in a renal carcinoma cell line. 786-O renal carcinoma cells, which do not normally express *ELF5* (Figure 3A) were stably transfected with a construct expressing *ELF5* (Figure 3A). The introduction of exogenous *ELF5* altered the growth of these cells in three-dimensional, anchorage-independent conditions compared to the mock transfected control. Approximately 90% of mock transfected 786-O cells formed colonies in soft agarose (Figure 3B, C). The *ELF5*-expressing 786-O cells showed a significant 71% decrease in the proportion of cells that formed colonies compared to mock transfectants ($p < .001$) and the colonies that formed were smaller in size (Figure 3B, C).

Next we inoculated our 786-O transfectants into SCID mice to analyse the tumor-forming capability of the cells. Group size is cumulative for the number of mice injected with two clones over two independent experimental replicates. Ninety per cent of mock transfectant-inoculated mice (9 of 10) established tumors, while tumors developed in only 10% of the of *ELF5*-transfectant inoculated mice (2 of 20) at 21 days post-inoculation. Analysis of the inoculation sites of mock transfected 786-O cells showed formation of solid tumors containing closely packed
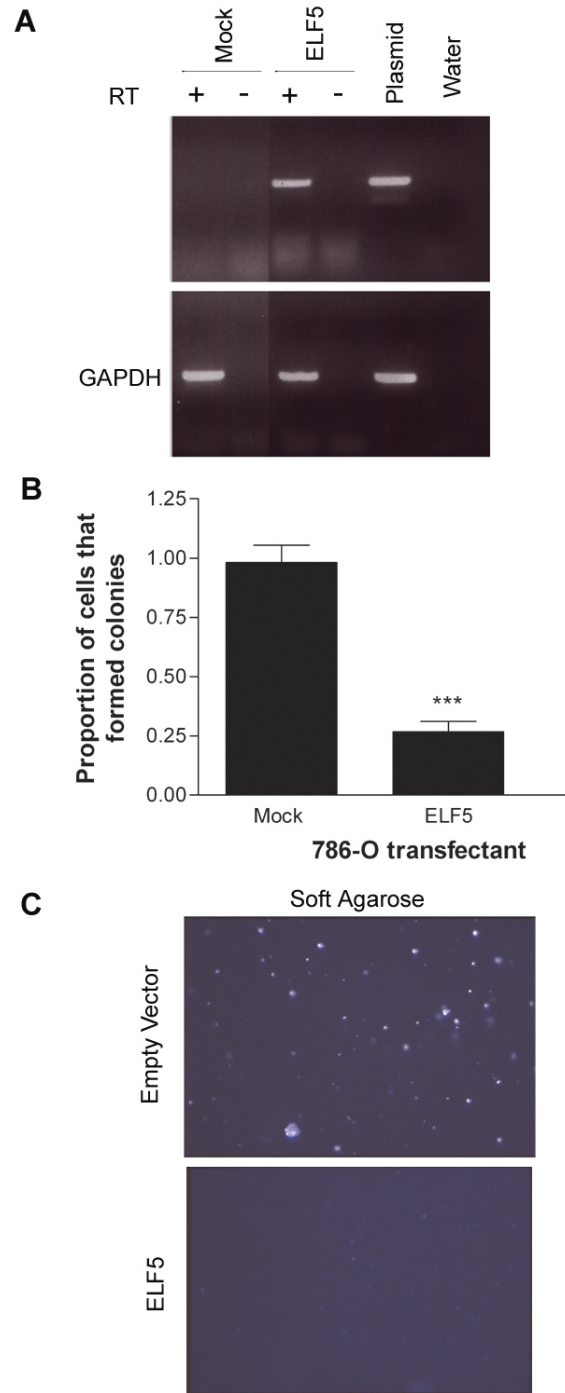

FIGURE 3

ELF5-expressing renal carcinoma cells show reduced colony formation in soft agarose.

Note: (A) Representative RT-PCR showing the absence of endogenous ELF5 in 786-O renal carcinoma cells (lane 1) and expression of exogenous ELF5 in stable transfectants (lane 3). Plasmid and no template (water) controls are shown. (B) Anchorage-independent colony formation of ELF5-expressing 786-O renal carcinoma cells is inhibited compared with mock transfected cells. Colony formation is reduced by 71% compared with mock transfected cells, where 90% of cells form colonies ($n = 3$; ***$p < .0001$). (C) Representative soft agar assay of 786-O cells transfected with the empty vector (top) and an ELF5-expression construct (bottom).

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epithelial-like cells and blood infiltrate. Small epithelial-like cell clusters, presumably of 786-O origin, dispersed among the subcutaneous fat were identified at the *ELF5*-expressing 786-O injection sites (Figure 4). Blood vessels and infiltrate were not observed at these sites.

Discussion

Ma and colleagues (2003) demonstrated decreased *ELF5* expression in premalignant, preinvasive and invasive breast carcinomas compared to normal tissue. *ELF5* mRNA expression was also decreased in some breast cancer cell lines (Zhou et al., 1998). In this study, we have shown in the kidney that *ELF5* mRNA was decreased in 17 of the 18 renal carcinomas examined. The majority of the samples were classified as renal cell carcinoma (15 of 18 samples), the most common form of kidney cancer. Too few samples were available to enable us to draw any correlations between either the type or grade of renal cell carcinoma and the level of *ELF5* mRNA. This reduction in *ELF5* message was consistent with the lack of immunoreactivity of the anti-*ELF5* antibody on kidney-derived

H & E

Empty Vector

ELF5

FIGURE 4

ELF5-expressing renal carcinoma cell lines show reduced tumor formation in SCID mice.

Note: Representative H&E stained sections of tumors taken from mock and *ELF5*-expressing 786-O SCID mouse tumors show densely packed epithelial-like cells in mock inoculants, with infiltrating blood cells, but sparse clumps of epithelial-like cells in the subcutaneous fat of *ELF5* inoculants.

carcinomas, whereas the normal kidney stained strongly throughout the tubules.

Re-expression of *ELF5* in renal carcinoma cells resulted in decreased three-dimensional, anchorage-independent growth and in tumorigenicity *in vivo*. Sites where the kidney-derived 786-O-*ELF5* cells are present in SCID mice lacked the tumor-associated blood vessels and blood cell infiltrations characteristic of aggressive tumors that promote formation of their own blood supply. Thus, *ELF5* in the kidney appears to have tumor suppressor function. The mechanism whereby the loss of *ELF5* expression contributes to tumorigenesis remains undefined but it is probable that like other Ets factors, aberrant expression of the *ELF5* transcription factor in carcinomas leads to dysregulation of its target genes, and thus contributes to cellular transformation. This could conceivably represent a role for *ELF5* in maintenance of growth suppression or terminal differentiation. Loss of *ELF5* expression in this context could result in a selective growth advantage for tumorigenic cells.

There is some evidence to support Ets factors having a role in kidney carcinoma. For instance, one study reported the up-regulation of an ETS-like protein in renal cell carcinoma versus normal kidney (Rae et al., 2000) and ETS-1 was identified as a potential culprit in renal cell carcinoma because of its involvement in the angiogenic and matrix remodeling processes associated with carcinogenesis. *ETS-1* is a target gene of VEGF and was found to be up-regulated and localized to carcinoma cells and endothelial cells in renal cell carcinoma (Mikami et al., 2006). ETS-1 regulates the expression of the matrix metalloproteinases *MMP-1*, *MMP-3* and *MMP-9* (Mizui et al., 2006), all of which have been found to be over-expressed in renal cell carcinoma (Bhuvarahamurthy et al., 2006). Suppression of the *Tsc2* tumor suppressor gene promoter by the Ets factor Elf-1 was also linked to renal cell carcinoma in a study using a rat model of tuberous sclerosis (Honda et al., 2003) and Pea3 was found to transactivate the Wilms' tumor gene, *WT1* (Discenza et al., 2004). Notably, all these studies invoke oncogenic properties of Ets transcription factors whereas our study supports a tumor suppressor function for *ELF5*. Although the mechanism whereby loss of *ELF5* contributes to renal cell carcinoma remains undefined, based on the requirement of *ELF5* for the differentiation of mammary epithelial cells and on its involvement in differentiated keratinocytes, we speculate that ELF5 is needed to maintain renal epithelial cells in a differentiated state. This role would also be consistent with its function as a transcription factor required for the expression of other tumor suppressor genes.

Dysregulation of a number of signal transduction pathways has been linked to the etiology of renal cell carcinoma. The most common pathway disrupted is centred around the loss of the *VHL* gene. The product of the *VHL* gene forms part of a ubiquitin ligase complex
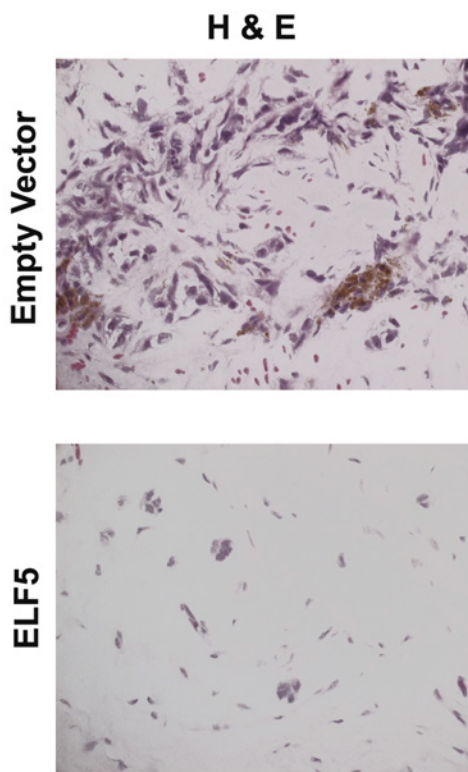
that targets proteins for degradation, so loss of *VHL* in renal cell carcinoma presumably disrupts normal cellular proteosome functions. Loss of *VHL* also increases the expression of hypoxia-inducible factor, HIF-1α which via its transcriptional regulation of vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), endothelial growth factor receptor (EGFR), glucose transporter protein 1 (GLUT1), erythropoietin and transforming growth factor-alpha (TGF α), creates a pseudohypoxic state in renal cells that promotes vascularisation of the tumor (Bratslavsky et al., 2007). A pathway known as the rapamycin-sensitive signal transduction pathway is also operative in renal cell carcinoma (Hidalgo & Rowinsky, 2000). The fungicide rapamycin exhibits its anti-tumorigenic properties by blocking the kinase activity of a protein called mTOR or mammalian target of rapamycin, which in turn leads to cell growth arrest. Recently, it was reported that inhibition of mTOR by rapamycin also inhibits tumor angiogenesis by blocking the mTOR-dependent activation of HIF-1 α and VEGF-A secretion (Land & Tee, 2007). How these pathways impinge on *ELF5* or conversely, how *ELF5* exerts its effects on these pathways, presently remains obscure. However, it is possible that the loss of *ELF5* expression may represent an alternative carcinogenic mechanism.

Conclusions

In summary, we have shown for the first time that an Ets transcription factor, *ELF5*, behaves in a fashion consistent with a tumor suppressor gene in renal cancer. Further work is required to examine LOH, promoter hypermethylation or somatic mutation of the gene as potential explanations for the loss of *ELF5* expression. Also, ascribing a role for *ELF5* in normal kidney development and maintenance of function will assist in elucidating how dysregulation of this tumor suppressor gene can result in carcinogenesis.

Acknowledgment

References

Audebert, M., Chevillard, S., Levalois, C., Gyapay, G., Vieillefond, A., Klijanienko, J., Vielh, P., El Naggar, A. K., Oudard, S., Boiteux, S., & Radicella, J. P. (2000). Alterations of the DNA repair gene OGG1 in human clear cell carcinomas of the kidney. *Cancer Research, 60,* 4740–4744.

Bhuvarahamurthy, V., Kristiansen, G. O., Johannsen, M., Loening, S. A., Schnorr, D., Jung, K., & Staack, A. (2006). In situ gene expression and localization of metalloproteinases MMP1, MMP2, MMP3, MMP9, and their inhibitors TIMP1 and TIMP2 in human renal cell carcinoma. *Oncology Reports, 15,* 1379–1384.

Bratslavsky, G., Sudarshan, S., Neckers, L., & Linehan, W. M. (2007). Pseudohypoxic pathways in renal cell carcinoma. *Clinical Cancer Research, 13,* 4667–1471.

Clifford, S. C., Prowse, A. H., Affara, N. A., Buys, C. H., & Maher, E. R. (1998). Inactivation of the von Hippel-Lindau (VHL) tumor suppressor gene and allelic losses at chromosome arm 3p in primary renal cell carcinoma: Evidence for a VHL-independent pathway in clear cell renal tumorigenesis. *Genes Chromosomes Cancer, 22,* 200–209.

Discenza, M. T., Vaz, D., Hassell, J. A., & Pelletier, J. (2004). Activation of the WT1 tumor suppressor gene promoter by Pea3. *FEBS Letters, 560,* 183–191.

Donaldson, L. W., Petersen, J. M., Graves, B. J., & McIntosh, L. P. (1994). Secondary structure of the ETS domain places murine Ets-1 in the superfamily of winged helix-turn-helix DNA-binding proteins. *Biochemistry*, 33, 13509–13516.

Gemmill, R. M., Bemis, L. T., Lee, J. P., Sozen, M. A., Baron, A., Zeng, C., Erickson, P. F., Hooper, J. E., & Drabkin, H. A. (2002). The TRC8 hereditary kidney cancer gene suppresses growth and functions with VHL in a common pathway. *Oncogene, 21,* 3507–3516.

Gnarra, J. R., Lerman, M. I., Zbar, B., & Linehan, W. M. (1995). Genetics of renal-cell carcinoma and evidence for a critical role for von Hippel-Lindau in renal tumorigenesis. *Seminars in Oncology, 22,* 3–8.

Gudmundsson, J., Barkardottir, R. B., Eiriksdottir, G., Baldursson, T., Arason, A., Egilsson, V., & Ingvarsson, S. (1995). Loss of heterozygosity at chromosome 11 in breast cancer: Association of prognostic factors with genetic alterations. *British Journal of Cancer, 72,* 696–701.

Harris, J., Stanford, P. M., Sutherland, K., Oakes, S. R., Naylor, M. J., Robertson, F. G., Blazek, K. D., Kazlauskas, M., Hilton, H. N., Wittlin, S., Alexander, W. S., Lindeman, G. J., Visvader, J. E., & Ormandy, C. J. (2006). Socs2 and elf5 mediate prolactin-induced mammary gland development. *Molecular Endocrinology, 20,* 1177–1187.

Hidalgo, M., & Rowinsky, E. K. (2000). The rapamycin-sensitive signal transduction pathway as a target for cancer therapy. *Oncogene, 19,* 6680–6686.

Honda, S., Kobayashi, T., Kajino, K., Urakami, S., Igawa, M., & Hino, O. (2003). Ets protein Elf-1 bidirectionally suppresses transcriptional activities of the tumor suppressor Tsc2 gene and the repair-related Nth1 gene. *Molecular Carcinogenesis, 37,* 122–129.

Karnik, P., Paris, M., Williams, B. R., Casey, G., Crowe, J., & Chen, P. (1998). Two distinct tumor suppressor loci within chromosome 11p15 implicated in breast cancer progression and metastasis. *Human Molecular Genetics, 7,* 895–903.

Land, S. C., & Tee, A. R. (2007). Hypoxia-inducible factor 1alpha is regulated by the mammalian target of rapamycin (mTOR) via an mTOR signaling motif. *Journal of Biological Chemistry, 282,* 20534–20543.

Lapinskas, E. J., Palmer, J., Ricardo, S., Hertzog, P. J., Hammacher, A., & Pritchard, M. A. (2004). A major site of expression of the Ets transcription factor Elf5 is epithe-

Erika J. Lapinskas, Suzanne Svobodova, Ian D. Davis, Jonathan Cebon, Paul J. Hertzog, and Melanie A. Pritchard

lia of exocrine glands. *Histochemistry and Cell Biology, 122,* 521–526.

Liou, L. S., Shi, T., Duan, Z. H., Sadhukhan, P., Der, S. D., Novick, A. A., Hissong, J., Skacel, M., Almasan, A., DiDonato, J. A. (2004). Microarray gene expression profiling and analysis in renal cell carcinoma. *BMC Urology, 4,* 9.

Ludwig, C. U., Raefle, G., Dalquen, P., Stulz, P., Stahel, R., & Obrecht, J. P. (1991). Allelic loss on the short arm of chromosome 11 in non-small-cell lung cancer. *International Journal of Cancer, 49,* 661–665.

Ma, X. J., Salunga, R., Tuggle, J. T., Gaudet, J., Enright, E., McQuary, P., Payette, T., Pistone, M., Stecker, K., Zhang, B. M., Zhou, Y. X., Varnholt, H., Smith, B., Gadd, M., Chatfield, E., Kessler, J., Baer, T. M., Erlander, M. G., Sgroi, D. C. (2003). Gene expression profiles of human breast cancer progression. *Proceedings of the National Academy of Sciences U.S.A.,* 100, 5974–5979.

Metzger, D. E., Xu, Y., & Shannon, J. M. (2007). Elf5 is an epithelium-specific, fibroblast growth factor-sensitive transcription factor in the embryonic lung. *Developmental Dynamics, 236,* 1175–1192.

Mikami, S., Oya, M., Mizuno, R., Murai, M., Mukai, M., & Okada, Y. (2006). Expression of Ets-1 in human clear cell renal cell carcinomas: Implications for angiogenesis. *Cancer Science, 97,* 875–82.

Mizui, M., Isaka, Y., Takabatake, Y., Sato, Y., Kawachi, H., Shimizu, F., Takahara, S., Ito, T., & Imai, E. (2006). Transcription factor Ets-1 is essential for mesangial matrix remodeling. *Kidney International, 70,* 298–305.

Mizushima, S., & Nagata, S. (1990). pEF-BOS, a powerful mammalian expression vector. *Nucleic Acids Research, 18,* 5322.

Morrissey, C., Martinez, A., Zatyka, M., Agathanggelou, A., Honorio, S., Astuti, D., Morgan, N. V., Moch, H., Richards, F. M., Kishida, T., Yao, M., Schraml, P., Latif, F., & Maher, E. R. (2001). Epigenetic inactivation of the RASSF1A 3p21.3 tumor suppressor gene in both clear cell and papillary renal cell carcinoma. *Cancer Research, 61,* 7277–7281.

Oettgen, P., Kas, K., Dube, A., Gu, X., Grall, F., Thamrongsak, U., Akbarali, Y., Finger, E., Boltax, J., Endress, G., Munger, K., Kunsch, C., & Libermann, T. A. (1999). Characterization of ESE-2, a novel ESE-1-related Ets transcription factor that is restricted to glandular epithelium and differentiated keratinocytes. *Journal of Biological Chemistry, 274,* 29439–29452.

Pavlovich, C. P., & Schmidt, L. S. (2004). Searching for the hereditary causes of renal-cell carcinoma. *Nature Reviews Cancer, 4,* 381–393.

Rae, F. K., Stephenson, S. A., Nicol, D. L., & Clements, J. A. (2000). Novel association of a diverse range of genes with renal cell carcinoma as identified by differential display. *International Journal of Cancer, 88,* 726–732.

Ramirez-Solis, R., Liu, P., & Bradley, A. (1995). Chromosome engineering in mice. *Nature, 378,* 720–724.

Rathmell, W. K. , & Godley, P. A. (2010). Recent updates in renal cell carcinoma. *Current Opinion in Oncology, 22,* 250–256.

Rogers, R. L., Van Seuningen, I., Gould, J., Hertzog, P. J., Naylor, M. J., & Pritchard, M. A. (2010). Transcript profiling of Elf5$^{+/-}$ mammary glands during pregnancy identifies novel targets of Elf5. *PLoS One, 5,* e13150.

Schlehofer, B., Pommer, W., Mellemgaard, A., Stewart, J. H., McCredie, M., Niwa, S., Lindblad, P., Mandel, J. S., McLaughlin, J. K., & Wahrendorf, J. (1996). International renal-cell-cancer study. VI. The role of medical and family history. *International Journal of Cancer, 66,* 723–726.

Seth, A., & Watson, D. K. (2005). ETS transcription factors and their emerging roles in human cancer. *European Journal of Cancer, 41,* 2462–2478.

Shipman, R., Schraml, P., Colombi, M., Raefle, G., & Ludwig, C. U. (1993). Loss of heterozygosity on chromosome 11p13 in primary bladder carcinoma. *Human Genetics, 91,* 455–458.

Tanimoto, K., Makino, Y., Pereira, T., & Poellinger, L. (2000). Mechanism of regulation of the hypoxia-inducible factor-1 alpha by the von Hippel-Lindau tumor suppressor protein. *EMBO Journal, 19,* 4298–4309.

Tummala, R., & Sinha, S. (2006). Differentiation-specific transcriptional regulation of the ESE-2 gene by a novel keratinocyte-restricted factor. *Journal of Cell Biochemistry, 97,* 766–781.

Winqvist, R., Hampton, G. M., Mannermaa, A., Blanco, G., Alavaikko, M., Kiviniemi, H., Taskinen, P. J., Evans, G. A., Wright, F. A., Newsham, I., & Cavenee, W. K. (1995). Loss of heterozygosity for chromosome 11 in primary human breast tumors is associated with poor survival after metastasis. *Cancer Research, 55,* 2660–2664.

Zhou, J., Ng, A.Y., Tymms, M. J., Jermiin, L. S., Seth, A. K., Thomas, R. S., & Kola, I. (1998). A novel transcription factor, ELF5, belongs to the ELF subfamily of ETS genes and maps to human chromosome 11p13–15, a region subject to LOH and rearrangement in human carcinoma cell lines. *Oncogene, 17,* 2719–2732.

Zhou, J., Chehab, R., Tkalcevic, J., Naylor, M. J., Harris, J., Wilson, T. J., Tsao, S., Tellis, I., Zavarsek, S., Xu, D., Lapinskas, E. J., Visvader, J., Lindeman, G. J., Thomas, R., Ormandy, C. J., Hertzog, P. J., Kola, I., & Pritchard, M. A. (2005). The mouse *Elf5* gene is essential for early embryogenesis and mammary gland development during pregnancy and lactation. *EMBO Journal, 24,* 635–644.