# The Role of sFRP1 in Conventional Renal Cell Carcinoma

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## ABSTRACT

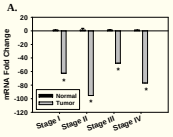
**Renal cell carcinoma** (RCC) is the sixth-leading cause of cancer death. Resistance to radiation and classical chemotherapy is often observed in metastatic RCC, a problem that is directly related to the fact that the molecular events leading to disease onset and progression are not well understood. Metastatic RCC typically results in death in less than a year. It is our long term goal to identify and characterize the molecular pathways leading to conventional RCC to enable the design of more effective, targeted therapies. One candidate is the **Wnt pathway**. Our clinical data indicate that loss of a negative regulatory protein, **secreted frizzled-related protein 1** (sFRP1), may be responsible for activation of Wnt signaling. Indeed, loss or repression of sFRP1 expression was observed in 100% of 53 patients tested. This is the first report of loss of sFRP1 expression in patients diagnosed with cRCC; this occurs in early stage cRCC, suggesting that it may be an important event in renal carcinogenesis. A human cRCC cell model recapitulated these observations and confirmed upregulation of many targets of the Wnt pathway. Treatment of cRCC cells with 5-aza-2′-deoxycytidine suggested that loss of sFRP1 is due to promoter hypermethylation. In order to test the hypothesis that sFRP1 acts as a tumor suppressor in cRCC, we have stably expressed sFRP1 in a cRCC cell line. UMRC3 cells represent a model of metastatic cRCC; sFRP1 expression in UMRC3 cells resulted in decreased growth in cell culture, inhibition of anchorage-independent growth, and decreased tumor volume in a nude mouse model. Together these data suggest an important role for sFRP1 as a **tumor suppressor** in cRCC.

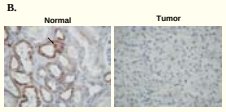
## INTRODUCTION

Renal cell carcinoma (RCC) is responsible for an estimated 12,000 deaths per year in the U.S. and more than 100,000 per year worldwide. Metastatic RCC is resistant to radiation and classical cytotoxic chemotherapies, a problem that is directly related to the fact that the molecular mechanisms of disease progression are poorly understood. In order to design more effective therapies, molecular events leading to carcinogenesis and metastasis must be determined. Development of effective therapies requires identification and characterization of molecular pathways that are important in renal cell carcinogenesis and progression. Consequently, the long-term goal of the laboratory is to identify and characterize the molecular mechanisms underlying the genesis and progression of human conventional or clear cell, RCC (cRCC), and to use this information to develop effective targeted therapies. Analysis of genomic profiling data revealed changes in the expression of several targets of the Wnt signaling pathway. The targets of this pathway regulate functions such as cell proliferation, extracellular matrix metabolism, cell survival, angiogenesis, and cell motility. We have confirmed that mRNA levels of at least nine Wnt targets are elevated in cRCC tissues. The mechanism responsible for activation of the Wnt pathway in cRCC has not been determined. The preliminary data presented here suggest that activation of the Wnt pathway in cRCC may be due to loss of secreted frizzled-related protein 1 (sFRP1), a protein involved in negative regulation of the Wnt pathway. Indeed, loss of sFRP1 has been reported in cancers of the colon, lung and ovary and may function as a tumor suppressor. It was the goal of the experiments presented here to test the hypothesis that sFRP1 functions as a tumor suppressor in cRCC.

## RESULTS

### sFRP1 Expression is Repressed in Conventional Renal Cell Carcinoma




C.

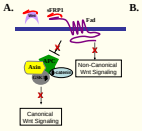
| Cells | sFRP1 ΔCt Value |
|---|---|
| hRE1S2hTERT | 21.59 +/-0.4 |
| UMRC3 | Undetermined |
| A498 | Undetermined |
| 769-P | Undetermined |
| 786-O | Undetermined |
| UMRC3-DAC 24 hr | 21.21 +/-2.7 |

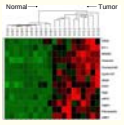
**A. sFRP1 is downregulated in cRCC patients.** Real time PCR was used to analyze sFRP1 mRNA levels in normal (black bars) and tumor (gray bars) samples in all four stages of cRCC; Stage I (n=10), Stage II (n=8), Stage III (n=10), Stage IV (n=5). Means are represented as +/- standard deviation. * p≤0.008

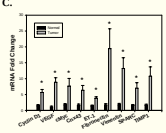
**B. sFRP1 protein expression is lost in cRCC.** Immunohistochemistry was performed to evaluate sFRP1 protein levels in normal and tumor tissue sections from a representative cRCC patient. Arrow indicates a proximal tubule, the region of the nephron from which cRCC is thought to arise.

**C. sFRP1 expression is undetectable in cRCC cell lines.** Several cRCC cell lines were evaluated for sFRP1 expression using real time PCR. After 40 amplification cycles the ΔCt value for sFRP1 was undetermined in the cRCC cell lines. To evaluate whether loss of sFRP1 is due to methylation-induced silencing, UMRC3 cells were treated for 24 hr with 5-aza-2′-deoxycytidine (DAC).

### Wnt Targets are Up-regulated in cRCC



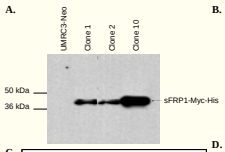



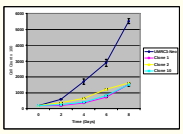

**A. Role of sFRP1 in blocking Wnt signaling.** Adapted from Kawano and Kypta *J. Cell Sci* 116:2627 2003

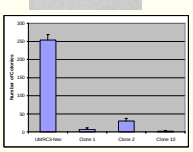
**B. Hierarchical cluster analysis reveals upregulation of Wnt targets in cRCC patients**. A heat map was generated from microarray data comparing expression of Wnt-regulated genes in patient matched normal and tumor tissue samples from Stage I cRCC. Color corresponds to the expression level of the transcript with low, intermediate and high expression represented by green, black, and red, respectively.

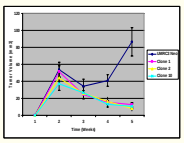
**C. Real Time PCR confirms upregulation of Wnt targets in cRCC**. Changes in expression between normal (black bars) and tumor tissue (gray bars) for all stages of cRCC are indicated. Means are represented as +/- standard error. * p≤0.001

### sFRP1 Acts as a Tumor Suppressor in cRCC




**A. Stable expression of sFRP1 in UMRC3 cells.** UMRC3 cells were transfected with pcDNA3.1-sFRP1-Myc-His (kind gift of J. Rubin) and selected with G418. Conditioned media was collected from UMRC3-Neo (emtpy vector control) and three representative clones, 1, 2, and 10. Media samples were concentrated and purified over a Ni-NTA column. Western blot analysis using an anti-myc tag antibody was used to confirm expression of sFRP1-Myc-His.

**B. sFRP1 clones are growth-inhibited**. Growth in culture of UMRC3-Neo, and three sFRP1-expressing clones was measured over the course of 8 days. Data are represented as the mean +/- standard error, n=3.

**C. sFRP1 expression results in inhibition of anchorage independent growth**. A soft agar assay was used to evaluate the ability of UMRC3-Neo or three sFRP1-expressing clones to form colonies. Data are represented as the mean +/- standard deviation, n=3.

**D. sFRP1 acts as a tumor suppressor in cRCC**. Athymic nude mice were injected ectopically with approximately two million cells per flank of each of the cell lines indicated. Tumor volumes were measured every 7 days and are represented as the mean, +/- standard error. N=8 mice per cell line.

## CONCLUSIONS

- **Loss of sFRP1 is an early event in cRCC and may be due to methylation-induced silencing**
- **Activation of the Wnt signaling pathway in cRCC patients may be the result of decreased sFRP1 expression**
- **sFRP1 demonstrates tumor suppressive activity in cRCC**