Type III TGFβ receptor is a tumor suppressor in human renal cell carcinoma

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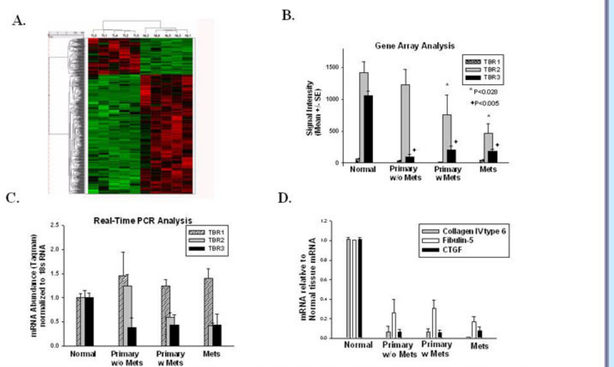
ABSTRACT

Using genomic profiling of patient matched tissue samples, we demonstrated that the type III TGFβ receptor (TβR3) expression is down-regulated in early stage conventional renal cell carcinoma (RCC)(*Oncogene* 22:8053, 2003). We further demonstrated that a subset of TGFβ regulated genes were suppressed, presumably related to decreased activity of the TGFβ pathway, in early stage RCC as compared to normals. Combined with published literature and laboratory data, demonstrating TGFβ inhibition of cell proliferation in normal renal epithelial cells, we hypothesized that TβR3 plays a critical role in TGFβ signaling in renal biology, and that this molecule (among others) must be down-regulated for tumorigenesis to occur in the kidney. Using normal renal epithelial cells, we have generated RNAi against TβR3 to demonstrate loss of TGFβ signaling and growth inhibitory responses. We further demonstrate that if TβR3 is stably re-expressed in human RCC cell lines that have lost expression of this gene, cell proliferation, soft agar colony formation, and in vivo tumorigenicity are inhibited significantly. Regulation of TβR3 expression is through the putative TβR3 promoter located upstream of the gene on chromosome 1. In our investigations, we discovered that TβR3 is upregulated by retinoic acid (RA) at the transcriptional level. We have cloned the TβR3 promoter (-1, -2, -5 kb DNA base sequences upstream of the transcriptional start site) and constructed deletion mutants to determine whether putative RA response elements are transcriptionally functional. From these data, we propose that TβR3 is necessary for TGFβ signaling in normal renal epithelial cells to maintain differentiated function and that loss of expression of this gene is a necessary event for tumorigenesis leading to RCC.

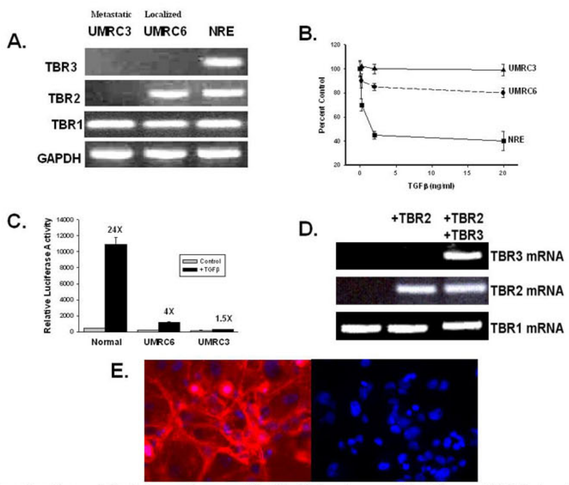
INTRODUCTION

Renal cell carcinoma represents a major health problem. The American Cancer Society predicts that there will be over 35,000 new cases of renal neoplasms in the coming year in the United States. Moreover, they predict that 12,500 patients will die as a consequence of disease progression associated with these renal neoplasms in the coming year. Using Affymetrix gene array technology, we compared differences in gene expression between normal renal epithelium, localized RCC, and metastatic RCC from patients with conventional histology, who had tissue harvested at the time of nephrectomy. Through these experiments, we identified that loss of TβR3 expression was an early event in RCC carcinogenesis, and that subsequent loss of TβR2 expression was associated with metastatic progression. We brought these clinical observations to the laboratory, confirmed them in our laboratory models of human RCC, and then manipulated the laboratory models with conventional molecular techniques to determine the importance of the TGFβ signaling pathway in human RCC and specifically, the significance of TβR3 as a tumor suppressor.

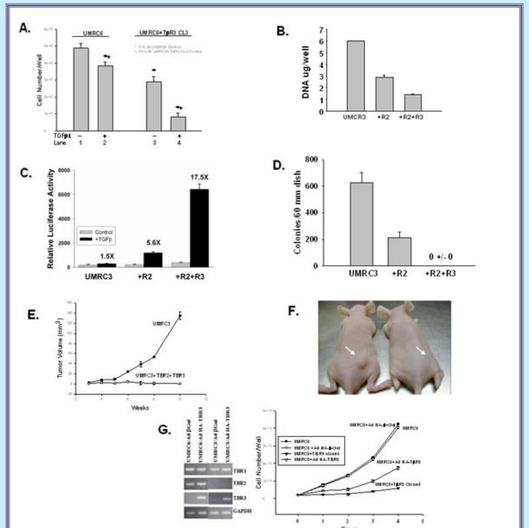
RESULTS



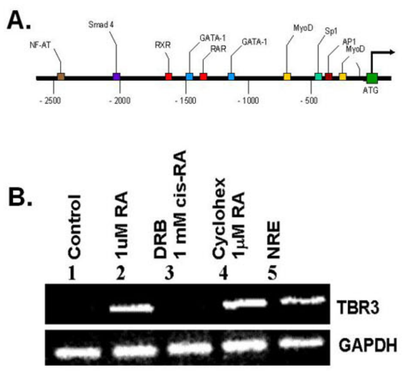
A. Heat map generated from gene array analysis comparing normal renal epithelium versus RCC demonstrated differential expression of 540 genes associated with RCC carcinogenesis and progression, including TβR3. **B.** TβR3 expression is lost early in carcinogenesis, while TβR2 expression is lost with metastatic progression. **C.** Gene Array data regarding TβR expression is confirmed in additional samples of normal-tumor matched RCC using Real-Time PCR analysis. **D.** Loss of TβR expression results in attenuation of TGFβ mediated gene expression with RCC carcinogenesis and progression.



A. Based on these clinical observations, we identified laboratory models of human RCC that replicated our clinical findings. UMRC3 was derived from a metastatic tumor and lacks TβR2 and TβR3. UMRC6 was derived from a localized tumor and lacks TβR3. **B, C.** These cell lines have predictable growth inhibitory responses and luciferase activity to exogenous TGFβ based on their respective receptor expression. **D.** We can reintroduce TβR expression into these lab models through the development of stable transfectants. **E.** Similarly, we can "knock-out" TβR expression using siRNA in NRE, such as TβR3 expression shown in this figure



A. Reintroduction of TβR3 expression into UMRC6 increases TGFβ responsiveness and inhibits cell growth. **B.** Reintroduction of TβR2 and TβR3 into UMRC3 increases TGFβ responsiveness and inhibits cell growth. **C.** Reestablishment of TGFβ signaling through TβR expression in human RCC increases TGFβ mediated gene expression as evidenced by increased luciferase activity. **D.** Reintroduction of TβR2 and TβR3 expression into UMRC3 inhibits colony formation in vitro. **E., F.** Reintroduction of TβR2 and TβR3 expression into UMRC3 inhibits tumor formation in vivo. **G.** Reintroduction of TβR3 alone, Either through adenovirus or stable transfection, independent of TβR2 expression and TGFβ signaling activity,demonstrates growth inhibitory activity in UMRC3



A. Using PCR, we cloned from 1000 to 5000 bp 5' upstream from the TβR3 gene start codon using gene sequencing information from the Celera database. Contained within this DNA fragment we believe lies the putative promoter for the human TβR3 gene. Our data suggest that TβR3 expression is altered at the level of transcription in human RCC. In analyzing the putative promoter sequence, we have identified many potential response element recognition sites in the promoter, including an RXR response element, which is activated through the retinoic acid pathway. Interestingly, we identified RXR as another gene downregulated early in human RCC. **B.** We incubated UMRC 6 cells with retinoic acid and demonstrated upregulation of TβR3 gene expression. This increased expression was inhibited by dichlororibofuranosylbenzimidazole (DRB), but not affected by cyclohexamide, indicating direct transcriptional activation of RXR by retinoic acid.

CONCLUSIONS

- The TGFβ pathway is an important signaling pathway in the biology of human RCC, as demonstrated through gene array analysis of clinical samples.
- Loss of TβR3 expression is an early event in RCC carcinogenesis and subsequent loss of TβR2 expression is associated with metastatic progression.
- TβR3 is a tumor suppressor gene in human RCC. Our data demonstrate that this tumor suppressive function appears to be mediated through TGFβ signaling in conjunction with TβR2 expression. In addition, TβR3 expression alone, independent of TβR2 expression and TGFβ signaling, results in a tumor suppressive phenotype in human RCC.