Neuronal Pentraxin2: A Novel Tumor-Specific Molecular Target That Mediates Clear Cell Renal Cell Carcinoma Malignancy

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BACKGROUND

RCC:
- Renal cell carcinomas (RCC) is the third most prevalent urological cancer, the 10th most common cause of cancer death in men and the 9th most common cause in women.
- The clear cell variant of RCC is the most common subtype accounting for 80% of all renal cancers.
- Due to its asymptomatic nature, it is estimated that up to 30% of patients present with metastatic ccRCC at time of diagnosis. Furthermore, due to its highly metastatic nature 20-30% of patients diagnosed with localized disease will relapse with metastatic ccRCC.
- For individuals presenting with advanced disease, treatment options are limited with no current drug therapy leading to long term survival with the exception of 6-7% of patients who respond to interlukin-2.
- ccRCC rarely responds to chemotherapy and radiation therapies, and drug resistance develops rapidly with application of targeted therapies.
- Genetic factors contributing to ccRCC development, progression, and metastasis are poorly defined.

NPTX2:
- Neuronal pentraxin 2 (NPTX2) belongs to a class of secreted proteins characterized by their pentraxin domain, and are related to C-reactive protein (CRP).
- NPTX2 is typically expressed in nervous system, testicular, pancreatic, skeletal muscle, and hepatic tissues, with little to no expression observed in normal renal tissue.
- NPTX2 is best characterized for its role in neutrophil outgrowth and synaptic plasticity of neuronal cells; mediates the clustering of the AMPA family of ionotropic glutamate receptors, forming ion permeable channels during excitatory synaptic transmission.

RESULTS

Figure 1: NPTX2 Expression Profile in ccRCC

Figure 2: Role of NPTX2 in ccRCC Viability and Invasion

Figure 3: GluR4 in ccRCC and Interaction with NPTX2

Figure 4: Loss of GluR4 Recapitulates NPTX2 Knockdown Phenotype

STUDY DESIGN

Translational Relevance:
Patients with metastatic ccRCC have poor prognoses, with an estimated 5 year overall survival of less than 10%. This is due to lack of remedial therapies that produce durable disease stabilization or tumor regression. Drug resistance is a hallmark of ccRCC and is linked to cancer cell heterogeneity. Additionally, there is a lack of known molecular factors that can be targeted pharmacologically. The clear cell variant of RCC also frequently manifests as metastatic disease, likely due to its disposition for increased migratory capacity and the formation of undetectable micrometastases. A focus on identifying both factors that contribute to ccRCC cell migration and those that are therapeutically targetable is paramount.

Methods:
We employed several high throughput screening methods to evaluate the pattern of NPTX2 expression in ccRCC samples derived from patient tumor tissues. This included high throughput gene array analysis, evaluation of published gene array datasets, meta-analysis screening, evaluation of patient tissue microarrays (TMA), comparative marker selection and pathway analysis. NPTX2 shRNAs were designed to target and abrogate NPTX2 expression in a cohort of established ccRCC cell lines, and resulting changes in proliferation, viability, and tumor cell migration were evaluated. We also investigated the expression of known NPTX2 binding partners in ccRCC, and evaluated their role in NPTX2 mediated tumorigenic activity. The nonnaptic glutamate receptor 4 (GluR4) subunit was identified to be expressed in ccRCC tissue, and we further demonstrate a tumorigenic role for this receptor in ccRCC.

CONCLUSIONS

- NPTX2 is highly over-expressed in ccRCC at the transcriptional and protein level.
- NPTX2 expression is specific for the clear cell subtype of RCC.
- NPTX2 consistently is the most differentially expressed gene transcript in ccRCC.
- NPTX2 expression is strongly correlated with advanced and metastatic ccRCC.
- Loss of NPTX2 expression leads to decreased tumor cell growth, loss of tumor cell viability, and decreased invasive capacity.
- We identify that GluR4 complexes with NPTX2 in ccRCC tumor cells.
- GluR4 is over-expressed in ccRCC tissue, and demonstrates the highest level of expression in metastatic tissues.
- Loss of GluR4 expression similarly leads to decreased cell proliferation, loss of tumor cell viability, and decreased invasive capacity of ccRCC cells.

CLINICAL IMPACT

- We demonstrate an oncogenic role for NPTX2 for the first time in a cancer system.
- As NPTX2 is a secreted protein that is consistently and specifically overexpressed in patient ccRCC samples, it presents as:
  - A candidate diagnostic biomarker for patients with the clear cell subtype of RCC.
  - A candidate prognostic marker for patients at risk of disease recurrence and/or the development of metastatic disease.
  - An optimal therapeutic target whose inhibition may clinically benefit a broad spectrum of patients.

FUTURE DIRECTIONS

- Development of a monoclonal antibody or small molecule inhibitor of NPTX2 to be tested for therapeutic efficacy in vivo.
- Evaluate NPTX2 expression in patient blood samples and correlate to disease staging and progression to evaluate efficacy as a diagnostic and prognostic biomarker.
- Define the signaling mechanism of NPTX2 oncogenic activity.
- Evaluate the efficacy of AMPA antagonists in vivo.

REFERENCES

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