Novel Urinary Markers in the Diagnosis and Management of Kidney Cancer

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• Washington University has a European patent (20120115747) and a US patent (9091690) for AQP1 as a “Method of Renal Cancer Detection”. PLIN2 patents pending.

• Member of the Scientific Advisory Board of Thrasos Therapeutics for the development of proprietary small peptides that act on the BMP/Smad pathway for the treatment of acute and chronic kidney diseases.

• Editorial Boards of: Kidney International American Journal Physiology-Renal
A Special Thanks

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Kidney Cancer Characteristics

• 3% of adult malignancies; 10th leading cause of cancer death.

• A “stealth” disease: silent/asymptomatic until advanced.

• Seventh among newly diagnosed cancers in men; more common than pancreatic cancer or leukemia.

• Tenth in women; as common as pancreatic or ovarian cancer, or leukemia.

• Survival (5 yr): <10% when symptomatically diagnosed (30-40% already metastatic).

>80% when diagnosed early (incidentally by imaging).

(American Cancer Society Facts and Figures 2015)
Kidney Cancer Characteristics

• Incidence varies from state-to-state and in counties within a state.

• Notoriously resistant to chemotherapy.

• Costs for treatment excessive.

• Early diagnosis enables:
  • Local vs metastatic disease.
  • Laparoscopic vs open nephrectomy.
  • Partial vs total nephrectomy.
  • Markedly improved survival.

(American Cancer Society Facts and Figures 2015)
Florida Incidence Rates of Kidney Cancer 2008-2012

All Races (includes Hispanic), Both Sexes, Ages 65+

Walton Co 96.7 (70.1, 130.3)/100,000

Hillsborough Co 63.5 (57.9, 69.6)/100,000

All Florida 57.5 (56.3, 58.7)/100,000

http://statecancerprofiles.cancer.gov/
The Cost of Treating Kidney Cancer

Two years after her husband's death, this cover story on 03Mar10 examines costs of keeping one man alive post nephrectomy.
Unmet Need for Kidney Cancer

• No diagnostic modality for early detection.

• Radiologic screening impractical.

• Unmet need: method for early detection of pre-metastatic kidney cancer.
  • Population screening.
  • Diagnosis of imaged renal masses.
Central Hypotheses

• Proteins upregulated in kidney cancer cells may be shed into urine.

• Urine proteins would be sensitive, specific biomarkers for detection/diagnosis.

• After preliminary testing of over 12 candidates, urine levels of aquaporin-1 (AQP1) and perilipin-2 (PLIN2), previously referred to as adipophilin (ADFP), were further evaluated.
Western blot Quantitation: AQP1

10 ug Ucr equivalent after Uprot ppt

Mayo Clin Proc 85:413-21, 2010
Western blot Quantitation: PLIN2

10 ug Ucr equivalent after Uprot ppt
Summary: Urinary AQP1 and PLIN2 as Biomarkers of Kidney Cancer

- Sensitive detection of clear cell and papillary subtypes of kidney cancer (almost 90% of malignant renal masses).
- Generally reflects tumor size and stage.
- Specificity:
  - Does not detect chromophobe (5%) or oncocytoma (4%).
  - Not confounded by non-cancerous kidney diseases.
  - Not confounded by bladder or prostate cancer.
Phases of Biomarker Discovery

• Phase 1: Identify promising biomarker(s). Pre-clinical exploratory.

• Phase 2: Identify clinical disease from potential confounders. Clinical assay and validation.

Pepe et al. JNCI J Natl Cancer Inst 2001; 93:1054-1061
Large Scale Evaluation of Biomarkers

- Measure urine AQP1 and PLIN2 *prospectively* in a large population of patients to evaluate absence/presence of renal cancer to determine sensitivity and specificity.

- Approach: Convenience sample of ~750 “normal” patients having abdominal CT at BJH. Collect urine and blood for AQP1 and PLIN2.

- Expect to discover 1-2 patients with kidney cancer.

- Supplement with ~ 20 patients with CT showing renal mass and pathologic confirmation of kidney cancer upon nephrectomy.
Screening Paradigm and Urine PLIN2

All Screened Patients

Urine PLIN2 (Units/mg Urine Creatinine) vs. Patient Number
Screening Paradigm and Urine PLIN2

Urine PLIN2 (Units/mg Urine Creatinine)

Patient Number

Known Kidney Cancer Patients
Screened Patients
Screening Paradigm and Urine PLIN2

- **Urine PLIN2 (Units/mg Urine Creatinine)**
  - **Screened Patients**
  - **Diagnosed Patients**

**Patient Number**

0 100 200 300 400 500 600 700 800
Screening Paradigm and Urine PLIN2

- **Diagnosed Patients**
- **Known Kidney Cancer Patients**
- **Screened Patients**

**Urine PLIN2 (Units/mg Urine Creatinine)**

**Patient Number**

0 100 200 300 400 500 600 700 800
Results of Screening Protocol

• Between AQP1 and PLIN2 assays, 3 patients were deemed suspicious of having kidney cancer.

• On retrospective review of these 3 patients, imaged renal masses were present.

• Two patients underwent partial nephrectomy and were found to have Grade 2, T1a clear cell carcinomas. The third patient died from other causes prior to a scheduled surgery.
Urine AQP1 Screening for Kidney Cancer

Sensitivity 0.92, Specificity 0.87, AUC 0.95
Urine PLIN2 Screening for Kidney Cancer

Sensitivity 0.85, Specificity 0.98, AUC 0.91
Statistical Resampling of Screened Patients

ROC Analysis possibly biased by 717 non-kidney cancer patients.

- Monte Carlo resampling method with a balanced dataset of the 21 active renal cancer patients and 21 randomly drawn patients from the 717 other patients.

- Calculate ROC AUC; repeat sampling 200 times.

- AQP1: Mean resampling AUC 0.95 (95% CI, 0.92-0.98)

- PLIN2: Mean resampling AUC 0.92 (95% CI, 0.90-0.93)
Phases of Biomarker Discovery

- Phase 1: Identify promising biomarker(s). Pre-clinical exploratory.
- Phase 2: Identify clinical disease from potential confounders. Clinical assay and validation.
- Phase 3: Identify early pre-clinical disease. Retrospective longitudinal.

Pepe et al. JNCI J Natl Cancer Inst 2001; 93:1054-1061
Positive Predictive Value vs Assay Specificity: Population screening paradigm

Incidence 65.3/100,000
Phases of Biomarker Discovery

• Phase 1: Identify promising biomarker(s). Pre-clinical exploratory.

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• Phase 3: Identify early pre-clinical disease. Retrospective longitudinal.

• Phase 4: Identify false positive, false negative rates. Prospective screening.
Clinical Management: Renal Mass Biopsy

• Increased but incidental detection of small renal masses by abdominal imaging. Patients asymptomatic.

• Between 70-80% of small renal masses malignant.

• Imaging alone cannot accurately diagnose small renal masses. (minimal fat angiomyolipomas).

• Recent studies support the use of renal mass biopsy to differentially diagnose small renal masses.
Clinical Management: Case For Renal Mass Biopsy

Data from SEER and J. Urology 193: 30-35, 2015
Clinical Management of Kidney Cancer

• The common approach to an imaged renal mass is partial or radical nephrectomy.


Clinical Management of Kidney Cancer

- Renal mass biopsy samples a small percentage of small renal masses.

- Each 2cm core of an 18 gauge biopsy samples only 0.26% of a 2cm tumor or 0.036% of a 4cm tumor.

- Documented intra-tumor heterogeneity of tumor grading then brings up the question of sampling in the biopsy.

- Need to improve pre-operative risk stratification.
Positive Predictive Value vs Assay Specificity: Differential Diagnosis of Imaged Renal Masses.

Assay Specificity

Positive Predictive Value

Incidence 85/100
Phases of Biomarker Discovery

- Phase 1: Identify promising biomarker(s). Pre-clinical exploratory.

- Phase 2: Identify clinical disease from potential confounders. Clinical assay and validation.

- Phase 3: Identify early pre-clinical disease. Retrospective longitudinal.

- Phase 4: Identify false positive, false negative rates. Prospective screening.

- Phase 5: Identify impact of screening on reducing burden of disease on population.

Pepe et al. JNCI J Natl Cancer Inst 2001; 93:1054-1061
Application of Urine Biomarkers for Kidney Cancer

- Differential diagnosis for imaged renal mass.
- Utility to monitor for post-surgical recurrence?
- Utility to monitor treatment of metastatic disease?
- Population screening for early disease diagnosis?
- Improved patient outcome: 80% cure with early discovery vs 90% fatality with late symptomatic discovery of disease.
- Preserve future kidney function by enabling partial nephrectomy or active surveillance.
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Renal Cell Carcinoma