Intraoperative Molecular Diagnostic Imaging Can Identify Renal Cell Carcinoma

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Abbreviations and Acronyms

CT = computerized tomography
FITC = fluorescein isothiocyanate
FRα = targeting folate receptor α
RCC = renal cell carcinoma
TBR = tumor-to-background ratio

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Purpose: Margin status can often be difficult to assess intraoperatively, particularly during partial nephrectomy given the time constraints related to renal hilar clamping. We hypothesized that a targeted molecular imaging approach could be used during surgery to identify tumor margins and confirm disease clearance.

Materials and Methods: EC17, a novel tracer targeting FRα, was used in murine models of renal cell carcinoma to identify positive margins after surgery. Positive margins were detected due to elevated tumor-to-background ratios of the tumor compared to surrounding normal tissues. We performed a pilot study in 4 patients using EC17 preoperatively with intraoperative imaging during the operation.

Results: FRα was highly expressed in 65% of clear cell renal cell carcinomas harvested from the operating room. In the murine model intraoperative imaging of renal cell carcinoma revealed a mean $\pm$ SD tumor-to-background ratio of 8.2 ± 1.1 in the RCC10, 11.2 ± 1.1 in the 786-0 and 4.3 ± 1.1 in the UMRC2 cell line. Compared to visual inspection intraoperative imaging of the surgical resection bed identified residual disease in 24% more animals. In the human pilot study targeted molecular imaging identified 2 of 4 renal cell carcinomas and had no false-positive results. In these 2 cases the tumor-to-background ratio was 3.7 and 4.6, respectively. In each case we confirmed disease clearance and tumor fluorescence did not correlate with nodule size or tumor grade.

Conclusions: To our knowledge this is the first demonstration in humans of identifying renal cell carcinoma during surgery using a targeted molecular contrast agent. This approach may lead to a superior method of identifying malignancy and tumor borders in the intraoperative setting.

Key Words: kidney; carcinoma, renal cell; nephrectomy; molecular imaging; fluorescein isothiocyanate

Clear cell RCC is the most common form of kidney cancer. There were 63,920 cases of kidney cancer diagnosed in 2014 in the United States.[1] The gold standard therapy for RCC remains surgical excision. However, visual inspection of the resection bed following partial nephrectomy can be inaccurate to ensure oncologic control. Additionally, the routine practice of intraoperative frozen section analysis at partial nephrectomy is controversial.[2]

Our group has been studying whether intraoperative molecular imaging has the potential to improve

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clear visualization of tumors and tumor borders at partial nephrectomy.[3] Molecular imaging is a technique by which small targeted molecules are used to generate or enhance visual information. Recent studies have shown that molecular imaging technology can be used in the operating room to detect cancer cells.[4–6] However, to our knowledge targeted intraoperative molecular imaging has not been demonstrated in humans for RCC, although several preclinical studies have been reported. For example, Yang et al recently synthesized the 2 heptamethine carbocyanine near infrared dyes IR-783 and MHI-148.[7,8] These contrast agents are taken up and retained by tumors due to differential expression of organic anion transporting peptides. In several preclinical studies human kidney cancer cells and circulating tumor cells preferentially accumulated these dyes, resulting in tumor fluorescence.

Recently a group from The Netherlands used a visible wavelength tagged molecular tracer to intraoperatively identify ovarian cancer nodules by targeting FRα.[4] Clear cell RCC is also known to be folate avid and express 10^3-fold to 10^4-fold FRα concentrations. Thus, we hypothesized that this tracer may also have clinical application to surgical resection of renal tumors.

The objective of our study was to describe the feasibility and preclinical application of a visible spectrum targeted, molecular contrast agent to detect RCC during surgery. Enhanced intraoperative visualization of RCC has potentially far-reaching applications. With superior tumor visualization such technology has the potential to allow for more efficient renal mass excision, limiting renal ischemia. While margin status is controversial in patients who undergo partial nephrectomy, this technology potentially enables more effective complete resection at surgery. Finally, it is possible that local recurrences and lymph node involvement may be identified using this technique.

First we confirmed that human renal adenocarcinomas have up-regulated FRα. We next performed several preclinical studies in cultured tumor cells and established xenograft models. Finally we translated our findings into a pilot study in 4 patients with RCC. We found that clear cell carcinoma has an up-regulated level of FRα and this targeted molecular imaging strategy can detect these tumors during surgery.

METHODS

Cell Lines

The cell lines 786-0 (CRL-1932), A498 (HTB-44, ATCC®), UMRC2 and RCC10 were used.[9] The human RCC cell line 786-0 was derived from a 58-year-old white male with a primary clear cell adenocarcinoma. The human RCC cell line A-498 was derived from a 52-year-old female. Cells were cultured in Dulbecco's modified Eagle's medium supplemented with penicillin/streptomycin, glutamine and 10% fetal bovine serum, deficient in folate (Mediatech, Washington, D.C.). Cultures were incubated at 37C in humidified air with 5% CO_2.

Folate-FITC

Folate-FITC (On Target Laboratories, West Lafayette, Indiana) is a conjugate between folate and FITC. This conjugate forms a negatively charged fluorescent molecule that binds weakly and nonspecifically to serum proteins at a level of approximately 75%. FITC is a synthetic organic compound that is excited in the 465 to 490 nm wavelength and emits light in the 520 to 530 nm range in the visible spectrum. For human studies a 0.1 mg/kg dose of this agent was dissolved in 10 ml normal saline and given to patients via peripheral vein injection 4 hours preoperatively.

Fluorescence Imaging

Intraoperative imaging was performed using the Artemis Fluorescence Imaging system (Quest Medical Imaging, Middenmeer, The Netherlands), the FloCam (BioVision, Exeter, Pennsylvania), a visualization system (Visionsense, Philadelphia, Pennsylvania) or a prototype system developed at our laboratory.[10] Positive and negative controls were used for all images. To quantitate tissue fluorescence we used region of interest software and the HeatMap plugin in ImageJ (http://rsb.info.nih.gov/ij/). A background reading was taken from adjacent normal lung tissue to generate the TBR.

Study Design

This study was approved by the University of Pennsylvania institutional review board and all patients provided informed consent. One and 3 patients scheduled for radical and partial nephrectomy, respectively, were eligible for study. All patients underwent CT with at least 0.1 cm slice thickness. Patients were asked to stop folate containing vitamins 1 week prior to surgery.

Four hours preoperatively patients were systemically infused with 0.1 mg/kg folate-FITC conjugate. Vital signs were obtained every 5 minutes following injection for 60 minutes after injection. Adverse events were monitored through the patient followup laboratory measurements 12 and 24 hours after injection. Additionally, patients were instructed to report any adverse events that developed up to 1 month after injection.

Surgery was performed via a retroperitoneal flank incision and the primary lesion was located using traditional methods. The fluorescence imaging system draped in sterile fashion was positioned above the retroperitoneum using a custom designed gantry device (BioMediCon, Moorestown, New Jersey). The operating room lights were switched off. The cancer was imaged and photodocumented by fluorescence and white light in situ. The remainder of the surgical cavity was imaged to detect any secondary lesions.

After removal frozen section biopsies of the tumor bed were performed as indicated. All specimens were sent for permanent histopathology evaluation. Final specimens were reviewed by a specialized genitourinary pathologist.