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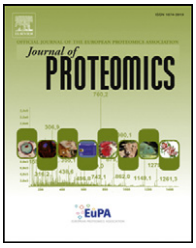

Discovery and validation of urinary biomarkers for detection of renal cell carcinoma

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ABSTRACT

IntroductionRenal cell carcinoma (RCC) is often accompanied by non-specific symptoms. The increase of incidentally discovered small renal masses also presents a diagnostic dilemma. This study investigates whether RCC-specific peptides with diagnostic potential can be detected in urine and whether a combination of such peptides could form a urinary screening tool.

Materials and methodsFor the discovery of RCC-specific biomarkers, we have employed CE–MS to analyze urine samples from patients with RCC (N = 40) compared to non-diseased controls (N = 68).

Results and discussion86 peptides were found to be specifically associated to RCC, of which sequence could be obtained for 40. A classifier based on these peptides was evaluated in an independent set of 76 samples, resulting in 80% sensitivity and 87% specificity. The specificity of the marker panel was further validated in a historical dataset of 1077 samples including age-matched controls (N = 218), patients with related cancer types and renal diseases (N = 859). In silico protease prediction based on the cleavage sites of differentially excreted peptides, suggested modified activity of certain proteases including cathepsins, ADAMTS and kallikreins some of which were previously found to be associated to RCC.

Abbreviations: ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; BMI, body mass index; DN, diabetic nephropathy; ECM, extracellular matrix, FDR, false discovery rate; LOOCV, leave one out cross-validation; MRI, magnetic resonance imaging; RCC, renal cell carcinoma; ROC, receiver operating characteristic; ST14, suppressor of tumorigenicity 14 protein; SVM, support vector machine; TCC, transitional cell carcinoma; TNM, tumor nodes metastases.

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ConclusionsRCC can be detected with high accuracy based on specific urinary peptides.

Biological significance
Clear cell renal cell carcinoma (RCC) has the highest incidence among the renal malignancies, often presenting non-specific or no symptoms at all. Moreover, with no diagnostic marker being available so far, almost 30% of the patients are diagnosed with metastatic disease and 30–40% of the patients initially diagnosed with localized tumor relapse. These facts introduce the clinical need of early diagnosis. This study is focused on the investigation of a marker model based on urinary peptides, as a tool for the detection of RCC in selected patients at risk. Upon evaluation of the marker model in an independent blinded set of 76 samples, 80% sensitivity and 87% specificity were reported. An additional dataset of 1077 samples was subsequently employed for further evaluation of the specificity of the classifier.

1. Introduction

Clear cell renal cell carcinoma (RCC) is the most common malignancy of the kidney. The conventional clear cell histological type is thought to arise from the proximal tubules and accounts for ~80% of RCC cases. Approximately 210,000 new cases of renal cancer are diagnosed each year worldwide, with over 100,000 deaths annually [1]. The standard treatment for locally advanced RCC is nephron-sparing or radical nephrectomy. In patients with small renal tumors and/or significant co-morbidity who are unfit for surgery, an ablative approach, e.g. cryotherapy and radiofrequency ablation remains an option [2,3]. To date no adjuvant therapy has been recommended. However several trials are currently investigating the efficacy of adjuvant tyrosine kinase inhibitors including sunitinib, pazopanib, axitinib and sorafenib in locally advanced disease [4]. Unfortunately in the metastatic situation this cancer is resistant to conventional chemotherapy or radiotherapy. In this case of poor prognosis, tyrosine kinase and mTOR inhibitors are approved as a second line option, increasing the sequential overall survival to 30 months [5–7].

The increase in disease rates, together with the fact that no diagnostic marker is available, has high socio-economic effects and underpins the demand, as recognized by the American Cancer Institute [8]. With either relatively non-specific or absent symptoms, about 30% of patients are diagnosed with distant or local metastatic disease. In addition, 30–40% of patients with initially clinically localized disease relapse during the follow-up period [9]. The 5-year survival for patients with metastatic disease is <10%. Earlier diagnosis would be beneficial. In parallel, through the wider use of abdominal imaging, an increasing number of tumors are found incidentally, many of which are small early stage tumors with up to 30% being benign. Although standard care is surgical excision/ablation, optimal management needs to balance the associated morbidity and risks of such procedures, particularly in elderly and/or frail patients with high levels of comorbidities, against the risk of the tumor progressing within the lifetime of the patient [4]. Markers which could be used in the differential diagnosis of the small renal masses and reduce the need for biopsy would be desirable.

Several recent reports demonstrated that urinary peptides and proteins can serve as specific biomarkers for e.g. chronic kidney diseases [10,11], bladder cancer [12,13], and prostate cancer [14]. Similarly to these previous studies and with the aim to identify early non-invasive urinary biomarkers of RCC, we have analyzed urine from RCC patients and age-matched controls using high resolution capillary electrophoresis coupled to mass spectrometry (CE–MS). This led to the identification and validation of a set of urinary peptide markers that enables detection of RCC with high sensitivity and specificity in a large study cohort that contains not only non-diseased controls, but also patients with similar diseases (other cancers), diseases affecting the same organ (kidney), or with systemic manifestations.

2. Materials and methods

2.1. Patients and samples

Clear cell RCC patients (N = 70) and center-specific non-RCC controls (N = 22) were recruited from those attending St James's University Hospital, Leeds, UK and University Hospital of Virginia, USA for investigation of suspected RCC during 2003 to 2006. An overall number of 1169 additional control samples were included from historical cohorts enrolled during 2003 to 2011 at 7 European clinical centers, namely Steno Diabetes Center Copenhagen, University of Glasgow, University of Groningen, Institut Universitaire de Recherche Clinique Montpellier, and the German hospital centers of Hamburg–Eppendorf, Hannover and Göttingen. Informed consent was obtained after local ethics committee approval. Studies were performed in accordance with the Helsinki Declaration. Urine samples were collected from patients with suspected RCC prior to any treatment, being later histologically confirmed to have clear cell RCC. All samples were spontaneously voided midstream urine and stored below −80 °C until CE–MS analyses. Table 1 summarizes the study design, describing the sample cohorts applied in the different phases. The cohorts of the patients used in the study as shown in Table 1 include a total of 1261 urine samples which were investigated for their peptide composition. Forty randomly selected patients with clear cell RCC and 68 non-diseased controls (containing 8 center specific controls and 60 from other clinical centers) were used as training set for RCC peptide marker discovery and marker panel establishment. The marker panel was subsequently validated in an independent set of 30 clear cell RCC patients and 46 non-diseased controls. Clinical characteristics of all patients and controls included in the training and validation cohort