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Original contribution

Profilin-1 expression is associated with high grade and stage and decreased disease-free survival in renal cell carcinoma☆,☆☆

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Summary Clear cell renal cell carcinoma (ccRCC) is associated with high mortality, although individual outcomes are highly variable. Identification of patients with increased risk of disease progression can guide customizing management plan according to disease severity. Profilin-1 (Pfn1) has been recently identified as overexpressed in metastatic ccRCC compared with primary tumors. We examined Pfn1 expression in a tissue microarray of 384 cases of histologically confirmed primary ccRCC with detailed clinical follow-up. Profilin-1 expression showed both cytoplasmic and nuclear staining patterns. The immunoexpression of Pfn1 was scored in a semiquantitative fashion. There was no significant difference in Pfn1 expression between normal kidney and kidney ccRCC. Our results show that strong cytoplasmic Pfn1 expression is associated with high-grade ($P < .001$) and high-stage (III-IV) ($P = .018$) disease. Univariate analysis of the data set showed that higher Pfn1 expression is associated with significantly shorter disease-free survival (hazard ratio 7.36, $P = .047$) and also lower overall survival. Kaplan-Meier analysis showed that high cytoplasmic expression of Pfn1 was also associated with a statistically

Abbreviations ccRCC, Clear cell renal cell carcinoma; Pfn1, Profilin 1; OS, Overall survival; DFS, Disease-free survival.

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significant lower disease-free survival (P = .018). It was also associated with lower overall survival, although this was not statistically significant. Profilin-1 lost its prognostic significance in the multivariate analysis when controlling for grade and stage. Profilin-1 expression was not associated with significant prognostic deference in the subgroup of patients with stage 1 disease. Our results suggest that the evaluation of Pfn1 by immunohistochemistry may help to identify patients with an increased risk of disease progression. We validated our results at the messenger RNA level on an independent patient cohort. Higher messenger RNA expression of Pfn1 is associated with significantly lower survival.

1. Introduction

Clear cell renal cell carcinoma (ccRCC) is the most common primary renal malignancy occurring in adults [1]. This tumor is associated with significant morbidity and mortality but, nonetheless, has a wide variance in individual patient outcomes—despite a reasonably uniform histologic appearance as compared with other malignancies with heterogeneous morphology and relatively uniform outcomes such as glioblastoma multiforme [2].

Until relatively recently, treating oncologists lacked useful therapeutic agents that demonstrated efficacy in patients with metastatic ccRCC, until the advent of sunitinib therapy [3] coupled with the recent development of several new second-generation small molecule kinase inhibitors approved for clinical use [4]. The need for pathologists to help identify patients with ccRCC at increased risk of disease progression and risk for metastatic spread is urgent because improved prognostic information now has the potential to better inform and optimize screening protocols and therapeutic regimens, heralding a new era of personalized medicine in kidney cancer [5]. The introduction of molecular profiling approaches that allow for simultaneous analysis of thousands of molecules [6] has significantly enhanced biomarker discovery, and independent additional validations are already showing the utility of leveraging a tumor's molecular signature to inform clinical practice [7,8].

Profilin-1 (Pfn1) is a 140 amino acid protein and major growth regulator of filamentous actin. It participates in many cellular activities, notably the polymerization of actin filaments for which it was originally recognized [9]. When human *profilin* was cloned in 1988, Northern blot analysis found the greatest concentration in human epithelial, muscle, and renal tissues [10]. Profilin-1 is now well established as a ubiquitously expressed actin monomer–binding protein involved in diverse cellular activities including actin monomer binding, actin polymerization, and transcriptional regulation [10,11]. Profilin-1 is required for cell survival, and double-knockout mouse embryos die before blastocyst formation [12].

Mutations in *PFN1* are known to be associated with human disease and have recently been identified as a cause of familial amyotrophic lateral sclerosis [13]. Profilin-1 has recently been implicated in the pathogenesis of several carcinomas, including breast [14] and bladder cancer [15]. Recent results by our laboratory and others have identified Pfn1 as being compara tively up-regulated in ccRCC as compared with normal kidney [16]. Using quantitative mass spectrometry analysis, we have recently identified several proteins, including Pfn1, that are dysregulated in metastatic as compared with primary ccRCC and demonstrated that they are involved in pathways related to tumor progression and metastasis and thus have the potential of being used as prognostic biomarkers [17,18].

Our current study aimed to evaluate the association between Pfn1 expression and pathologic (grade and stage) and clinical (disease-free survival [DFS] and overall survival [OS]) parameters and its potential utility as a prognostic marker for ccRCC.

2. Materials and methods

2.1. Tissue microarray construction

Pure areas from normal kidney cortex tissue and primary ccRCC were selected and circled from donor blocks by a pathologist. Tissue microarray (TMA) blocks containing duplicate 1.0-mm cores of 10% buffered formalin-fixed paraffin-embedded tissue blocks from each specimen were constructed with a manual tissue microarrayer (Beecher Instruments; Sun Prairie, WI). The TMAs contained 384 cases of primary ccRCC obtained from the surgical pathology archives of St. Michael's Hospital between 2001 and 2009. Each block contained 2 marker cores for TMA orientation. Included cases had clinical information that included sex, grade, stage, survival outcomes, and time to disease recurrence if applicable. The study was approved by the research ethics board of St. Michael's Hospital, Toronto. All cases were primary ccRCC and were reviewed by a pathologist. All new recognized entities including clear cell papillary, translocation carcinomas, etc, were excluded from the analysis. For 80 specimens, matched normal tissues from the same patient were also assessed.

2.2. Immunohistochemistry staining

Tissue microarray sections were cut 5-μm thick and placed on charged slides. Slides were deparaffinized in