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Original contribution

Immunoexpression status and prognostic value of mammalian target of rapamycin and hypoxia-induced pathway members in papillary cell renal cell carcinomas ☆

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Summary Dysregulation of the mammalian target of rapamycin and hypoxia-induced pathways has been consistently identified in clear cell renal cell carcinomas. However, experience with non–clear cell renal cell carcinoma subtypes is scant. In this study, we evaluated the immunohistochemical expression of upstream (PTEN and phosphorylated AKT) and downstream (phosphorylated S6 and 4EBP1) effectors of the mammalian target of rapamycin pathway, as well as related cell-cycle proteins (p27 and c-MYC), and a member of the hypoxia-induced pathway (HIF-1α) in 54 patients with papillary renal cell carcinoma treated by nephrectomy. PTEN was lower in tumor than in normal kidney, and loss of PTEN expression was found in 48% of the patients. In tumor tissues, phosphorylated S6, 4EBP1, and HIF-1α were higher than in normal kidney. Conversely, scores of p27 were lower in tumor than in normal kidney. Finally, scores of c-MYC and phosphorylated AKT were similar in tumor and in normal kidney. Overall mortality and cancer-specific mortality were 24% and 11%, respectively. Tumor progression was observed in 17% of the patients. None of the tested biomarkers predicted cancer-specific mortality or tumor progression. As expected, patients with high T-stage tumors had higher hazard ratios for cancer-specific mortality (hazard ratio, 6.9) and tumor progression (hazard ratio, 6.7). Patients with higher Fuhrman grades also had higher risks for cancer-specific mortality (hazard ratio, 11.4) and tumor progression (hazard ratio, 4.5). In summary, our study provides evidence of dysregulation of the mammalian target of rapamycin and hypoxia-induced pathways in papillary renal cell carcinoma. Immunohistochemistry for members of the mammalian target of rapamycin pathway and for HIF-1α lacked prognostic significance in our cohort.

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1. Introduction

Dysregulation of the mammalian target of rapamycin (mTOR) and hypoxia-induced pathways has been consistently identified in clear cell renal cell carcinomas (RCCs) [1,2]. Currently, inhibitors of the mTOR and vascular endothelial growth factor (VEGF) pathways are being used in patients with advanced clear cell RCC, either as first-line options or in refractory disease [3,4]. However, few clinical trials are addressing the role of mTOR inhibitors and VEGF antagonists in papillary RCC [3,5]. In addition, previous studies have analyzed the expression status and prognostic significance of members of the mTOR and hypoxia-induced pathways in RCC [2,6-8], but none has focused exclusively on papillary RCC. Considering that papillary RCC is the second most common subtype of RCC [9] with no established therapy for disseminated disease, such studies are needed.

In this study, we evaluated the immunohistochemical expression of upstream (phosthatase and tensin homolog [PTEN] and phosphorylated AKT [phos-AKT]) and downstream (phosphorylated S6 [phos-S6] and 4E-binding protein 1 [4EBP1]) effectors of the mTOR pathway, as well as related proteins (p27 and c-MYC), and a member of the hypoxia-induced pathway (hypoxia-inducible factor1 alpha [HIF1 α]) in patients with papillary RCC. First, we compared the expression of these biomarkers between normal kidney and tumor tissue. Second, we analyzed the associations between biomarkers and pathologic features of the primary tumor. Third, we evaluated the prognostic impact that these biomarkers may have on the outcome of patients with papillary RCC.

2. Materials and methods

The present study includes tissue samples from 54 consecutive patients with papillary RCC treated at the Johns Hopkins Medical Institutions (Baltimore, MD) between January 2004 and December 2006. All patients were treated by partial/radical nephrectomy without adjuvant therapy. After approval by the institutional review board, a retrospective study was performed with outcome assessment based on the chart review of clinical and pathologic data. Histologic slides were retrieved and reviewed by 2 urologic pathologists (R.A. and G.J.N.) for confirmation of the original diagnosis and pathologic staging, in compliance with the American Joint Committee on Cancer 2009 Classification [10]. Using a previously described procedure [11], 2 tissue microarrays were build. Four cores of tumor tissue and 4 cores of paired normal kidney tissue were spotted from each specimen. Patients were followed up from the date of surgery (mean, 55 months; median, 60 months; range, 10-91 months). For outcome analysis, end points included cancer-related death, tumor progression, and overall mortality. *Tumor progression* was defined as the presence of pelvic recurrence or metastasis to distant sites. Overall mortality refers to all-cause death.

2.1. Immunohistochemistry

Immunohistochemistry was performed for the following proteins: PTEN, phos-AKT, phos-S6, 4EBP1, c-MYC, p27, and HIF-1α. Immunostaining was performed on formalin-fixed, paraffin-embedded tissue sections using the PowerVision Poly-HRP IHC Detection Systems (Leica Microsystems, Bannockburn, IL). Sections were deparaffinized, rehydrated, and subjected to heat-induced antigen retrieval with a buffer solution using a steamer. Sections were then incubated with the appropriate primary antibody. After the application of an antirabbit or antimouse poly-HRP secondary (except for c-MYC, for which the Dako Catalyzed Signal Amplification System Kit was used [Dako, Carpinteria, CA]), the slides were developed using 3-3′-diaminobenzidine chromogen and counterstained with hematoxylin. Proper cell lines were used as external controls, and internal controls were checked for negative and positive immunohistochemical expression. For HIF-1α, the protocol described by Tickoo et al [12] was used. Table 1 lists information regarding antibodies and vendors.

2.2. Scoring system

Immunohistochemistry staining was evaluated using a previously validated methodology [2]. Both tumor cells and normal epithelial cells from proximal and distal renal tubules

Table 1 Summary of antibodies used for immunohistochemical analysis

	Vendor	Clone	Pretreatment	Dilution
PTEN	Cell Signaling (Beverly, MA)	D4.3	EDTA, 45 min	1:100
c-MYC	Epitomics (Burlingame, CA)	Y69	EDTA, 45 min	1:300
p27	Transduction Lab (Sparks, MD)	57	Citrate, 25 min	1:4000
phos-AKT [a]	Cell Signaling	736E11	EDTA, 45 min	1:50
phos-S6 [b]	Cell Signaling	Polyclonal	EDTA, 45 min	1:200
4EBP-1	ProSci (San Diego, CA)	Polyclonal	Citrate, 25 min	1:250
HIF-1α	Novus Biologicals (Littleton, CO)	NB100-123	Heat (oven) at 62°C, 60 min	1:1600

[a] Phosphorylation site at Ser473.
[b] Phosphorylation site at Ser235/236.