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Original article

Prognostic impact of concomitant loss of PBRM1 and BAP1 protein expression in early stages of clear cell renal cell carcinoma

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Abstract

Purpose: To evaluate the prognostic impact of immunohistochemical expression of BAP1 and PBRM1 in patients with early stage (pT1–pT2N0M0) clear cell renal cell carcinoma (ccRCC).

Patients and methods: A total of 441 consecutive patients treated surgically for stages I and II (TNM-AJCC 2010) ccRCC between 1990 and 2016 were selected. All cases were reviewed for uniform reclassification and the most representative tumor areas were selected for the construction of a tissue microarray. Sixty-two patients had frozen tumoral tissue available in the tumor bank of our institution for quantitative real-time reverse transcriptase polymerase chain reaction analysis.

Results: Of the 441-immunostained ccRCC specimens, 91 (20.6%) and 107 (24.3%) showed negative-expression of PBRM1 and BAP1, respectively. Fifty-eight (13.2%) showed negative-expression of both markers (PBRM1-/BAP-). There was an association between both markers expression pattern and classical parameters, such as pT stage ($P < 0.001$), tumor size ($P < 0.001$), and tumor grade ($P < 0.001$). Both independent PBRM1 and BAP1 negative-expression were associated with lower rates of disease-specific survival and recurrence-free survival. When patients were grouped into presence of positive expression of one or both markers vs. PBRM1-/BAP1- patients, disease-specific survival and rates were 95.3% vs. 77.6%, respectively ($P < 0.001$). PBRM1-/BAP1-group presented a higher risk of cancer specific death (hazard ratio = 2.722, $P = 0.007$) and disease recurrence (hazard ratio = 2.467, $P = 0.004$) in multivariate analysis.

Conclusion: Patients with early stage tumors that present concomitant loss of both PBRM1 and BAP1 demonstrated worse survival rates and represent a relevant risk group for tumor recurrence and death.

Keywords: Renal carcinoma; Molecular marker; Prognosis

1. Introduction

Renal cell carcinoma (RCC) is the second most common urologic malignancy and recent studies show an increase in its incidence in the United States [1]. At the same time RCC mortality has remained stable, suggesting improved survival for patients with RCC [1,2]. Clear cell renal cell carcinoma (ccRCC) is the most common and aggressive histologic subtype accounting for the most RCC-specific deaths [2]. Predictors of recurrence and prognosis have been evaluated in a series of prognostic nomograms in order to assist physicians to better select a treatment modality and decide when to start treatment [3]. However, there is room for improvement in the predictive power of such tools.

The increasing incidental detection rates of RCC, mostly due to the increased use of abdominal imaging led to a significant stage migration. Despite significant advances in the understanding of small renal masses, there is still no consensus about the ideal candidate for active treatment or

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observation. Therefore, improving the selection of candidates who will benefit from active treatment becomes necessary, and the identification of molecular biomarkers might help distinguish diseases with indolent or aggressive behavior [4]. Two recently published prospective trials addressing the impact of adjuvant tyrosine-kinase inhibitors (TKI) in high-risk localized RCC showed conflicting results [5,6]. Such results, in combination with ongoing trials using immune checkpoint inhibitors, have reinvigorated expectations concerning the use of adjuvant therapy in appropriately selected patients with a high risk of recurrence following surgical treatment. Identifying this high-risk group of patients remains a clinical challenge. The incorporation of molecular markers into conventional models is anticipated to enhance their predictive accuracy [4].

Recent analyses of ccRCC using next-generation sequencing revealed frequent mutations including PBRM1, BAP1, SETD2, and KDM5C [7]. These genes encode proteins that regulate chromatin and most reported somatic mutations result in loss of function, indicating that these proteins function as tumor suppressors [8]. PBRM1 is the second most commonly mutated gene in ccRCC. However, the biological consequences of PBRM1 mutations for kidney tumorigenesis are still poorly understood. PBRM1 gene encodes BAF180 protein, which is a subunit of the ATP-dependent complex of chromatin remodeling called SWI/SNF (SWItch/sucrose nonfermentable). Such complex plays a role in the mobilization of nucleosomes by promoting insertion or removal of histones from the chromatin [9]. Both PBRM1 and BAP1 genes are situated on chromosome 3p in a region that is deleted in more than 90% of RCCs. Mutations in BAP1 occur in 5% to 15% of sporadic ccRCC tumors, and germline BAP1 mutations occur in some cases of ccRCC. BAP1 functions as a deubiquinating enzyme that regulates multiple cellular pathways related to tumorigenesis [7]. Loss of BAP1 tissue expression has been reported as a poor prognostic event in ccRCC [10].

Small renal masses biopsy data are emerging and are becoming more common in centers that routinely incorporate this into clinical practice. The rationale of using molecular markers in this scenario seems reasonable [11]. The aim of the present study was to evaluate the prognostic impact of immunohistochemical expression of BAP1 and PBRM1 in patients with early stage (pT1–pT2N0M0) ccRCC.

2. Patients and methods

In all, 604 consecutive cases involving radical or partial nephrectomy for ccRCC between 1990 and 2016 were selected from the medical records of our institution. Abdominal computed tomography or magnetic resonance imaging were used as the standard imaging methods for diagnostic confirmation. In suspected cases of systemic metastases, chest computed tomography and bone scans were performed. Patients were evaluated quarterly during the first 2 years and every 6 months thereafter. Four uropathologists reviewed all of the cases for uniform reclassification according to the new renal tumor classification [12,13] and determined the selection of the most representative tumor areas for the construction of the tissue microarray (TMA). Our internal review board approved the present study. Samples were provided by our institution biobank with patient's informed consent.

The following variables were included in the data bank: age, sex, time since diagnosis, type of surgery, Eastern Cooperative Oncology Group (ECOG) status, staging (TNM American Joint Committee on Cancer [AJCC]/Union Internationale Contre le Cancer [UICC] 2010), UCLA Integrated Staging System (UISS) [14], ISUP grade, histological subtype, lymphovascular invasion (LVI), perinephric fat invasion, lymph node involvement, presence of tumor necrosis, presence of metastases, and analysis of PBRM1 and BAP1 immunohistochemical expression patterns. During radical nephrectomy, retroperitoneal lymphadenectomy was restricted to the renal hilum and was performed for staging purposes only. In nephron-sparing procedures lymph node dissection was not performed. For the purpose of this study, patients with metastatic disease at presentation or with lymph node metastases were excluded and only confirmed cases of stages I and II (pT1–pT2N0M0) ccRCC were selected, resulting in 441 patients.

2.1. TMA construction

Two cylinders measuring 1 mm in diameter taken from the original paraffin blocks from different parts of the tumor were used to build a TMA. Sequential 4-μm sections were obtained for the immunohistochemical study. A Hematoxylin and Eosin (HE) was also performed to check the quality of the TMA and the presence of the tumor in the spots.

2.2. Immunohistochemistry

The sections were mounted on positively charged glass slides and dried for 30 minutes at 37°C. The sections were deparaffinized in xylene and rehydrated via a series of graded alcohols. Sections were then incubated with a primary rabbit polyclonal antibody against BAF180 (Methyl, Montgomery, TX) at a 1:500 dilution for 60 minutes. For BAP1 reactions, sections were incubated with a primary mouse monoclonal antibody against BAP1—clone C4 (Santa Cruz, Dallas, TX) at a 1:100 dilution for 60 minutes. All immunohistochemical procedures were performed automatically in the auto-stainer Benchmark ULTRA (VENTANA), using the Flex Plus visualization system according to the supplier′s specifications. The same pathologists "blinded" to the outcome of the cases, semiquantitatively scored the nuclear staining intensity of PBRM1 and BAP1 in all specimens according to the number of positive cells and it ranged from 0% to 100%. For immunohistochemical score assessment, all spots were evaluated in duplicate and then a mean of the 2 spots for