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Original article

Loss of BAP1 protein expression in the first metastatic site predicts prognosis in patients with clear cell renal cell carcinoma

Yuji Miura, M.D.[a,*], Naoko Inoshita, M.D., Ph.D.[b], Masaomi Ikeda, M.D., Ph.D.[c], Yu Miyama, M.D., Ph.D.[b], Ryosuke Oki, M.D.[a], Suguru Oka, M.D.[c], Chihiro Kondoh, M.D.[a], Yukinori Ozaki, M.D.[a], Yuko Tanabe, M.D.[a], Kazuhiro Kurosawa, M.D.[c], Shinji Urakami, M.D., Ph.D.[c], Tadasu Kohno, M.D., Ph.D[d], Toshikazu Okaneya, M.D., Ph.D.[c], Toshimi Takano, M.D.[a]

[a] *Department of Medical Oncology, Toranomon Hospital, Tokyo, Japan*
[b] *Department of Pathology, Toranomon Hospital, Tokyo, Japan*
[c] *Department of Urology, Toranomon Hospital, Tokyo, Japan*
[d] *Department of Thoracic Surgery, Toranomon Hospital, Tokyo, Japan*

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Abstract

Objectives: To investigate the intratumoral heterogeneity of BAP1 and PBRM1 expression at the primary site and metastatic sites and to evaluate whether BAP1 and PBRM1 expression in metastatic sites of clear cell renal cell carcinoma (ccRCC) has prognostic value.

Methods and materials: We collected paired samples from the primary site and the first metastatic site in 41 patients with ccRCC. Immunohistochemistry analyses were performed for the expression of BAP1 and PBRM1 proteins. We retrospectively analyzed the associations between the expression of BAP1 and PBRM1 and overall survival (OS).

Results: The most common first metastatic sites were lung (68.3%) and lymph node (12.2%). BAP1 protein expression was negative in 8 (19.5%) primary sites and in 11 (26.8%) metastatic sites. PBRM1 protein expression was negative in 9 (22.0%) primary sites and in 11 (26.8%) metastatic sites. The incidences of intratumoral heterogeneity for BAP1 and PBRM1 protein expression in primary/metastatic sites were 9.8%/2.4% and 24.4%/7.3%, respectively. The concordance rates between primary and metastatic sites for BAP1 and PBRM1 protein expression were 82.9% and 63.4%, respectively. Median OS from the first occurrence of metastasis in patients with BAP1-positive and BAP1-negative metastatic sites were 97 months (95% CI: 58–136) and 51 months (95% CI: 13–82), respectively ($P = 0.0077$). Median OS in patients with PBRM1-positive and PBRM1-negative metastatic sites were 82 (95% CI: 42–97) and 120 (95% CI: 52–120) months, respectively ($P = 0.25$).

Conclusion: Intratumoral heterogeneity of BAP1 protein expression is more frequent in primary tumor than in metastatic sites. The loss of BAP1 protein expression in metastatic sites predicts poor prognosis in patients with ccRCC.

Keywords: BAP1; PBRM1; Immunohistochemistry; Intratumoral heterogeneity

1. Introduction

Renal cell carcinoma (RCC) accounts for 80% to 85% of all primary kidney neoplasms [1]. The incidence rates of RCC in Japan were 8.2 and 3.6 per 100,000 population for men and women, respectively [2]. Among patients newly diagnosed with RCC, 25% to 30% have metastases at initial presentation. Additionally, approximately 30% of patients with localized RCC who undergo radical or partial nephrectomy relapse at a median of 1.9 years after surgery [1,3]. Common metastatic sites include lung, bone, and liver. The most common subtype of RCC is clear cell RCC (ccRCC), which accounts for approximately 70% to 80% of all RCC [4]. The von Hippel-Lindau tumor suppressor (*VHL*) gene

* Corresponding author: Yuji Miura, M.D. Tel.: +81-3-3588-1111; fax: +81-3-3560-7673.
E-mail address: yujmiura@mac.com (Y. Miura).

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on chromosome 3p25.3 appears to be important in the pathogenesis of sporadic ccRCC. *VHL* gene inactivation by somatic mutation or methylation is detected in 82% to 92% of ccRCC cases [5,6]. This gene inactivation leads to stabilization of hypoxia-inducible factors *HIF-1α* and *HIF-2α*. Furthermore, recent studies using whole-genome and exome sequencing technology identified additional genetic alterations associated with ccRCC tumor behavior, e.g., mutations in BRCA1-associated protein-1 (*BAP1*), polybromo-1 *(PBRM1*), and SET domain-containing 2 (*SETD2*) [7–9]. *BAP1*, *PBRM1,* and *SETD2* are chromatin-modifying genes located on chromosome 3p21, and modifications of these genes are detected in approximately 10% to 15% [7], 41% [8], and 10% to 15% [9] of ccRCC cases, respectively. Patients with *BAP1*-mutant ccRCC had shorter overall survival (OS) than those with *PBRM1* mutations [10]. BAP1 protein expression can be assessed by an immunohistochemistry assay with positive and negative predictive values of 100% and 98.6%, respectively [7]. The loss of BAP1 protein expression, as determined by this immunohistochemistry assay, was associated with poor OS in patients with ccRCC [11]. However, all the samples in these studies were collected from the primary tumor site. We hypothesized that the presence of BAP1 mutations in metastatic sites might predict prognosis more accurately than those in the primary site because intratumoral heterogeneity is often observed in the primary site of ccRCC [12]. The purpose of this study was to investigate the intratumoral heterogeneity of BAP1 expression at the primary site and metastatic sites and to evaluate whether the loss of BAP1 expression at metastatic sites is an effective prognostic marker.

2. Materials and methods

Data collection and analysis were approved by the Institutional Review Board of Toranomon hospital.

2.1. Patients

Using the RCC database in the Department of Urology, we performed a retrospective survey of 504 patients with RCC who underwent either radical or partial nephrectomy at our institution between April 1988 and January 2013. Our survey identified 103 (20.4%) patients in whom RCC recurred. The Pathological Diagnosis Database in the Department of Pathology was cross-referenced for patients who underwent biopsy or surgical resection of the first metastatic site, and 50 (9.9%) patients were extracted. Formalin-fixed paraffin-embedded tissue samples of the primary tumors and matched first metastatic sites were retrieved from the archives of our hospital. In total, 41 paired samples were pathologically evaluable. The pathologic T stage of tumors was classified according to the 2010 American Joint Committee of Cancer TNM classification [13]. Tumor grade was determined by the Fuhrman grading system [14]. A pathologist (N.I.) reviewed representative hematoxylin and eosin–stained sections and evaluated the evidence of necrosis, sarcomatoid features, and Fuhrman grade. At least 2 regions of the primary site and 1 region of the metastatic site were evaluated to construct a tissue microarray (TMA) of pathologic features.

2.2. Detection of BAP1 and PBRM1 by immunoassay

Immunohistochemistry analyses were performed for BAP1 (Santa Cruz Biotechnology Inc., Dallas, TX, USA) and PBRM1 (Bethyl Laboratories Inc., Montgomery, TX, USA). Positive staining was defined as uniform staining on all tumor nuclei (Fig. 1A for BAP1, Fig. 1C for PBRM1). Negative staining was defined as all tumor nuclei uniformly stained negative. Intratumoral inflammatory cells or

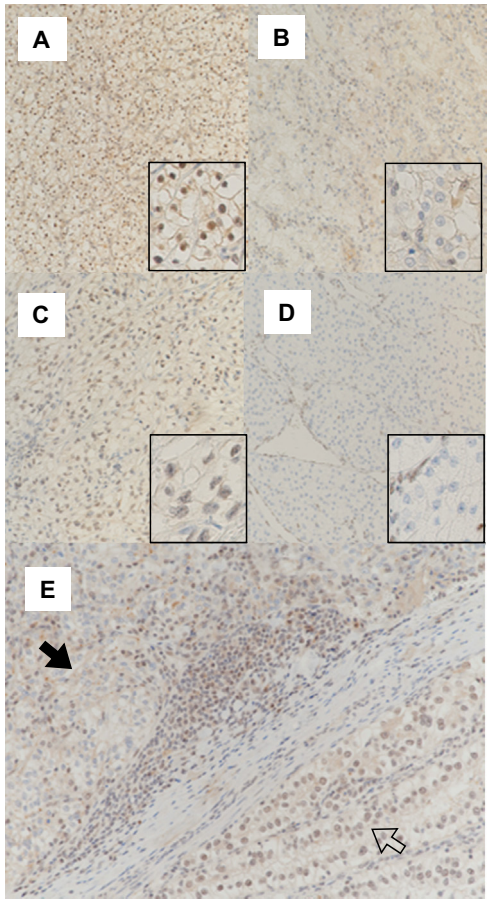

Fig. 1. Representative photomicrographs of immunohistochemistry-stained ccRCC tumor samples. (A) BAP1-positive tumor. All tumor nuclei are uniformly stained positive for BAP1. (B) BAP1-negative tumor. All tumor nuclei are uniformly stained negative for BAP1. Inflammatory cells or vascular endothelial cells serve as positive controls. (C) PBRM1-positive tumor. All tumor nuclei are uniformly stained positive for PBRM1. (D) PBRM1-negative tumor. All tumor nuclei are uniformly stained negative for PBRM1. Inflammatory cells or vascular endothelial cells serve as positive controls. (E) BAP1 heterogeneous tumor. Open arrow, BAP1-positive tumor cells; solid arrow, BAP1-negative tumor cells. (A) to (D) are ×100. (E) and the small boxes in (A) to (D) are ×200.