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Original Article

Loss of BAP1 expression in metastatic tumor tissue is an event of poor prognosis in patients with metastatic clear cell renal cell carcinoma

Walter Henriques da Costa, M.D., Ph.D.*, Aline Fusco Fares, M.D., Ph.D., Stephania M. Bezerra, M.D., Mariana A Morini, M.D., Ligia Alencar de Toledo Benigno, M.D., Diego Abreu Clavijo, M.D., Lucas Fornazieri, M.D., Maurício Murce Rocha, M.D., Isabela Werneck da Cunha, M.D, Ph.D., Stenio de Cassio Zequi, M.D., Ph.D.

A.C. Camargo Cancer Center, Urology Division, Sao Paulo, Brazil

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Abstract

Purpose: To evaluate the prognostic impact of the protein expression of both PBRM1 and BAP1 in metastatic tissue of patients with metastatic clear cell renal cell carcinoma (ccRCC).

Patients and methods: In all 124 consecutive cases of metastatic ccRCC, who underwent metastasectomy or biopsy of metastatic tumor tissue between 2007 and 2016 were selected from the medical records of our institution. Additionally, 38 paired cases with tissue from the primary tumor involving radical or partial nephrectomy for ccRCC were also selected. All cases were reviewed for uniform reclassification and the most representative tumor areas were selected for the construction of a tissue microarray.

Results: PBRM1 nuclear staining of the 124-immunostained metastases of ccRCC specimens showed that 98 (79.0%) had negative expression and 26 (21.0%) positive expression of PBRM1. Regarding BAP1 expression, we observed that 77 (62.1%) specimens were negative and 47 (37.9%) showed positive nuclear staining. When we compared the expression of both markers on primary tumor and tumor metastasis, we found disagreement in half of the cases. Five-year overall survival rates in patients with positive expression and negative expression of BAP1 were 53.2% and 35.1%, respectively ($P = 0.004$). Five-year progression-free survival rates in patients with positive expression and negative expression of BAP1 were 14.9% and 3.9%, respectively ($P = 0.003$). Conversely, PBRM1 expression did not significantly influence either overall survival or progression-free survival rates. In multivariate analysis, negative expression of BAP1 tumors also presented higher risks of death (hazard ratio (HR) = 1.913, $P = 0.041$) and disease progression (HR = 1.656, $P = 0.021$).

Conclusion: The use of prognostic biomarkers identified in the primary tumor tissue might be not reliable in the metastatic disease scenario. Patients with metastatic ccRCC that present loss of BAP1 expression in metastatic tissue demonstrated poor survival rates and represent a relevant risk group for tumor recurrence and death.

Keywords: Renal carcinoma; Molecular marker; Metastasis; Prognosis

1. Introduction

Kidney cancer accounts for 5% of all cancers in men and 3% of all cancers in women, and approximately 15% of these patients present metastatic disease at diagnosis [1]. About 70% of renal cell carcinomas (RCC) present with localized disease and about 30% of patients who undergo surgery with curative intent may experience a recurrence. Clear cell renal cell carcinoma (ccRCC) is the most common histological subtype and accounts for the most RCC-specific deaths [2]. Recent analyses of ccRCC using next-generation sequencing revealed novel, frequent mutations including PBRM1, BAP1, SETD2, and KDM5C [3]. Such genes encode proteins involved in chromatin regulation and function as tumor suppressors.

PBRM1 is the second most commonly mutated gene in ccRCC. PBRM1 encodes BAF180 protein, which is a

*Corresponding author. Tel.: +55-5511-3021-4155.
E-mail addresses: drwalterdacosta@gmail.com, walter.costa@accamargo.org.br (W.H. da Costa).

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subunit of the adenosine triphosphate (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 [4]. 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 deubiquitinating enzyme that regulates multiple cellular pathways related to tumorigenesis [3]. Both PBRM1 and BAP1 are situated on chromosome 3p in a region that is deleted in more than 90% of RCCs.

In spite of some studies with conflicting results, the loss of tissue expression of both markers has been associated with worse outcomes and lower survival rates in patients with ccRCC. Previous studies have described the loss of gene and protein expression of PBRM1 in primary tumors as an adverse event in patients with ccRCC [5,6,7] while a previous study did not observe such finding [8]. The prognostic role of loss of BAP1 protein expression is also well recognized as a poor prognostic event in patients with ccRCC [9,10]. Our group recently evaluated the prognostic impact of both markers in pT1-pT2 ccRCC patients and confirmed that tumors with concomitant loss of both PBRM1 and BAP1 represented a relevant risk group for tumor recurrence and death [11].

Despite extensive literature describing the prognostic role of the tissue expression pattern of both markers in primary ccRCC tumors, there is little evidence of the potential prognostic and predictive power of response to systemic therapies of the pattern of BAP1 and PBRM1 expression in metastatic tissue. In view of the intratumor heterogeneity in metastatic RCC, a better understanding of the molecular pathways of carcinogenesis and of potential reliable biomarkers for both tyrosine kinase inhibitors (TKI) and checkpoint inhibitors is critical [12].

The aim of our study was to evaluate the prognostic impact of the protein expression of both PBRM1 and BAP1 in metastatic tissue of patients with metastatic ccRCC. In addition, we sought to investigate the role of both markers as predictive markers to systemic therapy.

2. Patients and methods

In all, 124 consecutive cases of metastatic ccRCC who underwent metastasectomy or biopsy of metastatic tumor tissue between 2007 and 2016 was selected from the medical records of our institution. Additionally, 38 paired cases with tissue from the primary tumor involving radical or partial nephrectomy for ccRCC were also selected from the medical records our center cohort (member of the LARCG-Latin American Renal Cancer Group: http://www.larcg.org). Abdominal CT or MRI was used as the standard imaging methods for diagnostic confirmation. In suspected cases of systemic metastases, chest CT, and bone scans were performed. Patients were evaluated quarterly during the first 2 years and every 6 months thereafter. Two uropathologists reviewed all cases for uniform reclassification according to the new renal tumor classification [13,14] 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, gender, type of nephrectomy, Karnofsky performance scale, the International Society of Urological Pathology (ISUP) grade, histological subtype, metastasis site, metastasectomy, and AJCC staging [15]. Regarding laboratory tests, we collected initial hematocrit and hemoglobin, neutrophil count, platelet count, total corrected calcium, and lymphocytes. Patients were categorized into risk groups according to the IMDC (International Metastatic Renal Cell Carcinoma database) criteria at low risk (0 positive variables), intermediate (1−2 positive variables), and high (3 or more positive variables) [16]. 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.

2.1. TMA construction

Two cylinders measuring 1 mm in diameter taken from the original paraffin blocks from metastatic samples 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, USA) 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, USA) at a 1:100 dilution for 60 minutes. All immunohistochemical procedures were performed automatically in the autostained 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, semi-quantitatively 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 each case was used for analysis. As a positive control of the

Table 1
Patients and pathological characteristics and the association between different variables and PBRM1 and BAP1 immunohistochemical expression.

Variable	PBRM1 Negative expression *n* (%)	PBRM1 Positive expression *n* (%)	*P* value	BAP1 Negative expression *n* (%)	BAP1 Positive expression *n* (%)	*P* value
Gender						
Male	70 (71.4)	18 (69.2)	0.827	53 (68.8)	35 (74.5)	0.502
Female	28 (28.6)	8 (30.8)		24 (31.2)	12 (25.5)	
Risk group						
Good	20 (22.7)	7 (31.8)	0.355	18 (26.5)	9 (21.4)	0.397
Intermediate	49 (55.7)	13 (59.1)		35 (51.5)	27 (64.3)	
Poor	19 (21.6)	2 (9.1)		15 (22.1)	6 (14.3)	
Cytoreductive nephrectomy						
Yes	89 (90.8)	25 (96.2)	0.374	68 (88.3)	46 (97.9)	0.088
No	9 (9.2)	1 (3.8)		9 (11.7)	1 (2.1)	
Metastasectomy						
Yes	37 (37.8)	12 (46.2)	0.436	26 (33.8)	23 (48.9)	0.094
No	61 (62.2)	14 (53.8)		51 (66.2)	24 (51.1)	
Systemic treatment						
No	16 (21.1)	9 (19.1)	0.637	20 (20.4)	5 (20.0)	0.550
Sunitinib	41 (53.9	25 (53.2)		51 (52.0)	15 (60.0)	
Sorafenib	2 (2.6)	0 (0)		1 (1.0)	1 (4.0)	
Pazopanib	9 (11.8)	10 (21.3)		16 (16.3)	3 (12.0)	
Temsirolimus	5 (6.6)	1 (2.1)		6 (6.1)	0 (0)	
Interferon	2 (2.6)	1 (2.1)		3 (3.1)	0 (0)	
Interleukin (IL-2)	1 (1.3)	1 (2.1)		1 (1.0)	1 (4.0)	
pT stage						
pT1a	9 (13.2)	2 (13.3)	0.966	9 (18.4)	2 (5.9)	0.446
pT1b	13 (19.1)	3 (20.0)		10 (20.4)	6 (17.6)	
pT2a	17 (25.0)	3 (20.0)		9 (18.4)	11 (32.4)	
pT2b	8 (11.8)	2 (13.3)		5 (10.2)	5 (14.7)	
pT3a	13 (19.1)	4 (26.7)		11 (22.4)	6 (17.6)	
pT3b	4 (5.9)	0 (0)		3 (6.1)	1 (2.9)	
pT4	4 (5.9)	1 (6.7)		2 (4.1)	3 (8.8)	
ISUP grade						
Low grade	28 (47.5)	6 (40.0)	0.605	20 (45.5)	14 (46.7)	0.918
High grade	31 (52.5)	9 (60.0)		24 (54.5)	16 (53.3)	
Primary tumor PBRM1 expression pattern						
Negative	15 (46.9)	2 (33.3)	0.540	12 (52.2)	5 (33.3)	0.254
Positive	17 (53.1)	4 (66.7)		11 (47.8)	10 (66.7)	
Primary tumor BAP1 expression pattern						
Negative	14 (43.8)	2 (33.3)	0.635	9 (39.1)	7 (46.7)	0.646
Positive	18 (56.3)	4 (66.7)		14 (60.9)	8 (53.3)	

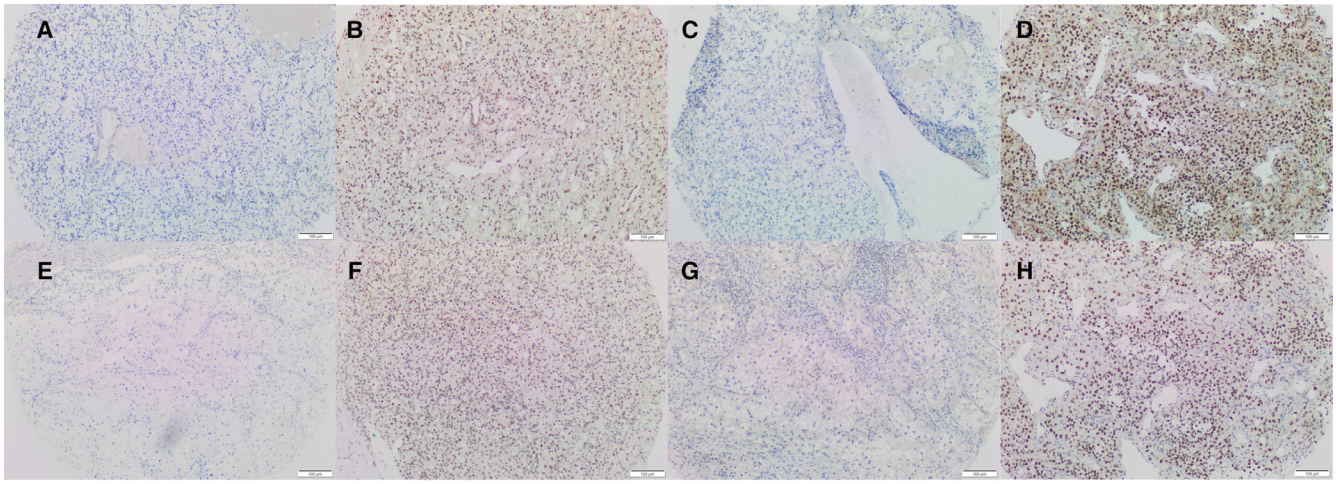

Fig. 1. Photomicrographs of immunohistochemical expression of PBRM1 and BAP1. (A) Negative expression of PBRM1 in a primary tumor. (B) Positive expression of PBRM1 in a primary tumor. (C) Negative expression of PBRM1 in metastatic tissue. (D) Positive expression of PBRM1 in metastatic tissue. (E) Negative expression of BAP1 in a primary tumor. (F) Positive expression of BAP1 in a primary tumor. (G) Negative expression of BAP1 in metastatic tissue. (H) Positive expression of BAP1 in metastatic tissue.

reaction, nonneoplastic renal tissue with positive staining for both markers was used. As a negative control, the standard staining protocol was used except for suppression of the primary antibody. The positivity criterion used was the finding of PBRM1 or BAP1 nuclear positivity of any intensity. Nonprimary control antibody was used. For quantitative analysis of BAP1 and PBRM1 expression, we determined 2 groups of observations with respect to a simple cut-point (Negative or Positive), which was estimated using the maximum of the standardized log-rank statistic proposed by Lausen and Schumacher [17].

2.3. Statistical analysis

To verify the association between PBRM1 and BAP1 immunohistochemical expression and the other variables, Pearson chi-square tests were used. Fisher exact test was applied for those cases in which the expected frequencies were <5. The Mann-Whitney test and Kruskal-Wallis tests were used to compare means among different expression levels of PBRM1 and BAP1 groups.

Overall survival (OS) was defined as the interval between primary surgery and the last follow-up visit or disease-related death. Progression-free survival (PFS) was defined as the interval between primary surgery and the last follow-up visit without disease or evidence of recurrence. To study DSS and PFS, Kaplan-Meier curves and the log-rank test were used. Cox proportional hazards model was used to determine which variables influenced survival. The confidence interval was 95%.

3. Results

The majority of patients were male (71.0%). In all, 114 (91.9%) patients had cytoreductive nephrectomy. Their mean (range) age was 57.4 (30–85) years. The median follow-up period was 42.8 months. It was observed that 44 (35.5%) patients presented synchronous metastases and 80 (64.5%) patients had metachronous metastases. 48 (56.5%) patients had pT1-pT2 disease while the majority of patients had high ISUP grade tumors. Regarding therapeutic options, 49 (39.5%) patients were submitted to isolated metastasectomy and 67 (54.0%) received systemic treatment only. The most commonly used first-line systemic therapeutic options were sunitinib (53.7%), followed by pazopanib (15.3%) and temsirolimus (4.9%). At the end of the study, 64 (51.6%) patients were alive and 60 (48.4%) patients had died of ccRCC (Table 1).

PBRM1 nuclear staining of the 124-immunostained metastases of ccRCC specimens showed that 98 (79.0%) had negative expression and 26 (21.0%) positive expression of PBRM1. Regarding BAP1 expression, we observed that 77 (62.1%) specimens were negative and 47 (37.9%) showed positive nuclear staining. Although also seen in the cytoplasm, the expression pattern was predominantly shown in the nucleus. An even distribution of the staining instead of the presence of hot spots was seen (Fig. 1).

In addition, 38 patients had available tissue samples from the primary renal tumor and we chose to analyze the immunohistochemical expression of BAP1 and PBRM1 also in the primary renal tumor samples. It was observed discordant tissue expression patterns between primary tumor and metastatic sites of BAP1 and PBRM1 in 17/38 (44.7%) patients and in 19/38 (50.0%) patients, respectively (Table 1).

Regarding survival analysis, the 5-year OS and PFS rates were 51.6% and 8.1%, respectively. Classical parameters such as IMDC risk groups stratification, cytoreductive nephrectomy, complete metastasectomy, and high ISUP tumor grade influenced both OS and PFS in univariate analyzes. OS rates in patients with positive expression and negative expression of BAP1 were 53.2% and 35.1%, respectively ($P = 0.004$). BAP1 tissue expression was

also associated with PFS rates. PFS rates in patients with positive expression and negative expression of BAP1 were 14.9% and 3.9%, respectively ($P = 0.003$). Conversely, PBRM1 expression did not significantly influence either OS or PFS rates. OS rates in patients with positive expression and negative expression of PBRM1 were 65.4% and 48.0%, respectively ($P = 0.173$). PFS rates in patients with positive expression and negative expression of BAP1 were 11.5% and 7.1%, respectively ($P = 0.322$). In multivariate analysis, IMDC prognostic classification and complete metastasectomy remained as independent predictors of OS and PFS in the multivariate analysis ($P < 0.001$ and $P < 0.001$). Negative expression of BAP1 tumors also presented higher risks of death (HR = 1.913, $P = 0.041$) and disease progression (HR = 1.656, $P = 0.021$; Fig. 2).

In a subgroup analysis containing only patients undergoing systemic treatment with TKI ($n = 87$), BAP1 expression pattern also showed a statistically significant predictive impact (Fig. 3). In multivariate analysis, negative expression of BAP1 metastases presented higher risk of death (HR = 2.017, $P = 0.045$) and disease progression (HR = 1.586, $P = 0.012$) (Supplementary material).

4. Discussion

Intratumoral heterogeneity leads to important consequences in personalized medicine, which is commonly based on single tumor biopsies for the molecular evaluation of biomarkers [2]. It is known that 63%–69% of somatic mutations are not detected in all regions of biopsied tumors. Only 31% of the mutations in the primary tumor detected by sequencing are present in all regions of the same tumor (including metastases) [12]. There is also evidence that the

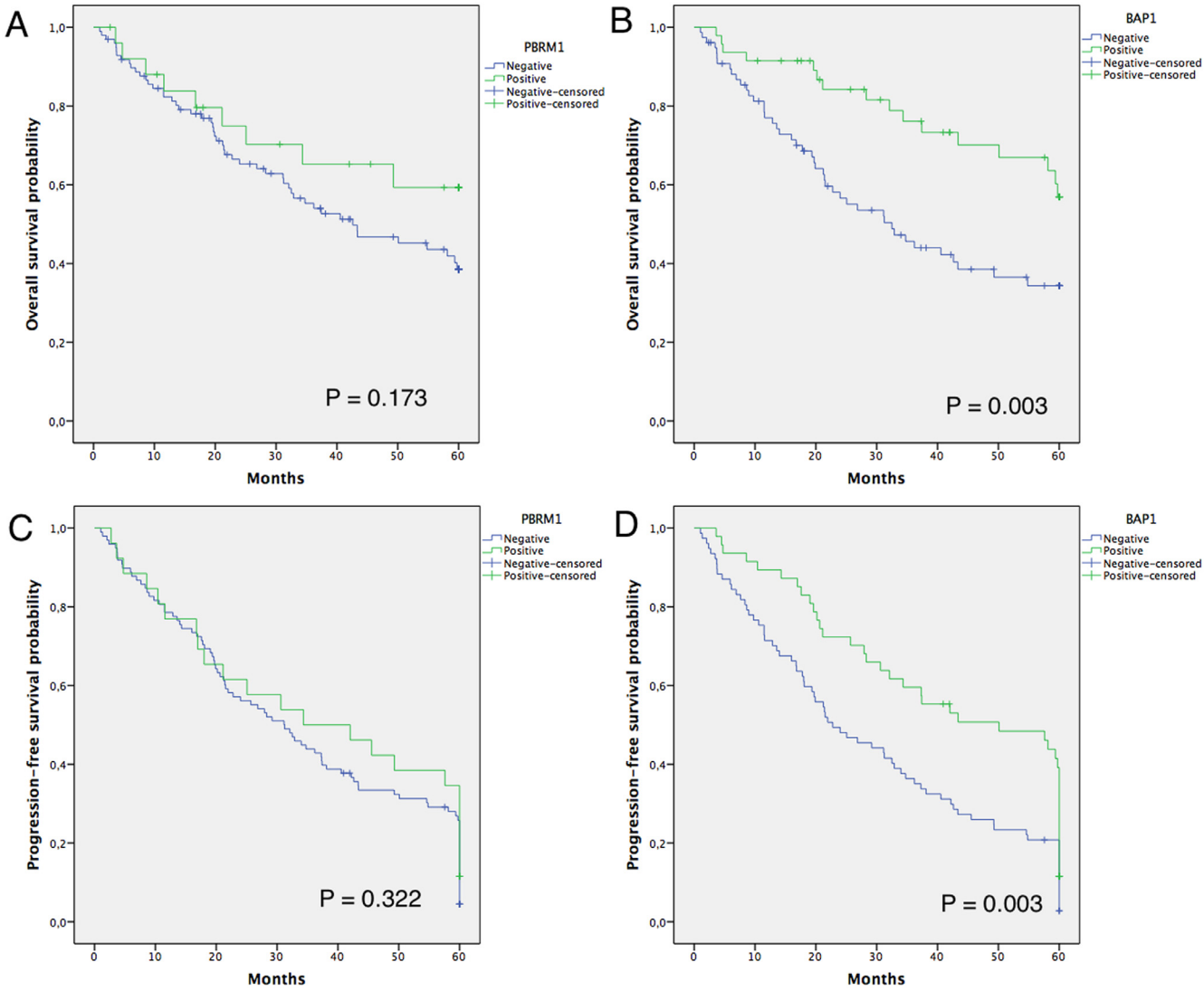

Fig. 2. Survival analysis based on BAP1 and PBRM1 expression. (A) Overall survival (OS) with BAP1 grouped into positive expression vs. negative expression levels. (B) OS with PBRM1 grouped into positive expression vs. negative expression levels. (C) Progression-free survival (PFS) with PBRM1 grouped into positive expression vs. negative expression levels. (D) PFS with BAP1 grouped into positive expression vs. negative expression levels.

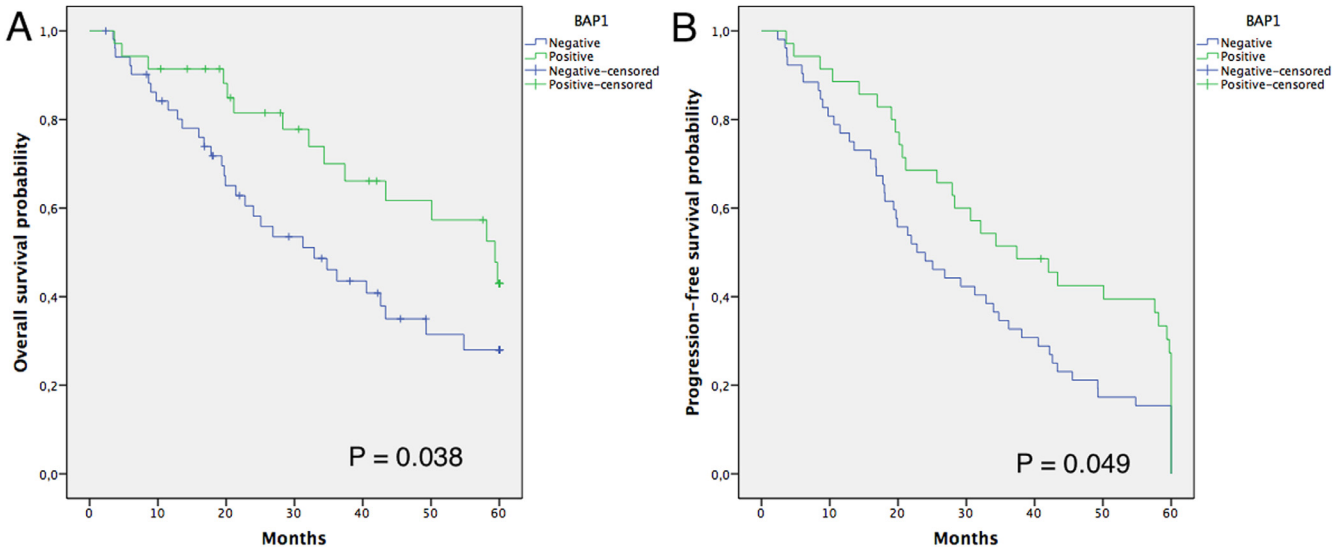

Fig. 3. Survival analysis based on BAP1 expression in a subgroup of 87 patients who received TKI as systemic treatment. (A) OS with BAP1 grouped into positive expression vs. negative expression levels. (B) PFS with BAP1 grouped into positive expression vs. negative expression levels.

use of systemic medications increases intratumor heterogeneity [18]. Such heterogeneity makes it even more difficult to search for prognostic and predictive biomarkers in mRCC [12,18]. Additionally, there are few studies evaluating metastatic tissue in RCC which reinforces the need to have a better understanding of the molecular pathways of carcinogenesis and of potential biomarkers for both TKI and immunotherapy.

In our cohort, the impact of tumor heterogeneity on the evaluation of the immunohistochemical pattern of both BAP1 and PBRM1 was very clear. When we compared the expression of both markers on primary tumor and tumor metastasis, we found disagreement in half of the cases. We consider this finding one of the most relevant of our work. The strategy of using tissue markers of the primary tumor in the prediction of response of metastatic disease is not reliable. Such fact reinforces the imminent need for identification and validation of tumor markers in metastatic tissue.

Regarding the prognostic role of the tissue expression of the markers, we noticed that the expression pattern of PBRM1 was not a significant prognostic event. Patients with positive or negative PBRM1 expression did not present a significant difference in the survival rates. PBRM1 gene regulates a number of signaling pathways such as angiogenesis, hypoxia, and cell adhesion. The prognostic role of PBRM1 in ccRCC is still controversial. Our group previously described that the loss of gene and protein expression of PBRM1 was associated with worse survival rates in patients with ccRCC [5,11]. Other groups have confirmed the loss of PBRM1 expression as an adverse event in patients with ccRCC

Table 2
Multivariate analysis of disease-specific survival (DSS) and progression-free survival (PFS).

Variable	5-year OS HR (95% CI)	*P* value	5-year PFS HR (95% CI)	*P* value
Risk group				
Low	Referent		Referent	
Intermediate	1.685 (0.757–3.751)	0.201	3.310 (1.059–8.248)	0.376
Poor	6.922 (2.846–16.835)	<0.001	4.600 (2.409–8.781)	<0.001
Metastasectomy				
Yes	Referent		Referent	
No	3.541 (1.715–7.164)	<0.001	2.289 (1.475–3.554)	<0.001
Cytoreductive nephrectomy				
Yes	Referent		Referent	
No	1.196 (0.441–3.246)	0.725	1.534 (0.633–3.690)	0.340
ISUP grade				
1 or 2	Referent		Referent	
3 or 4	1.891 (0.891–2.987)	0.209	1.312 (0.518–2.045)	0.437
BAP1 status				
Positive	Referent		Referent	
Negative	1.913 (1.027–3.565)	0.041	1.656 (1.077–2.544)	0.021

[6,7] while previous studies did not observe such finding [8,19].

The loss of BAP1 expression, however, was shown to be a significant finding of poor prognosis in patients with metastatic ccRCC. In our cohort, negative expression of BAP1 tumors presented higher risks of death (HR = 1.913, $P = 0.041$) and disease progression (HR = 1.656, $P = 0.021$; Table 2). A number of studies confirm the prognostic role of loss of BAP1 expression in primary tumor tissue in ccRCC patients [7–11]. However, to our knowledge, our study is the largest series to evaluate the significant prognostic impact of loss of BAP1 expression exclusively on metastatic tissue. A previous study by Miura et al. analyzed the prognostic impact of the loss of BAP1 expression on metastatic tissue of 41 patients with ccRCC. Despite a negative impact on OS rates in univariate analysis, BAP1 expression pattern was not an independent predictor of survival in multivariate analysis in part because of the reduced cohort size [20].

BAP1 functions as a deubiquinating enzyme that regulates multiple cellular pathways related to tumorigenesis. How BAP1 loss is associated with a more aggressive biology and poorer outcomes is not well understood. Interestingly, there is a correlation between BAP1 inactivation and activation of mammalian target of rapamycin complex 1. There are evidences that show the mammalian target of rapamycin complex 1 pathway activation in BAP1-mutated ccRCC tumors, which might explain the poorer outcomes of such patients [21]. Our findings corroborate such prior data. In the subgroup analysis of patients undergoing systemic treatment with TKI, patients with loss of BAP1 expression presented worse rates of OS and PFS (Fig. 3). Such finding has the potential to generate hypotheses. Patients with negative BAP1 expression (and consequent activation of the m-TOR pathway) might benefit from m-TOR inhibitors as first-line systemic treatment. As we had only 6 patients receiving temsirolimus it was not possible to evaluate the systemic response of these patients based on the expression of BAP1.

Our study has limitations that should be mentioned. As mentioned, significant genetic heterogeneity has been reported in primary ccRCC as major limitation of studies involving immunohistochemical analysis with TMA, as it is possible that different staining patterns occur in other parts of the tumor. That might occur also in metastatic tissue. However, since the genetic alterations of both PBRM1 and BAP1 are truncal events in ccRCC, a significant degree of heterogeneity is not expected. There is evidence that in this situation, immunohistochemical analysis with TMA is reliable [22]. Additionally, this is a single-center retrospective analysis with a relatively low number of events due to the characteristics of our cohort, which included only patients with metastatic disease. Besides, the immunohistochemical procedure itself may have had problems posed by inadequate technique of fixation in formalin material. Despite such limitations, we believe that we have added relevant information regarding the best understanding of the molecular biology of metastatic ccRCC, especially regarding the role of PBRM1 and BAP1.

5. Conclusions

The pattern of immunohistochemical expression of both PBRM1 and BAP1 was shown to be significantly discordant when comparing the expression of primary tumor and metastatic tumor tissue. The use of prognostic biomarkers identified in the primary tumor tissue might be not reliable in the metastatic disease scenario. Patients with metastatic ccRCC that present loss of BAP1 expression in metastatic tissue demonstrated poor survival rates and represent a relevant risk group for tumor recurrence and death.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.urolonc.2018.10.017.

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