

**ESTUDO DO VALOR PROGNÓSTICO E EXPRESSÃO
IMUNO-HISTOQUÍMICA DO GENE *SETD2* EM CARCINOMA
DE CÉLULAS RENAIS DO TIPO CÉLULAS CLARAS**

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DEDICATÓRIA

À minha amada mãe Denise Porto Espinheira, a quem eu devo tudo que hoje sou, aquela que sempre vibrou com cada vitória minha, muitas vezes, mais até do que eu mesmo. Tenho certeza que onde ela estiver, estará feliz e orgulhosa, com mais essa etapa em minha vida.

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RESUMO

Santos VE. **Estudo do valor prognóstico e expressão imuno-histoquímica do gene *setd2* em carcinoma de células renais do tipo células claras.** [Dissertação]. São Paulo: Fundação Antônio Prudente; 2021.

Objetivo: Avaliar o impacto prognóstico da expressão imuno-histoquímica de SETD2 em pacientes com carcinoma renal do tipo células claras (ccRCC). **Pacientes e métodos:** Um total de 662 pacientes com ccRCC primário ou metastático foram avaliados. Dois patologistas revisaram todos os casos, e determinaram a seleção das áreas tumorais mais representativas para a construção do *tissue microarray* (TMA). **Resultados:** A coloração nuclear de SETD2 mostrou que 101 (15,3%) tiveram expressão negativa e 561 (84,7%) expressão positiva de SETD2. A expressão da proteína do SETD2 foi associada ao estágio clínico ($p < 0,001$), estágio pT ($p < 0,001$), tamanho do tumor ($p < 0,001$), invasão de gordura peri-renal ($p < 0,001$), ECOG ($p = 0,004$), tipo de cirurgia ($p < 0,001$), grau de ISUP ($p < 0,001$) e necrose tumoral ($p < 0,001$). O padrão de expressão IHQ de SETD2 influenciou as taxas de sobrevida câncer específica (SCE) e a sobrevida global (SG). As taxas de SCE em pacientes com expressão positiva e negativa de SETD2 foram 90,2% e 58,4%, respectivamente ($p < 0,001$). As taxas de SG em pacientes com expressão positiva e negativa de SETD2 foram 87% e 55,4%, respectivamente ($p < 0,001$). Em uma análise multivariada de Cox, a expressão negativa de SETD2 foi um preditor independente de SCE (HR 1,690, IC 95% 1,058 - 2,700, $p = 0,031$) e SG (HR 1,641, IC 95% 1,039 - 2,593, $p = 0,037$). **Conclusão:** Nosso estudo mostrou que a expressão negativa de SETD2 associou-se a um pior prognóstico, sendo um preditor independente de SG e SCE em pacientes com ccRCC.

Descritores: Carcinoma de Células Renais. Prognóstico. Biomarcadores. Imuno-histoquímica

SUMMARY

Santos VE. [Study of the prognostic impact and immunohistochemical expression of the *setd2* gene in clear cell renal cell carcinoma]. [Dissertação]. São Paulo: Fundação Antônio Prudente; 2021.

Purpose: To evaluate the prognostic impact of immunohistochemical expression of SETD2 in patients with clear cell renal cell carcinoma (ccRCC). Patients and methods: A total of 662 patients with primary or metastatic ccRCC were evaluated. Two genitourinary pathologist reviewed all of the cases for uniform reclassification and determined the selection of the most representative tumor areas for the construction of the tissue microarray (TMA). Results: SETD2 nuclear staining showed that 101 (15,3%) had negative expression and 561 (84,7%) positive expression of SETD2. The protein expression of SETD2 was associated with clinical stage ($P < 0.001$), pT stage ($P < 0.001$), tumour size ($P < 0.001$), perinephric fat invasion ($P < 0.001$), ECOG status ($P = 0.004$), surgery type ($P < 0.001$), ISUP grade ($P < 0.001$) and tumor necrosis ($P < 0.001$). SETD2 influenced disease specific survival (DSS) and overall survival (OS). DSS rates in patients with positive and negative expression of SETD2 were 90,2% and 58,4%, respectively ($P < 0.001$). OS rates in patients with positive and negative expression of SETD2 were 87% and 55,4%, respectively ($P < 0.001$). In a Multivariate Cox analysis negative SETD2 expression was a independent predictor of DSS (HR 1.690, 95% CI 1.058 - 2.700, $p = 0.031$) and OS (HR 1.641, 95% CI 1.039 - 2.593, $p = 0.037$). Conclusion: Our study showed that the negative expression of SETD2 was associated with a worse prognosis, being an independent predictor of survival in patients with ccRCC.

Keywords: Carcinoma, Renal Cell. Prognosis. Biomarkers. Immunohistochemistry

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LISTA DE ABREVIATURAS

AJCC	American Joint Committee on Cancer
CCR	Carcinoma de células renais
ccRCC	Carcinoma renal de células claras
ECOG	Eastern Cooperative Oncologic Group
HR	Hazard Ratio
IC95%	Intervalo de confiança de 95%
IHQ	Imuno-histoquímica
ISUP	International Society of Urologic Pathologists
SCE	Sobrevida câncer específica
SG	Sobrevida global
TMA	Tissue microarray
TNM	Tumor-node-metastasis
UISS	University of California - Los Angeles Integrated Staging System
VHL	Von Hippel Lindau

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1 INTRODUÇÃO

1.1 O CARCINOMA DE CÉLULAS RENAIIS (CCR)

O carcinoma de células renais (CCR) é uma neoplasia comum, sendo mundialmente o sexto câncer diagnosticado com mais frequência em homens e o décimo em mulheres, isto corresponde a até 5% de todas as malignidades em adultos (Capitanio et al. 2019). Nas últimas décadas, houve um aumento em sua incidência global, devido ao envelhecimento populacional e principalmente a uma maior detecção radiológica incidental (Bandini et al. 2018). Somente nos Estados Unidos, ocorrem cerca de 76.000 novos casos e quase 14.000 mortes por CCR a cada ano (Siegel et al. 2021). Em todo o mundo, em 2018, houve cerca de 403.000 novos casos de CCR e 175.000 mortes devido ao câncer renal (Bray et al. 2018).

O CCR acomete aproximadamente duas vezes mais homens do que mulheres, ocorrendo predominantemente na sexta à oitava década de vida (Siegel et al. 2021). Alguns fatores de risco como o consumo tabaco, obesidade e hipertensão já estão bem estabelecidos. Já existem evidências que evitar o tabagismo e reduzir a obesidade sejam as formas mais eficazes de profilaxia do CCR (Tahbaz et al. 2018). Uma recente revisão de literatura, mostrou que exercícios regulares, consumo de frutas e vegetais e o uso moderado de álcool possam, também, ter algum papel benéfico da prevenção do CCR (Al-Bayati et al. 2019).

O carcinoma de células renais de células claras (ccRCC) é a variante histológica mais comum, correspondendo até a 75% dos casos de CCR, além disso, é responsável pela maioria das mortes relacionadas ao câncer renal (Palsdottir 2012). Outros subtipos histológicos incluem tumores papilíferos (15%), cromófbos (5%) e variantes mais raras como carcinoma de ductos coletores e o carcinoma medular (Dunnick 2016).

A maioria dos pacientes com CCR apresentam-se assintomáticos ao diagnóstico, tendo em vista que, mais de 50% são diagnosticados incidentalmente (Jayson e Sanders 1998). Para pacientes não diagnosticados acidentalmente, os sinais e sintomas geralmente estão relacionados à invasão de estruturas adjacentes ou metástases a distância. Uma análise retrospectiva de 721 pacientes identificou 43% de casos sintomáticos, sendo os sintomas mais comuns, hematúria macro ou microscópica, dor abdominal, edema em membro inferiores,

síndromes paraneoplásicas ou combinações destes (Lee et al. 2002). A tríade clássica de dor no flanco, hematúria visível e massa abdominal palpável é rara e se correlaciona com histologia agressiva e doença avançada (Patard et al. 2003).

Historicamente a nefrectomia radical foi o tratamento padrão para todos casos de CCR localizado (Robson et al. 1969), porém, nas últimas décadas a cirurgia poupadora de néfrons vem ganhando cada vez mais espaço. Atualmente a nefrectomia parcial é a abordagem de escolha para tumores renais T1a (<4cm) e sempre que tecnicamente factível para tumores T1b (< 7cm), uma vez que quando comparada a nefrectomia radical apresenta resultados oncológicos semelhantes, preserva a função renal e pode reduzir o risco de complicações cardiovasculares (Huang et al. 2009; Kates et al. 2011).

A vigilância ativa com realização periódica de exames de imagem pode ser uma opção factível, sobretudo para pequenas massas renais em pacientes idosos e com comorbidades, haja vista que, a maioria dos pequenos tumores crescem lentamente e possuem baixo potencial de tornarem-se metastáticos (Wehle et al. 2004; Lamb et al. 2004). Neste mesmo grupo de pacientes, as terapias ablativas como crioablação e radiofrequência, também aparecem como uma abordagem interessante, uma vez que, evitam a morbidade cirúrgica e oferecem um tratamento potencialmente curativo (Andrews et al. 2019).

Para tumores maiores ($\geq T2$) e localmente avançados, a nefrectomia radical laparoscópica ainda aparece como a melhor opção, apresentando menor tempo de internação, menos dor no pós-operatório e com tempo de convalescença significativamente menor, quando comparada a técnica convencional aberta (Hemal et al. 2007; Steinberg et al. 2004). A nefrectomia radical laparoscópica com auxílio do robô apresenta resultados oncológicos semelhantes a laparoscopia simples, todavia, com um maior custo hospitalar (Asimakopoulos et al. 2014; Jeong et al. 2017).

O papel da linfadenectomia permanece um tema controverso o EORTC 30881 é o único estudo randomizado que avaliou a linfadenectomia em combinação com a nefrectomia radical no tratamento do CCR, e nenhuma vantagem de sobrevida pode ser demonstrada (Blom et al. 2009). Outros estudos retrospectivos, sugerem que a linfadenectomia pode ter algum efeito benéfico, sobretudo, em casos localmente avançados e com características clínicas e patológicas desfavoráveis (Studer e Birkhäuser 2009; Capitanio et al. 2011).

Não há ainda indicação para o uso de terapia adjuvante após ressecção cirúrgica completa do CCR, não existindo na literatura evidências que suportem tal terapia fora de

estudos clínicos (Haas et al. 2017; Motzer et al. 2017). O estudo de fase III S-TRACT, randomizou 615 pacientes com ccRCC operados, comparando Sunitib adjuvante, com placebo. Trata-se do único trial que demonstrou algum benefício em sobrevida livre de doença, mas não em sobrevida global (SG), e á custa de elevada toxicidade (Ravaud et al. 2016).

No cenário da doença metastática o progresso ocorre principalmente no ccRCC, onde imunoterapia vem mostrando resultados superiores quando comparadas ao uso isolado de inibidores de tirosina quinase (ex: Sunitib, Sorafenib, Pazopanib). A escolha do tratamento é baseada na estratificação de pacientes em grupos de risco, favorável, intermediário e alto risco, utilizando os critérios criados pelo *IMDC - International Metastatic Renal Cell Carcinoma Database Consortium* (Heng et al. 2013). A combinação de nivolumab/cabozantinib, bem como, pembrolizumab/axitinib ou pembrolizumab/lenvatinib são hoje consideradas o tratamento padrão para todos os grupo de risco do *IMDC* (Rini et al. 2019; Choueiri et al. 2021; Motzer et al. 2021). Além destas, a associação de nivolumab/ipilimumab aparece como mais uma opção nos grupos intermediário e alto risco (Motzer et al. 2018a).

Levando em consideração que o CCR pode ter um amplo espectro de apresentação clínica, desde uma pequena massa renal indolente, até uma doença agressiva e metastática, muitos autores tem se concentrado em identificar características clínicas e patológicas associadas ao seu prognóstico. A extensão anatómica da doença, avaliada através do TNM (Tumor-Node-Metastasis), é o fator que mais influencia o prognóstico. Uma série americana utilizando 87.225 pacientes do banco de dados do *National Cancer Institute*, identificou uma SCE em 5 anos de 97,4%, 89,9%, 77,9% e 26,7% para pacientes estágio I, II, III e IV respectivamente (Cheaib et al. 2020).

Alguns fatores patológicos também são relevantes no desfecho do CCR. Desde o estudo clássico realizado por Fuhrman et al. em 1982, o grau nuclear tumoral tem sido um dos mais importantes. Tumores de alto grau (III/IV) tem um comportamento mais agressivo, com maior propensão a evoluírem para doença mestastática, quando comparados a tumores de baixo grau. O grau nuclear de Fuhrman amplamente utilizado por décadas, atualmente foi substituído pelo grau nuclear de ISUP (Delahunt et al. 2013). Outros fatores como presença de necrose tumoral e degeneração sarcomatóide, também estão associadas a uma pior sobrevida (Sengupta et al. 2005; Kim et al. 2015).

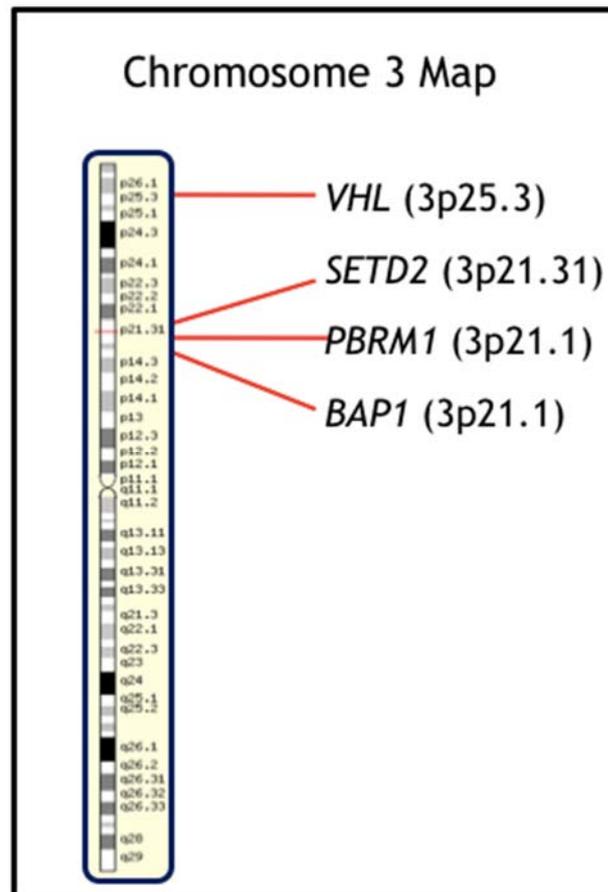
Além da extensão anatómica da doença e dos critérios patológicos, diversos fatores clínicos também podem impactar a evolução da doença. Um performance status ruim e a

presença de sinais e sintomas como caquexia, perda de peso, trombocitopenia e anemia, estão associados a um prognóstico adverso (Motzer et al. 1999; Bensalah et al. 2006; Kim et al. 2004). Alguns modelos prognósticos tem sido desenvolvidos para integrar as informações do estadiamento anatômico, com parâmetros histopatológicos e clínicos, como por exemplo, o sistema de estadiamento integrado da Universidade da Califórnia, Los Angeles (UCLA) (UISS), que utiliza o estadiamento T, o grau nuclear de ISUP e o status performance ECOG, para estratificar pacientes e predizer a SCE em 5 anos (Zisman et al. 2002).

O avanço na identificação de marcadores prognósticos biomoleculares relevantes, tem sido mais limitado e a crescente disponibilidade de drogas direcionadas molecularmente para pacientes com ccRCC ressaltou a necessidade de verdadeiros biomarcadores que predizem o comportamento tumoral (Hakimi et al. 2013b; Piva et al. 2015).

Dentre as alterações genéticas presentes no ccRCC, a deleção do gene supressor de tumor Von Hippel Lindau (*VHL*) foi a primeira a ser estudada, tal mutação pode estar presente em até 90% dos casos esporádicos de ccRCC, sendo considerada um evento precoce e com importante papel na carcinogênese (Yao et al. 2002). No entanto, sua inativação isolada é insuficiente para desencadear esse processo, sugerindo que mutações em outros genes são necessárias. Além disto, a mutação do *VHL* não demonstrou afetar o comportamento clínico do ccRCC, não sendo portanto, um bom biomarcador prognóstico da doença (Hakimi et al. 2013b; Alves et al. 2014; da Costa et al. 2014).

Estudos recentes de sequenciamento de exoma completo identificaram mutações recorrentes em uma série de genes próximos ao gene *VHL* (Figura 1), como proteína-1 associada a *BRCA-1* (*BAP1*), Polibromo-1 (*PBRM1*) e conjunto de domínio contendo 2 (*SETD2*). Apesar de parecem ser eventos secundários, possuem potencial prognóstico relevante, contribuindo para o desenvolvimento de fenótipos de maior agressividade (Mitchell et al. 2018). Estes genes codificam proteínas que estão envolvidas na remodelação da cromatina e metilação de histonas, e a maioria das mutações relatadas resulta em perda de função, indicando que essas proteínas funcionam como supressores de tumor (da Costa et al. 2018).



Fonte: Hakimi et al. (2013b)

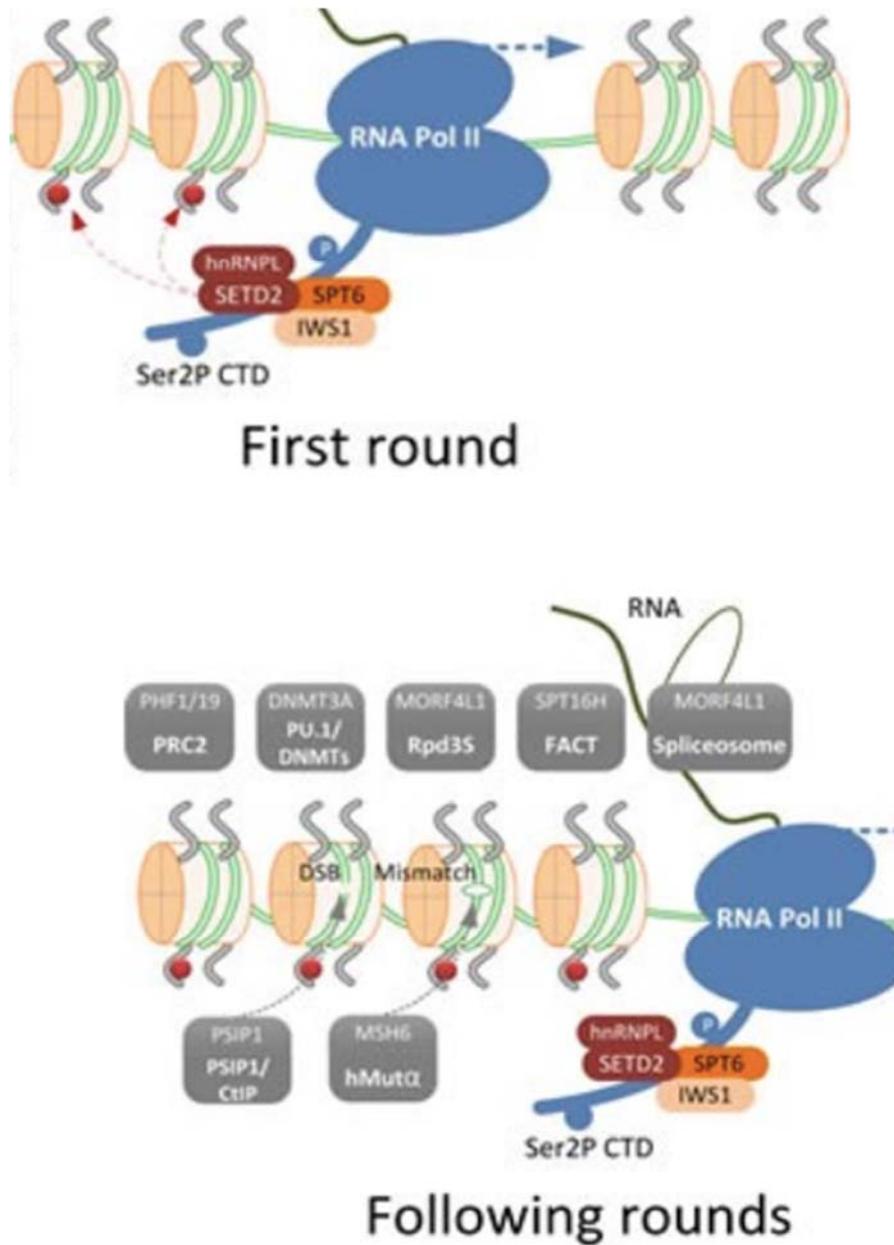
Figura 1 - Mapa do cromossomo 3p, evidenciando a proximidade entre o *VHL* e o *SETD2*.

Uma revisão de literatura realizada em 2015, ressaltou o papel prognóstico de mutações em genes como *BAP1*, *PBRM1* e *SETD2* (Piva et al. 2015). Um estudo realizado em 2018 por da Costa et al., demonstrou que pacientes com ccRCC em estágio inicial e que apresentavam perda concomitante da expressão de *PBRM1* e *BAP1*, evoluíam com piores taxas de sobrevida e um maior risco de recorrência tumoral e óbito. Outros estudos corroboram o promissor papel prognóstico destes genes (Hakimi et al. 2013a e b, Morris e Latif 2016).

1.2 O MARCADOR

O gene *SETD2* está localizado no braço curto do cromossomo 3p, e nos últimos anos vem atraindo atenção por seu potencial papel como supressor tumoral. Sua mutação ou perda funcional está relacionada a surgimento e progressão de diversos tumores como ccRCC, mama, pulmão, leucemia, entre outros (Forbes et al. 2015). O gene codifica a proteína SETD2 que pertence a família das *histone lysine methyltransferases* (KMTs), responsável pela tri-metilação da histona H3K36, gerando então a histona modificada H3K36m3. Estudos recentes revelam que a metilação de histonas desempenha um papel crucial em mecanismos regulatórios intracelulares (Li et al. 2016).

Os mecanismos moleculares exatos nos quais o SETD2 parece atuar na supressão tumoral não estão totalmente esclarecidos. A função mais proeminente estudada é a regulação da transcrição e correção de danos do DNA exercida diretamente pela proteína SETD2, mas principalmente pela H3K36m3 (Figura 2). Além disto a proteína SETD2 parece ligar-se também, diretamente em receptores do gene *P53* ativando processos de apoptose celular (Li et al. 2016; Chen et al. 2020).



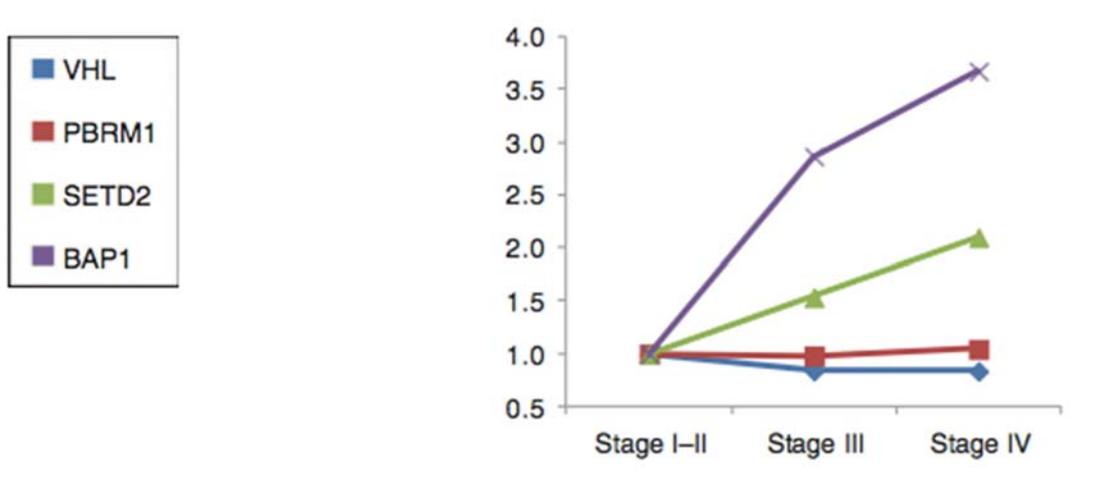
Fonte: Li et al. (2016)

Figura 2 - Representação esquemática da tri-metilação da H3K36, mediada por SETD2 e uma visão geral dos leitores H3K36me3. *First round* = ocorre a tri-metilação (em vermelho) mediada pela SETD2. *Following rounds* = Recrutamento dos leitores de H3K36me3 (mostrados nas caixas cinzas), responsáveis por vários processos biológicos de regulação da transcrição e reparos ao DNA.

A primeira associação entre SETD2 e câncer renal ocorreu em 2010, quando Dalglish et al. sequenciaram 407 amostras tumorais de pacientes com ccRCC e identificaram 12 (3%) casos com mutações no SETD2, desde então, outros autores seguem a mesma linha de pesquisa. Hakimi et al. em 2013b avaliaram 185 amostras de DNA extraídas de tecido congelado de ccRCC e descreveram 7,6% de mutações do SETD2.

De acordo com diferentes estudos, a incidência de mutações do SETD2 em tumores ccRCC esporádicos, varia de 3% a 16%, estando entre os genes mais frequentemente mutados da doença (Gossage et al. 2014, Wang et al. 2016). Notadamente, a maioria dessas mutações levam a perda da expressão proteica de SETD2 (Liu et al. 2017).

Além de relativamente frequente, a mutação do SETD2 tem sido associada a um pior prognóstico em pacientes com ccRCC. Um estudo de 2013 avaliou uma coorte de 421 pacientes oriundos do banco de dados genômico e clínico do The Cancer Genome Atlas (TCGA) e evidenciou que, quando presente, a mutação do SETD2 associou-se a piores taxas de sobrevida câncer específica [HR 1.68; 95% CI 1.04–2.73; p=0.036;] e estádios clínicos mais avançados (Hakimi et al. 2013a) (Figura3).



Fonte: Hakimi et al. (2013a)

Figura 3 - Associação entre mutações do *SETD2*, *VHL*, *PBRM1* e *BAP*, com os diversos estádios do carcinoma renal de células claras

Tendo em vista que a maioria das alterações genéticas do *SETD2*, levam a perda da expressão proteica, alguns autores, tem utilizado análise imuno-histoquímica (IHQ) para avaliar a expressão da proteína SETD2 e correlacionar com dados clínicos e patológicos. Em 2017 Liu et al., estudaram 135 pacientes com ccRCC e identificaram que a baixa expressão proteica de SETD2 associou-se significativamente a estágio tumoral mais avançado, metástase à distância, maior grau nuclear de ISUP e piores taxas de SG.

Wang et al. em 2016, avaliaram retrospectivamente 111 pacientes com ccRCC metastático tratados com Sunitinib ou Sorafenib e identificaram que a baixa expressão proteica do SETD2 associou-se a redução da SG e sobrevida livre de progressão.

Outro estudo em 2015, realizado em um único centro na China, analisou retrospectivamente 192 pacientes com ccRCC não metastático, e identificou que pacientes com baixa expressão de SETD2 eram propensos a possuir tumor de tamanho maior e estágio pT mais avançados (Liu et al. 2015).

Embora já existam na literatura evidências descrevendo o padrão de expressão proteica do SETD2 como um potencial biomarcador prognóstico no ccRCC, ainda não se trata de um marcador bem estabelecido, não sendo usado rotineiramente na prática clínica, devido à disponibilidade limitada de estudos, ao pequeno número de pacientes nos estudos atuais e à falta de estudos externos de validação (Hakimi et al. 2013b; Liu et al. 2015; Wang et al. 2016; Liu et al. 2017).

2 OBJETIVOS

2.1 OBJETIVO GERAL

Analisar a incidência da perda de expressão imuno-histoquímica de SETD2 e seu impacto prognóstico em amostras de tumor primário de pacientes portadores de ccRCC.

2.2 OBJETIVO ESPECÍFICO

Analisar o impacto dos níveis de expressão do SETD2 nas taxas de SG e SCE.

3 ARTIGO ACEITO PARA PUBLICAÇÃO

 Prognostic Impact of Loss of SETD2 in Clear Cell Renal Cell Carcinoma

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> Clin Genitourin Cancer. 2021 Mar 17;S1558-7673(21)00066-5. doi: 10.1016/j.clgc.2021.03.003.
Online ahead of print.

Prognostic Impact of Loss of SETD2 in Clear Cell Renal Cell Carcinoma

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Abstract

The purpose of this study was to evaluate the prognostic impact of immunohistochemical expression of SETD2 in 662 patients with clear cell renal cell carcinoma (ccRCC). In a multivariate Cox analysis, negative SETD2 expression was an independent predictor of disease-specific survival and overall survival. The protein expression of SETD2 is an important biomarker in the management of patients with ccRCC.

Purpose: To evaluate the prognostic impact of immunohistochemical expression of SETD2 in patients with clear cell renal cell carcinoma (ccRCC). **Patients and Methods:** A total of 662 patients with primary or metastatic ccRCC were evaluated. Two genitourinary pathologist reviewed all of the cases for uniform reclassification and determined the selection of the most representative tumor areas for construction of the tissue microarray (TMA). **Results:** SETD2 nuclear staining showed that 101 areas (15.3%) had negative expression, and 561 areas (84.7%) had positive expression of SETD2. The protein expression of SETD2 was associated with clinical stage ($P < .001$), pathological stage ($P < .001$), tumor size ($P < .001$), perinephric fat invasion ($P < .001$), Eastern Cooperative Oncology Group status ($P = .004$), surgery type ($P < .001$), International Society of Urologic Pathologists grade ($P < .001$), and tumor necrosis ($P < .001$). SETD2 influenced disease-specific survival (DSS) and overall survival (OS). DSS rates in patients with positive and negative expression of SETD2 were 90.2% and 58.4%, respectively ($P < .001$). OS rates in patients with positive and negative expression of SETD2 were 87% and 55.4%, respectively ($P < .001$). In a multivariate Cox analysis, low SETD2 expression was an independent predictor of DSS (hazard ratio [HR], 1.690; 95% confidence interval [CI], 1.0582-2.700; $P = .031$) and OS (HR, 1.641; 95% CI, 1.039-2.593; $P = .037$). **Conclusion:** Our study showed that the negative expression of SETD2 was associated with a worse prognosis, and it was an independent predictor of survival in patients with ccRCC. We believe that the protein expression of SETD2 is an important biomarker in the management of patients with ccRCC.

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Introduction

Renal cell carcinoma is a common neoplasm, accounting for up to 3% of all adult malignancies.¹ In the past several decades, there has been an increase in its global incidence, due primarily to the rise in incidental radiological detection and the aging population.² Clear cell renal cell carcinoma (ccRCC) is the most common histological variant, accounting for most kidney cancer-related deaths.³ Many

researchers have focused on identifying the clinical and pathological characteristics that are associated with the prognosis of patients with ccRCC. To this end, tumor size, advanced pathological stage, high International Society of Urologic Pathologists (ISUP) nuclear grade, and the presence of tumor necrosis are related to lower survival rates. But, progress in identifying relevant biomolecular markers has been limited, and the increasing availability of molecularly targeted drugs for ccRCC patients has underscored the need for true biomarkers that predict tumor behavior.^{4,5}

Mutation in the tumor suppressor gene von Hippel-Lindau (*VHL*) is present in up to 90% of sporadic cases of ccRCC and plays an important role in carcinogenesis; however, its isolated inactivation is insufficient to trigger this process, suggesting that mutations in other genes are necessary.^{4,6} Although frequent, the *VHL* mutation has not been shown to affect the clinical behavior of

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the disease.¹ Recent complete exome sequencing studies have identified recurrent mutations in a series of genes near the *VHL* gene, such as BRCA1-associated protein-1 (*BAP1*), polybromo-1 (*PBRM1*), and SET domain-containing 2 (*SETD2*), rendering them potential prognostic markers.^{1,3,4,7}

SETD2 is an active tumor suppressor in breast cancer and leukemia; it lies on chromosome 3p, an area that is frequently deleted in ccRCC.⁵ Its mutation occurs in up to 16% of sporadic ccRCC cases, and it is among the most frequently mutated genes after *VHL*.^{4,6,8,9} *SETD2* mediates chromatin remodeling by regulating the transcription of genes and modifying histones, and it plays a significant role in DNA damage repair. *SETD2* is responsible for trimethylation of lysine-36 of histone H3, generating its canonical histone modification product, H3K36me3.^{8,9-12}

Mutation in *SETD2* is related to a worse prognosis in ccRCC.^{4,13} However, it is not a well-established prognostic marker, and it is not used routinely in clinical practice due to the limited availability of studies, the small number of patients in current studies, and the lack of external validation studies.^{6,8,9} The aim of our study was to assess the prognostic impact of immunohistochemical expression of *SETD2* in a large cohort of patients with primary or metastatic ccRCC.

Patients and Methods

Initially, 732 patients who were treated surgically for ccRCC between 1985 and 2016 were retrospectively assessed in the Department of Urology of our institution. A total of 662 patients were included; 70 were excluded due to the unavailability of tissue samples.

Clinicopathological parameters and data on treatment, response, and survival were collected from medical records and electronic databases. Ethical approval was granted by our internal review board. Samples were provided by our institution with the patient's informed consent.

Two genitourinary pathologists reviewed all cases for uniform reclassification according to the new renal tumor classification^{14,15} and selected the most representative tumor areas for construction of a tissue microarray (TMA). Initial tumor stage was classified per radiographic reports and postoperative pathological data, based on the eighth edition of the American Joint Committee on Cancer (AJCC) TNM classification.¹⁶

The following variables were included in the databank: age, sex, time since diagnosis, type of surgery, Eastern Cooperative Oncology Group (ECOG) status, staging (AJCC/Union for International Cancer Control staging system), ISUP grade, tumor size, microscopic vascular invasion, microscopic lymphatic invasion, margin status, presence of sarcomatoid component, presence of tumor necrosis, presence of metastases, and analysis of *SETD2* immunohistochemical expression pattern. The University of California-Los Angeles Integrated Staging System (UISS)¹⁷ for renal cell carcinoma was used to stratify patients with localized disease into low-risk, intermediate-risk, and high-risk subgroups.

Tissue Microarray and Immunohistochemistry

Two cylinders, 1 mm in diameter, were captured from the donor paraffin blocks and mounted in a new paraffin block (recipient

block), with their 2-dimensional position specified and recorded. From this block, histological sections were prepared on slides that had been treated with cling film, for immunohistochemistry reactions. Hematoxylin and eosin staining was also performed to determine the quality of the TMA and the presence of tumor in the cores.

TMAs of ccRCCs were sectioned on a microtome at a thickness of 4 μ m. The reactions were carried out on a Ventana BenchMark XT (Ventana Medical Systems, Oro Valley, AZ), and immunostaining was performed with the Ventana Ultraview detection kit (cat. no. 760-500). The slides were dewaxed using Ventana EZ Prep Concentrate solution (cat. 950-102) and subjected to antigenic recovery for 30 minutes with Ventana Cell Conditioning Reagent (cat. 950-224) at 95°C. Then, the slides were incubated for 30 minutes with anti-*SETD2* (ab113642, 1:500; Abcam, Cambridge, UK).

The same pathologists who were blinded to the outcome of the cases performed the histopathological analysis. The nuclear expression of the antibody was evaluated according to the technique described by Jiang et al.^{18,19} We defined negative expression as only those cases in which there was no immunohistochemical expression or cases that were weakly stained in less than 5% of cells (Figure 1). If one of the two spots was classified as having negative expression, the case was considered as such.

Statistical Analysis

Pearson's χ^2 test and Fisher's exact method were used to verify the association between *SETD2* immunohistochemical expression and the other variables. Disease-specific survival (DSS) was defined as the interval between the primary surgery and the last follow-up visit or disease-related death, overall survival (OS) was considered the time from surgery to death as a result of any cause, and recurrence-free survival (RFS) was defined as the interval between the surgery and the last follow-up, recurrence date, or cancer-related death. The impact of *SETD2* expression on DSS, OS, and RFS was estimated by the Kaplan-Meier method and log-rank test. A Cox proportional hazards model was used to determine the variables that influenced survival in the univariate and multivariate analysis. The confidence interval (CI) was 95%.

Results

The study sample was comprised of 420 men (63.4%) and 242 women (36.6%). Their ages ranged from 21 to 88 years, with a mean of 54 years. Radical nephrectomy was performed in 326 patients (49.3%), whereas 336 patients underwent partial resection (50.8%). The mean follow-up time was 55.7 months. The tumor size varied between 0.6 and 25 cm, with an average of 5.3 cm. Regarding pathological T (pT) staging, there was a predominance of pT1 (451, 68.1%), most of which were pT1a (292, 44%); 43 cases (6.5%) were pT2, and 168 (25.4%) were \geq pT3. The surgical margin was compromised in 33 patients (5.1%). At the end of the study, 97 patients (14.7%) had died from ccRCC (Table 1).

SETD2 nuclear staining of the 662 immunostained ccRCC specimens showed that 101 specimens (15.3%) had negative expression, and 561 specimens (84.7%) had positive expression. There was an association between *SETD2* expression and clinical stage ($P < .001$), pT stage ($P < .001$), tumor size ($P < .001$), perinephric fat

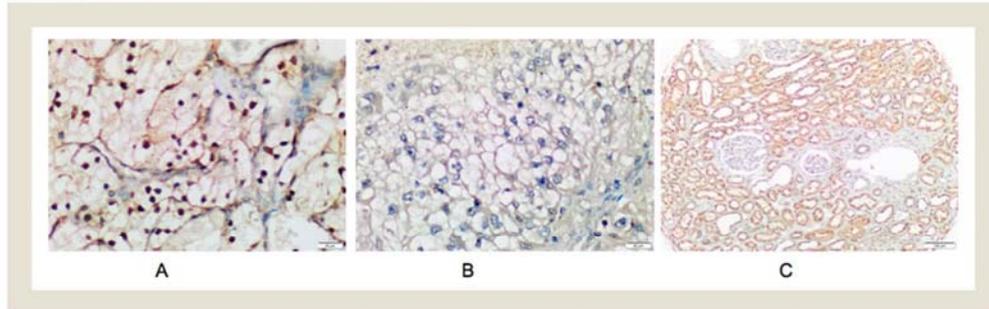
Table 1 Patients and Pathological Characteristics and Associations Among Variables and SETD2 Expression.

Variable	Negative Expression, n (%)	Positive Expression, n (%)	P
Gender			.911
Male	65 (64.4)	355 (63.3)	
Female	36 (35.6)	206 (36.7)	
ECOG status			.004
0	47 (51.1)	317 (70.8)	
≥1	45 (48.9)	131 (29.2)	
Clinical stage			<.001
I, II	42 (41.6)	439 (78.2)	
III, IV	59 (58.4)	122 (21.7)	
pT stage			<.001
pT1a	9 (8.9)	283 (50.4)	
pT1b	26 (25.7)	133 (23.7)	
pT2a	13 (12.9)	23 (4.1)	
pT2b	3 (3.0)	4 (0.7)	
pT3a	35 (34.7%)	97 (17.3)	
pT3b	3 (3.0)	10 (1.8)	
pT4	12 (11.9)	11 (2.0)	
Tumor size (cm)			<.001
0-4	11 (10.9)	290 (52.1)	
>4	90 (89.1)	267 (47.9)	
Surgery			<.001
Partial nephrectomy	15 (14.9)	321 (57.2)	
Radical nephrectomy	86 (85.1)	239 (42.6)	
Surgical margins			.793
Negative	81 (94.2)	520 (94.9)	
Positive	5 (5.8)	28 (5.1)	
ISUP grade			<.001
Low (I, II)	29 (28.7)	329 (58.6)	
High (III, IV)	72 (71.3)	232 (41.4)	
Necrosis			<.001
No	58 (58.0)	431 (77.0)	
Yes	42 (42.0)	129 (23.0)	
Perinephric fat invasion			<.001
No	63 (66.3)	483 (89.6)	
Yes	32 (33.7)	56 (10.4)	
Microscopic vascular invasion			<.001
No	70 (69.3)	519 (93.5)	
Yes	31 (30.7)	36 (6.5)	
Synchronous metastasis			<.001
No	80 (79.2)	522 (93.0)	
Yes	21 (20.8)	39 (7.0)	
UISS classification			<.001
Low risk	11 (15.3)	196 (45.5)	
Intermediate risk	36 (50.0)	206 (47.8)	
High risk	25 (35.7)	29 (6.7)	

Abbreviations: ECOG = Eastern Cooperative Oncology Group; ISUP = International Society of Urologic Pathologists; pT = pathological T; UISS = University of California-Los Angeles Integrated Staging System.

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Figure 1 Photomicrographs of Immunohistochemical Expression of SETD2. A, Positive Expression. B, Negative Expression. C, Positive Expression in Normal Renal Tissue.



invasion ($P < .001$), lymphovascular invasion ($P < .001$), ECOG status ($P = .004$), surgery type ($P < .001$), ISUP grade ($P < .001$), and tumor necrosis ($P < .001$) (Table 1). There was no correlation between SETD2 expression and the presence of a positive margin or a sarcomatoid component. The 10-year DSS and OS rates were 85.3% and 82.2%, respectively. Negative SETD2 expression was linked to a shorter DSS and OS by Kaplan–Meier analysis ($P < .001$) (Figure 2).

The DSS rates in patients with positive and negative expression of SETD2 were 90.2% and 58.4%, respectively ($P < .001$). The OS rates in patients with positive and negative expression of SETD2 were 87% and 55.4%, respectively ($P < .001$). Using a univariate Cox regression model, we determined that classical parameters, such as age, ECOG status, clinical stage, tumor size, ISUP grade, microscopic vascular invasion, microscopic lymphatic invasion, tumor necrosis, and margins, were also associated with lower survival rates. Multivariate Cox analysis of these parameters indicated that low SETD2 expression was an independent predictor of DSS (hazard ratio [HR], 1.690; 95% CI, 1.058–2.700; $P = .031$) and OS (HR, 1.641; 95% CI, 1.039–2.593; $P = .037$). Age, grade of ISUP, clinical staging, and ECOG status also remained independent markers of survival in the multivariate analysis (Table 2).

For a subgroup analysis, we selected patients with high-risk localized disease per UISS criteria and assessed DSS and RFS for 54 patients. The 10-year DSS was 63%, and the RFS was 48.1%. Negative expression of SETD2 was associated with a shorter DSS ($P = .012$) and RFS ($P = .001$) by Kaplan–Meier analysis (Figure 2). The estimated average survival in the negative-expression SETD2 group was 42 months, whereas in the positive-expression group, the median was not reached.

Discussion

In this study, we evaluated the influence of the immunohistochemical expression of SETD2 in patients with ccRCC who were treated by surgery. Our findings show that negative expression is closely associated with worse results, with an impact on DSS and OS, strengthening the findings of previous studies.^{6,8,9} SETD2 is a tumor suppressor that acts through various intracellular mechanisms, including the regulation of transcription elongation,

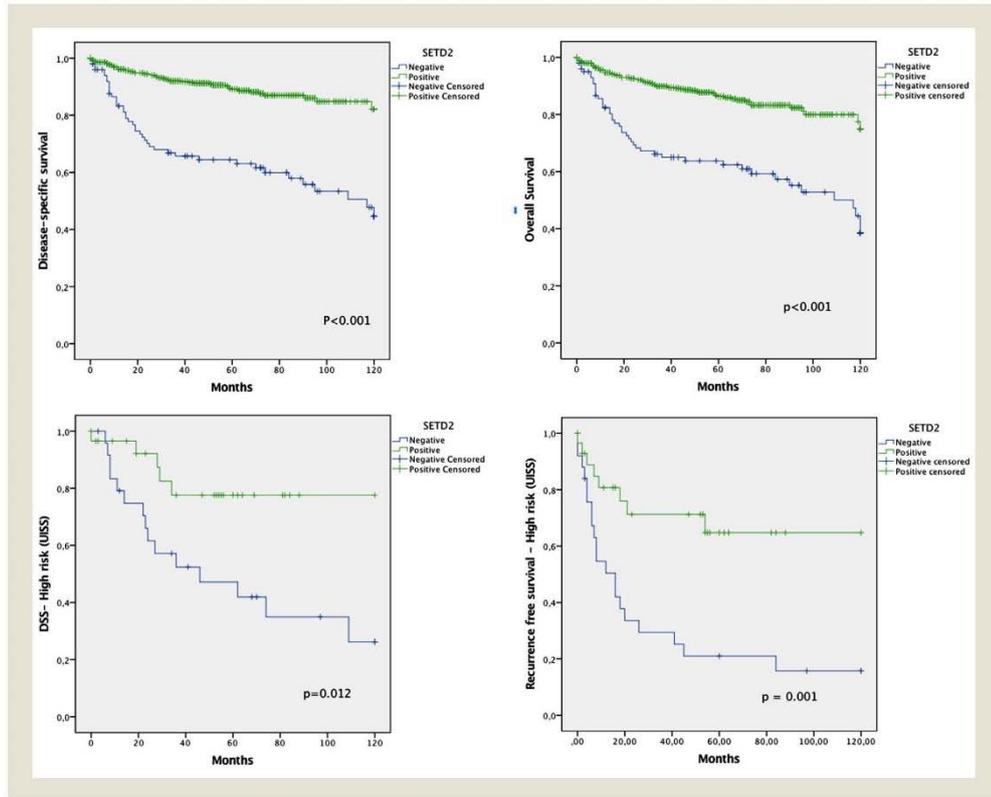
RNA processing, and double-stranded DNA break repair. Its mutation ranges in incidence from 3% to 16%.^{9,20,21} In addition to a loss of copy number, most mutations in SETD2 are nonsense or frameshift truncations, invariably causing a loss of the protein product or function.^{6,9} Our finding that 15.3% of patients lack expression are consistent with the expected mutation rate, suggesting that our method is adequate for immunohistochemical analyses. Other groups that have also used immunohistochemical analysis have reported a higher percentage of negative or low expression of SETD2, likely due to differences in the immunohistochemical analysis method.^{6,8}

We observed that negative expression of SETD2 had important clinical significance, being associated with several known factors of a worse prognosis, such as a high degree of ISUP ($P < .001$), more advanced clinical stage ($P < .001$), greater incidence of metastasis at diagnosis ($P < .001$), larger tumor size ($P < .001$), greater presence of tumor necrosis ($P < .001$), and invasion of perinephric fat ($P < .001$). Moreover, in the multivariate analysis, it was an independent prognostic factor of DSS and overall survival, prompting us to conclude that the use of this marker, associated with other traditional prognostic information such as those above, can help better define the outcome of patients.

The expression of SETD2 was also a good prognostic marker when we evaluated the subgroup with high-risk localized disease. This subgroup alone already carries a great risk of unfavorable progression; however, they evolved even worse when SETD2 was absent, with shorter survival, higher recurrence rate, and an earlier recurrence. This association can help stratify possible candidates for adjuvant therapy trials, given that there remains a lack of sufficient evidence to encourage the use of adjuvant therapy in ccRCC.^{22,23} The mechanism by which SETD2 leads to a worse phenotype in ccRCC is unclear. Tumor cells that lack SETD2 show microsatellite instability and have a high number of mutations that can accelerate the progression of the disease.⁹

A controversial and interesting point is the relationship between SETD2 and its canonical histone modification, H3K36me3, which mediates genomic stability and DNA damage repair, being closely linked to the role of tumor suppressor function of the SETD2 gene. However, several groups have evaluated the protein expression of

Figure 2 Impact of SETD2 Expression on A, Disease-Specific Survival; B, Overall Survival; C, Disease-Specific Survival in UISS High-Risk Patients; and D, Recurrence-Free Survival in UISS High-Risk Patients. Abbreviation: UISS = University of California-Los Angeles Integrated Staging System (UISS).



both, side by side, and found that 30% of cases have asymmetry: low SETD2 and high H3K36me3 or high SETD2 and low H3K36me3. This pattern suggests that not all types of disruptions in the SETD2 gene have a significantly proportional effect on the change in H3K36me3 and that other pathways, such as other H3K36 demethylases (JHDM3/JMJD2 family), are involved in maintaining H3K36me3 levels.⁶ In addition, Liu et al.⁸ showed that only the loss of SETD2 expression, not H3K36me3, correlates with aggressive clinicopathological features in ccRCC patients. Thus, the prognostic and functional significance of H3K36me3 status requires further investigation.^{8,9}

Our study has several limitations. The tumor heterogeneity in ccRCC is a limiting factor in studies by TMA, but our immunohistochemical analysis allowed us to evaluate a large number of patients (662) at a lower cost compared with other techniques. In addition, the immunohistochemical procedure itself might have encountered problems due to inadequate formalin fixation

of the material. Finally, its retrospective and single-center design could have generated errors, such as missing data in the medical records.

In conclusion, negative expression of SETD2 is associated with a worse prognosis and is an independent predictor of survival in patients with ccRCC. This finding confirms and validates previous studies^{6,8,9} in a larger population and with a different ethnicity. Further studies should be performed to determine how mutations in chromatin remodeling genes such as SETD2 alter the treatment of metastatic ccRCC, especially in the current era of increasingly personalized oncology.

We encourage other groups to maintain this line of research, and we believe that the protein expression of SETD2 is an important biomarker in the management of patients with ccRCC; however, we understand that further studies using genetic sequencing or analysis using quantitative reverse transcription polymerase chain

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Table 2 Cox Regression Analysis of DSS and OS.

Feature	10 year-DSS				10 year-OS			
	Univariate	Multivariate		Univariate	Multivariate			
	P	HR	95% CI	P	P	HR	95% CI	P
SETD2 (Negative x Positive)	< 0.001	1,690	(1,058-2,700)	0,028	< 0.001	1.641	(1,039-2,593)	0,034
ISUP grade	< 0.001	2,040	(1,208-3,445)	0,008	< 0.001	1.982	(1,205-3,260)	0,007
Clinical stage	< 0.001	2,191	(1,794-2,676)	<0,001	< 0.001	1,684	(1,390-2,040)	< 0.001
Age	< 0.001	1,432	(1,100-1,865)	0,008	< 0.001	1,624	(1,256-2,100)	< 0.001
ECOG status	< 0.001	NE	NE	NE	< 0.001	1,523	(1,162-1,995)	0,002
Microscopic vascular invasion	< 0.001	1,079	(0,615-1,893)	0,792	< 0.001	1,248	(0,695-2,240)	0,459
Microscopic lymphatic invasion	< 0.001	1,399	(0,616-3,176)	0,422	< 0.001	1,710	(0,773-3,779)	0,185
Necrosis	< 0.001	1,024	(0,621-1,690)	0,925	< 0.001	1,400	(0,875-2,241)	0,160
Margin	0,007	1,026	(0,491-2,144)	0,946	0,013	0,741	(0,347-1,582)	0,439
Tumor size	< 0.001	1,096	(0,822-1,460)	0,533	< 0.001	0,907	(0,678-1,213)	0,510
Sarcomatoid features	0,003	1,236	(0,555-2,754)	0,633	0,004	1,289	(0,5742,893)	0,538

Abbreviations: CI = confidence interval; DSS = disease-specific survival; ECOG = Eastern Cooperative Oncology Group; HR = hazard ratio; ISUP = International Society of Urologic Pathologists; NE = NOT EVALUATED; OS = overall survival.

reaction may help to corroborate our findings and confirm the role of SETD2 as a potential prognostic marker of the disease.

Clinical Practice Points

- The use of this biomarker, associated with other already known criteria, such as ISUP grade and necrosis, among others, can help to predict the results in patients with clear cell renal cell carcinoma.

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Disclosure

The authors have stated that they have no conflicts of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.clgc.2021.03.003.

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4 CONCLUSÕES

- A expressão negativa de SETD2 ocorreu em 15,3% dos casos de ccRCC, estando associada a fatores clínico e patológicos de pior prognóstico .
- A expressão negativa de SETD2 foi um fator prognóstico independente de SCE e SG, levando-nos a concluir que o uso desse marcador, associado a outras informações prognósticas, pode ajudar a definir melhor o desfecho dos pacientes com ccRCC.

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Anexo 1 - Classificação de performance/status do ECOG: Eastern Cooperative Oncologic Group Performance Status Classification.

0 – Assintomático.

1 – Sintomático, mas completamente ambulatorial.

2 – Sintomático, permanece no leito <50% do dia.

3- Sintomático, permanece > 50% do dia no leito, mas não restrito ao leito.

4 – Restrito ao leito.

5 – Morto.

Fonte: Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol. 1982 Dec;5(6):649-55.

Anexo 2 - Classificação TNM de 2009 da “American Joint Committee on Cancer”

Tx	Tumor primário não pode ser avaliado.
T0	Ausência de evidência de tumor primário.
T1	Tumor ≤ 7cm no maior diâmetro, limitado ao rim.
T1a	Tumor ≤ 4cm no maior diâmetro, limitado ao rim.
T1b	Tumor > 4cm e ≤ 7cm no maior diâmetro, limitado ao rim.
T2	Tumor > 7cm no maior diâmetro, limitado ao rim.
T2a	Tumor > 7cm e ≤ 10cm no maior diâmetro, limitado ao rim.
T2b	Tumor > 7cm no maior diâmetro, limitado ao rim.
T3	Tumor com extensão até grandes vasos ou tecido perinefrético e não ultrapassa fáscia de Gerota.
T3a	Tumor invade seio e/ou gordura perinéfretica, mas não ultrapassa fáscia de Gerota e/ou com extensão até veia renal ou seus ramos segmentares.
T3b	Tumor com extensão até veia cava infradiafragmática
T3c	Tumor com extensão até veia cava supra-diafragmática
T4	Tumor com invasão além da fáscia de Gerota (inclusive invasão contígua da glândula adrenal ipsilateral).
NX	Linfonodos regionais não podem ser avaliados.
N0	Ausência de metástases linfonodais.
N1	Presença de metástase em linfonodos regionais.
M0	Ausência de metástases a distância.
M1	Presença de metástases a distância.

Fonte: Novara G, Ficarra V, Antonelli A, Artibani W, Bertini R, Carini M, Cosciani Cunico S, Imbimbo C, Longo N, Martignoni G, Martorana G, Minervini A, Mirone V, Montorsi F, Schiavina R, Simeone C, Serni S, Simonato A, Siracusano S, Volpe A, Carmignani G; SATURN Project-LUNA Foundation. Validation of the 2009 TNM version in a large multi-institutional cohort of patients treated for renal cell carcinoma: are further improvements needed? Eur Urol. 2010 Oct;58(4):588-95. doi: 10.1016/j.eururo.2010.07.006.

Anexo 3 - Estadiamento clínico de 2009 da “American Joint Committee on Cancer”

Estadio clínico	T	N	M
I	T1	N0	M0
II	T2	N0	M0
III	T1ouT2	N1	M0
	T3	N0 ou N1	M0
IV	T4	Qualquer N	M0
	Qualquer T	Qualquer N	M1

Fonte: Novara G, Ficarra V, Antonelli A, Artibani W, Bertini R, Carini M, Cosciani Cunico S, Imbimbo C, Longo N, Martignoni G, Martorana G, Minervini A, Mirone V, Montorsi F, Schiavina R, Simeone C, Serni S, Simonato A, Siracusano S, Volpe A, Carmignani G; SATURN Project-LUNA Foundation. Validation of the 2009 TNM version in a large multi-institutional cohort of patients treated for renal cell carcinoma: are further improvements needed? Eur Urol. 2010 Oct;58(4):588-95. doi: 10.1016/j.eururo.2010.07.006.

Anexo 4 - Classificação UISS - University of California - Los Angeles Integrated Staging System / localized disease

Localized disease (Any T, N0M0)			
T stage	Nuclear grade	ECOG PS	Risk
T1	Fuhrman 1-2	0	Low
		≥1	
	Fuhrman 3-4	Any	Intermediate
T2	Any	Any	
T3	Fuhrman 1	0	
		≥1	
	Fuhrman >1	0	High
		≥1	

Fonte: Zisman A, Pantuck AJ, Wieder J, Chao DH, Dorey F, Said JW, deKernion JB, Figlin RA, Belldegrun AS. Risk group assessment and clinical outcome algorithm to predict the natural history of patients with surgically resected renal cell carcinoma. J Clin Oncol. 2002 Dec 1;20(23):4559-66.

Anexo 5 - Critérios estratificação risco doença metastática IMDC

International Metastatic Renal Cell Carcinoma Database Consortium criteria

Karnofsky performance status score <80
Time from original diagnosis to initiation of targeted therapy <1 year
Hemoglobin less than the lower limit of normal
Serum calcium greater than the upper limit of normal
Neutrophil count greater than the upper limit of normal
Platelet count greater than the upper limit of normal

- Favorable risk: None of the above risk factors present.
- Intermediate risk: 1 or 2 of the above risk factors present.
- Poor risk: 3 or more risk factors present.

Fonte: Heng DY, Xie W, Regan MM, Harshman LC, Bjarnason GA, Vaishampayan UN, Mackenzie M, Wood L, Donskov F, Tan MH, Rha SY, Agarwal N, Kollmannsberger C, Rini BI, Choueiri TK. External validation and comparison with other models of the International Metastatic Renal-Cell Carcinoma Database Consortium prognostic model: a population-based study. **Lancet Oncol.** 2013;14(2), 141–148

Anexo 6 - Carta de aceitação do Artigo (03/03/2021)

Dear Dr. Espinheira Santos,

Congratulation! We are pleased to accept your manuscript for publication in Clinical Genitourinary Cancer. I encourage you to cite this interesting article of yours in your future relevant publications. Furthermore, I would ask for your help to serve as an expert reviewer of Clinical Genitourinary Cancer when the occasion arises.

Currently we publish less than 15% of submissions. Your manuscript will appear in one of our future issues. You will receive galley proofs when the manuscript has been placed in a line of content and production has begun.

The editor reserves the right to schedule your manuscript for print or online only publication.

All manuscripts will appear in a printed table of contents; however, some papers may only be available online due to print page restrictions (NOTE: the publication of your paper could be delayed if you are not the corresponding author); please let us know as soon as possible if any change in e-mail address is necessary.

If any changes other than author queries, typographical errors or printer errors are made to the proofs, the proofs will be returned to the editorial office for re-review and publication will be delayed.

If you have any questions, please contact our editorial office at clgc@elsevier.com. Thank you for submitting your manuscript to Clinical Genitourinary Cancer.

Sincerely,

Dr. James J Hsieh , MD PhD
Editor, Clinical Genitourinary Cancer
<https://www.editorialmanager.com/cguc>



APROVAÇÃO

Os membros do Comitê de Ética em Pesquisa da Fundação Antonio Prudente – A.C. Camargo Cancer Center, em sua última reunião de 18/06/2019, após analisarem as respostas aos questionamentos realizados em reunião de 23/04/2019, aprovaram a realização do projeto nº 2693/19 intitulado: "ESTUDO DO VALOR PROGNÓSTICO E EXPRESSÃO IMUNO-ISTOQUÍMICA DO GENE SETD2 EM CARCINOMA DE CÉLULAS RENAIIS DO TIPO CÉLULAS CLARAS."

Pesquisador Responsável: Walter Henriques da Costa
Aluno: Victor Espinheira Santos (Mestrado)

Informações a respeito do andamento do referido projeto deverão ser encaminhadas ao CEP dentro de 06 meses em relatório (modelo CEP).

São Paulo, 25 de junho de 2019.

Atenciosamente,

Dra. Sandra Caires Serrano
1ª. Vice-Coordenadora do Comitê de Ética em Pesquisa

Apêndice 1 - Ficha levantamento de dados

Nome: _____

Registro: _____

Lâmina: _____

Data de admissão: _____

Idade (anos): _____

Raça: (1) Branco (2) Não branco

Sexo: (1) Masculino (2) Feminino Tabagismo: (1) Fumante (2) Não fumante

ASA:(1)1 (2)2 (3)3 (4)4

Sintomas: (0) Ausência (1) Hematúria (2) Dor lombar (3) Emagrecimento (4) Febre (5) Outros
(6) Massa palpável (9) Ignorado

Tempo de Evolução (meses): _____

Tumor incidental: (0) Sim (1) Não

Metástase ao diagnóstico: (0) Não (1) Sim

Hemoglobina: _____ Hematócrito: _____

Cirurgia: (1) Radical (2) Parcial

Incisão: (1) Abdominal (2) Lobotomia

Ressecção: (0) Irressecável (1) Ressecção total (2) Margem microscópica (3) Margem
macroscópica

Tempo de Cirurgia: _____

Data da Cirurgia: _____

Transfusão: (0) Não (1) Sim Volume de transfusão: _____

Complicações Precoces: (0) Não (1) Insuficiência renal aguda (2) Íleo paralítico (3) Infecção (4)
Cardiovascular (5) Tromboembolismo (6) Fistula urinaria (7) Hemorragia (8) Outras

Internação (dias): _____

Complicações Tardias: (0) Não (1) Insuficiência renal aguda (2) Suboclusão intestinal (3)

Parede abdominal (4) Hipertensão arterial sistêmica (5) Outras Estadio T: (1) T1a (2) T1b (3)

T2a (4) T2b (5) T3a (6) T3b (7) T3c (8) T4

Estadio N: (0) N0 (1) N1 (2) N2 (3) Nx

No de Linfonodos: _____ No de Linfonodos +: _____

Estadio clínico: (1) I (2) II (3) III (4) IV

Grau de Fuhrman / ISUP: (1) I (2) II (3) III (4) IV

Tipo Histológico: (1) Células claras (2) Papilífero (3) Cromóforo (4) TTE3 Gene (5) Ductos coletores (6) Inclassificável (7) Outros

Diâmetro tumoral (cm): _____

Lateralidade: (1) Direito (2) Esquerdo (3) Bilateral

Tumor bilateral: (0) Não (1) Sincrônico (2) Metacrônico

Multifocal: (0) Não (1) Sim

Topografia: (1) Polo superior (2) Terço médio (3) Polo inferior (4) Mais de um polo

Invasão vascular: (0) Não (1) Sim

Invasão linfática: (0) Não (1) Sim

Invasão de veia renal: (0) Não (1) Sim

Invasão de veia cava: (0) Não (1) Sim

Invasão de pelve e/ou ureter: (0) Não (1) Sim

Invasão de gordura: (0) Não (1) Sim

Necrose tumoral: (0) Não (1) Sim

Invasão de Adrenal: (0) Não (1) Sim

Falha: (0) Não (1) Sim Data da falha: _____

Falha: (0) Não (1) Local (2) Pulmão (3) Osso (4) Fígado (5) SNC (6) Rim contralateral (7) Outro

Tratamento: (0) Não (1) Cirurgia (2) Radioterapia (3) Cirurgia + RT (4) Imunoterapia (5)

Imunoterapia + quimioterapia (6) Outro (7) Quimioterapia (8) Inibidor tirosina quinase

Data da última consulta: _____

Status: (1) Vivo sem doença (2) Vivo com doença (3) Morte outras causas (5) Morte por câncer

(6) Perda de vista Óbito: (1) Sim (2) Não

Sobrevida global (meses): _____

Sobrevida livre de doença (meses): _____

Seguimento atualizado (meses): _____