The New Journal of Urology
eISSN 3023-6940
doi: https://doi.org/10.33719/nju1594236

RESEARCH ARTICLE

The Importance of MTHFD2 Expression in Renal Cell Carcinoma

Onur Ceylan [1], Remzi Arslan [1]

[1] Department of Pathology. Ataturk University, Faculty of Medicine, Erzurum, Türkiye

Submitted: *2024-12-02*
Accepted: *2025-07-05*

Corresponding Author;
Onur Ceylan, Assoc.Prof., MD

Address: Ataturk University
Faculty of Medicine Department of Medical Pathology
Erzurum, Türkiye

E-mail: dr.onurceylan@gmail.com

ORCID
O.C. 0000-0001-7025-0521
R.A. 0000-0002-3198-4706

Abstract

Objective: Renal cell carcinoma (RCC) carries a poor prognosis at advanced stages. Identifying reliable prognostic biomarkers is essential for improved clinical management. Methylenetetrahydrofolate dehydrogenase 2 (MTHFD2), a key mitochondrial enzyme in the folate cycle, is overexpressed in various rapidly proliferating malignancies. However, its prognostic value in RCC remains underexplored. For this reason, we purposed to search the prognostic role of MTHFD2 expression in RCC.

Materials and Methods: This study included 124 RCC patients who applied radical nephrectomy between 2015 and 2020. Immunohistochemical analysis of MTHFD2 expression was performed on paraffin-embedded tumor samples. Expression levels were classified using a histoscore-based system: low (grades 0–1) and high (grades 2–3). Correlations between MTHFD2 expression and clinical/pathological parameters were evaluated, and survival analysis was conducted.

Results: MTHFD2 overexpression was detected in 53% of tumors and was absent in adjacent non-tumor tissues. High expression was significantly associated with adverse prognostic features, including higher histological grade, sarcomatoid differentiation, advanced pT stage, and presence of distant metastases (all $p < 0.05$). Patients with high MTHFD2 expression had significantly reduced overall survival ($p < 0.001$). Remarkably, early-stage tumors (pT1–2) with high MTHFD2 expression were linked to shorter survival compared to more advanced tumors (pT3–4) with low expression.

Conclusion: Our results pointed out that high expression of MTHFD2 is associated with poor prognosis in RCC and may function as an independent prognostic biomarker. These findings underscore the potential of MTHFD2 in risk stratification and as a therapeutic target in RCC.

Keywords: MTHFD2, renal cell carcinoma, prognostic marker, immunohistochemical study

Cite; Ceylan O, Arslan R. The Importance of MTHFD2 Expression in Renal Cell Carcinoma. New J Urol. 2025;20(3):121-129. **doi:** https://doi.org/10.33719/nju1594236

INTRODUCTION

Renal cell carcinoma (RCC) ranks ninth among all cancers (1), with its incidence increasing by approximately 2% in recent years (2). Around one-third of RCC cases metastasize, and metastases are often already present at the time of diagnosis (3). Despite slight improvements in the five-year survival rate, the prognosis for advanced-stage RCC remains poor (1). Recently, therapies targeting vascular endothelial growth factor (VEGF) and specific immunotherapy agents have been introduced as standard treatments for RCC. However, the emergence of resistance to these targeted therapies has become an increasing concern. Therefore, novel treatment strategies are urgently needed, particularly for patients with advanced disease (4).

Folic acid metabolism controls nucleotide synthesis, methylation, and repair, and is involved in the development of many tumors. A single carbon unit is transferred from serine to tetrahydrofolate (THF) by serine hydroxymethyl transferases to form methylenetetrahydrofolate (MTHF). This single carbon unit is then transferred between different types of THF to complete the folate cycle. This cycle consists of separate parallel reactions: cytoplasmic, mitochondrial and nucleus (5). In mitochondria, these reactions take place via two different methylenetetrahydrofolate dehydrogenase 2 (MTHFD2), consisting of MTHFD2 and MTHFD2L (6) (Figure 1). Among these, MTHFD2 is more highly expressed and plays a predominant role in supporting mitochondrial folate metabolism and in responding to growth factor stimulation (7, 8). MTHFD2 is essential for cancer cell proliferation and tumor progression. While it is minimally or not expressed in most normal adult tissues, high levels of MTHFD2 expression have been observed in various malignancies and in developing embryos (6). Previous studies have demonstrated that MTHFD2 overexpression correlates with poor prognosis in some cancers, including colorectal carcinoma (9), breast carcinoma (10), RCC (11), and hepatocellular carcinoma (HCC) (12). However, limited data exist on its specific prognostic role in RCC. Therefore, in this study, we aimed to evaluate the clinical significance of MTHFD2 expression in RCC and explore its association with established prognostic parameters.

MATERIALS AND METHODS

Patients' General Information and Features of Their Tissues

This study included 124 radical nephrectomy materials from patients diagnosed with renal cell carcinoma (RCC) at our institution between January 2015 and 2020. Of these, 86 cases were clear cell RCC, 22 were chromophobe

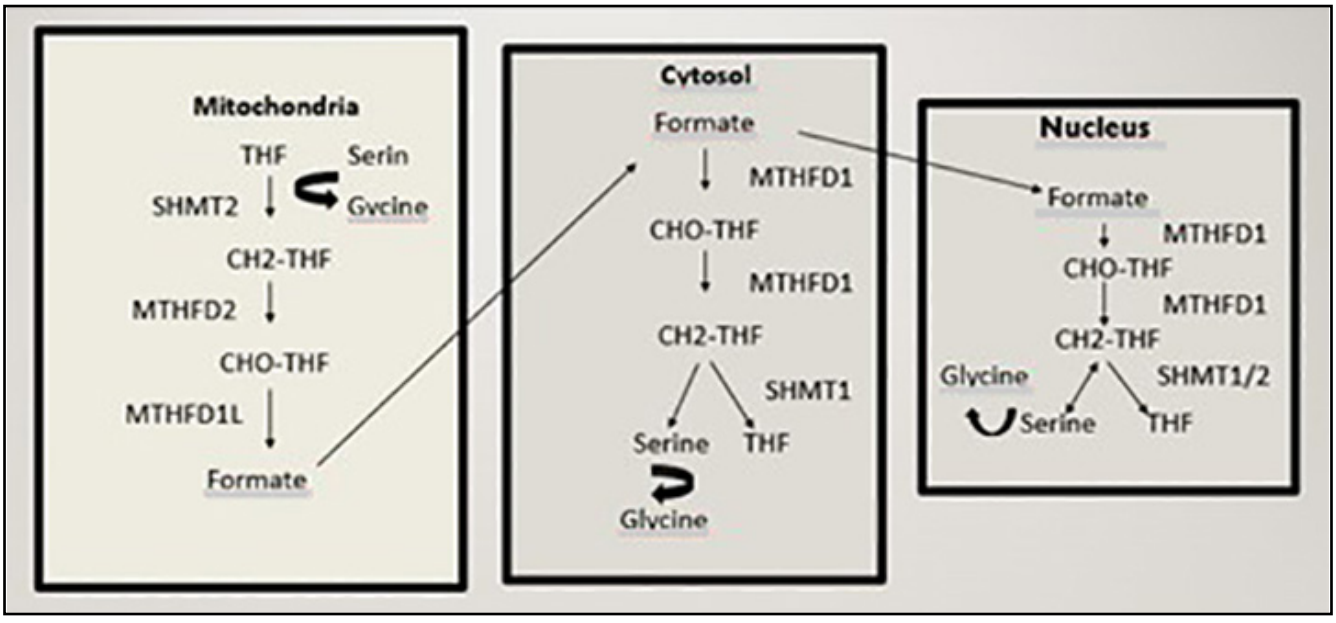

Figure 1. Schematic representation of folate one-carbon metabolism.

RCC, and 16 were papillary RCC. Prognostic parameters such as lymphovascular invasion, histological subtype, histological grade (according to the 2016 The World Health Organisation/International Society of Urological Pathology [WHO/ISUP]), macroscopic tumor diameter, presence of sarcomatoid and rhabdoid features, and pathological staging (pTNM) were recorded. Survival data were also collected. This study was approved by Ethics Committee of Atatürk University (Approval number: B.30.2.ATA.0.01.00/388, Date: 26.06.2020).

Histological grading was not applicable to chromophobe RCC cases; thus, grading evaluation was conducted on 102 cases. Tumor staging including primary tumor (pT), regional lymph nodes (pN), and distant metastases (pM) was based on the 8th edition of the American Joint Committee on Cancer (AJCC) staging manual (13). For many cases, pN and pM statuses were indeterminate and recorded as pNx and pMx, respectively (Table 1, 2).

The mean follow-up period was 37 ± 17 months (1–71 months). Overall survival was calculated from the date of surgery to either death or the last follow-up. Only RCC-related mortality was included in the survival analysis; deaths due to unrelated causes were excluded. For the purpose of analysis, MTHFD2 expression was categorized as low (histoscore grades 0–1) or high (grades 2–3).

Formalin-fixed, paraffin-embedded blocks containing both tumor and non-tumor tissues were selected from each case for immunohistochemical analysis.

Immunohistochemical Study

Blocks with the highest tumor density were selected and sections of four microns were taken. These materials laid in the Ventana automated immunohistochemistry staining device after being kept on charged slides in a 70-degree drying oven for 15 minutes. Following deparaffinization, dehydration, hydrogen peroxide processes, tissues were treated with MTHFD2 antibody (Leica, United Kingdom). Cytoplasmic staining was considered positive for MTHFD2. For MTHFD2, a staining rate of 0% was classified as Grade 0, 1-10% as Grade 1, 11-49% as Grade 2, and ≥50% as Grade 3. Staining intensity was evaluated as follows: no staining: Grade 0; weak staining: Grade 1; moderate staining: Grade 2; and strong staining: Grade 3. The immunoreactivity score was calculated by multiplying staining intensity and staining rate. And it was evaluated as follows: (negative) 0: Grade 0; 1-3: Grade 1; 4-6: Grade 2; 7-9: Grade 3 (Figure 2).

Table 1. Histopathological and demographic features of the patients

Age ± SD	**Patients (n = 124) (%)**
	58 ± 13.7
Gender n (%)	
Male	70 (56)
Female	54 (44)
Tumor Macroscopic Diameter (cm)	
n (%)	6.3 ± 2.6
≤ 4 cm	26 (21)
4<x≤7 cm	64 (52)
7<x≤10 cm	24 (19)
>10 cm	10 (8)
Histological Type n (%)	
Clear cell	86 (69)
Papillary	16 (13)
Chromophobe	22 (18)
pT n (%)	
pT1	62 (50)
pT2	20 (16)
pT3	40 (32)
pT4	2 (2)
pN n (%)	
pN0, x	100 (81)
pN1,2, 3	24 (19)
pM n (%)	
pM0,x	104 (84)
pM1	20 (16)
Recurrence n (%)	
Absent	118 (95)
Present	6 (5)
Sarcomatoid Features n (%)	
Absent	118 (95)
Present	6 (5)
Rhabdoid Features n (%)	
Absent	116 (94)
Present	8 (6)
Outcome n (%)	
Survived	94 (76)
Died	30 (24)

pT: *Primary Tumor,* **pN:** *Lymph Node Metastasis,* **pM:** *Distant Metastasis*

Table 2. Correlation between prognostic factors and MTHFD2 expression

	Histoscore				P
	Grade 0 (n = 57)	**Grade 1 (n = 51)**	**Grade 2 (n = 6)**	**Grade 3 (n = 10)**	
Histological Type n					
Clear cell	39	35	6	6	*0.6485*
Papillary	2	12	0	2	
Chromophobe	16	4	0	2	
pT (n)					
pT1, 2	51	25	4	2	***0.0001***
pT3, 4	6	26	2	8	
pN (n)					
pN, x	55	49	6	8	*0.4102*
pN1, 2, 3	2	2	0	2	
pM (n)					
pM0,x	55	39	4	6	***0.0046***
pM1	2	12	2	4	
Recurrence n					
Absent	55	47	6	10	*0.8443*
Present	2	4	0	0	
Sarcomatoid Features n					
Absent	57	49	4	8	***0.0184***
Present	0	2	2	2	
Rhabdoid Features n					
Absent	55	49	4	8	*0.1307*
Present	2	2	2	2	
Outcome n					
Survived	53	45	**7**	6	***< 0.001***
Died	4	6	**1**	4	

pT: *Primary Tumor,* **pN:** *Lymph Node Metastasis,* **pM:** *Distant Metastasis*

Statistical Analysis

The relationship between MTHFD2 expression and prognostic factors was evaluated with the Spearman correlation test. For survival analysis Kaplan-Meier survival analysis and log-rank test were used. The Cox regression multivariate analysis was applied to determine independent prognostic factors. Descriptive information is stated as mean and deviation for continuous measurements and n as percentage for categorical variables. For the two-tailed p value, <0.05 was received as significant. Hazard rate rates obtained as a result of Cox regression analysis presented. In addition, overall survival rate and standard error values reported with 95% confidence intervals (Figure 4). MedCalc software was used for statistical analysis.

RESULTS

Patients' Demographic and Histopathological Features

A total of 124 patients were included in the study, with a mean age of 58 ± 13.7 years (range: 17–85). The male-to-female ratio was 1.3. The histological subtypes of RCC were distributed as follows: clear cell RCC in 69% of cases, papillary RCC in 13%, and chromophobe RCC in 18%. The mean tumor diameter was 6.3 ± 2.6 cm (1.3–13 cm) (Table 1). Regarding tumor grade, 12 cases were grade 4, 32 were grade 3, 42 were grade 2, and 16 were grade 1. During follow-up, 30 patients died due to RCC-related complications. Among the deceased patients, 18 had clear cell RCC, 8 had papillary RCC, and 4 had chromophobe RCC.

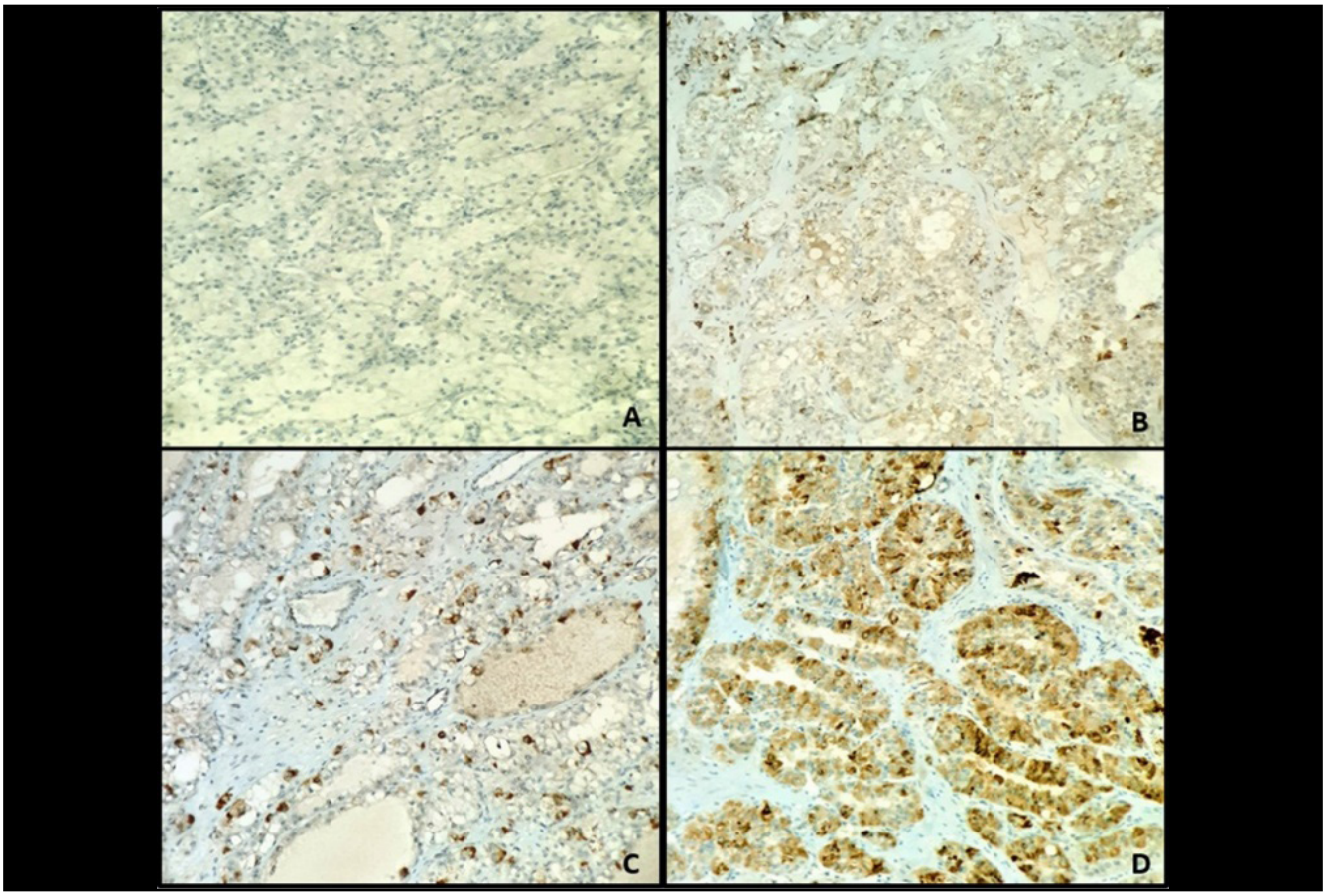

Figure 2. Histological images of MTHFD2 staining (x200) **A:** no MTHFD2 staining, **B:** weak MTHFD2 staining, **C:** moderate MTHFD2 staining, **D:** strong MTHFD2 staining

Prognostic Significance of MTHFD2 expression in RCC

MTHFD2 overexpression was observed in tumor tissues in 66 (53%) of the 124 cases. No MTHFD2 expression was detected in adjacent non-neoplastic tissues. Stronger expression was particularly noted in areas exhibiting rhabdoid and sarcomatoid morphology (Figure 3).

MTHFD2 overexpression was significantly associated with adverse pathological features including advanced pT stage, presence of distant metastasis, and sarcomatoid differentiation (all $p < 0.05$). Moreover, high MTHFD2 expression correlated significantly with key determinants of pT staging such as invasion into the renal pelvis and perirenal adipose tissue ($p < 0.05$ for all). Additionally, an important association was observed between MTHFD2 expression and histological grade in clear cell and papillary RCC ($p = 0.037$). Significant associations weren't found between MTHFD2 expression and histologic subtype, pN stage, recurrence, or rhabdoid features ($p > 0.05$) (Table 2). Likewise, no significant correlations were identified with age ($p = 0.37$), gender ($p = 0.64$), tumor size ($p = 0.98$), lymphovascular invasion ($p = 0.30$), or perineural invasion ($p = 0.31$).

Kaplan–Meier survival analysis revealed 1-, 3-, and 5-year overall survival rates of 85%, 83%, and 80%, respectively. High MTHFD2 expression was significantly associated with decreased survival compared to low expression, as confirmed by the log-rank test ($p < 0.001$) (Figure 4).

In multivariate Cox regression analysis—including MTHFD2 expression, pT stage, and presence of metastasis—MTHFD2 overexpression remained an independent prognostic factor for overall survival (Hazard Ratio = 5.25; 95% CI: 1.30–21.23; $p = 0.0019$).

To further evaluate the prognostic value of MTHFD2, subgroup survival analyses were conducted based on pT and metastasis status. pT stage was dichotomized into early

(pT1–2) and advanced (pT3–4). Patients were stratified into the following subgroups:

1- low expression/ no distant metastasis, low expression/ distant metastasis, high expression/no distant metastasis, and high expression/distant metastasis
2- low expression/early pT, low expression/advanced pT, high expression/early pT, and high expression/advanced pT

Patients with high MTHFD2 expression and distant metastasis had the poorest survival outcomes (p = 0.0004), as did those with high MTHFD2 expression and advanced pT stage (p = 0.0031). Notably, patients with early-stage tumors (pT1–2) but high MTHFD2 expression had shorter survival than those with more advanced tumors (pT3–4) and low expression, highlighting its independent prognostic impact (Figures 4).

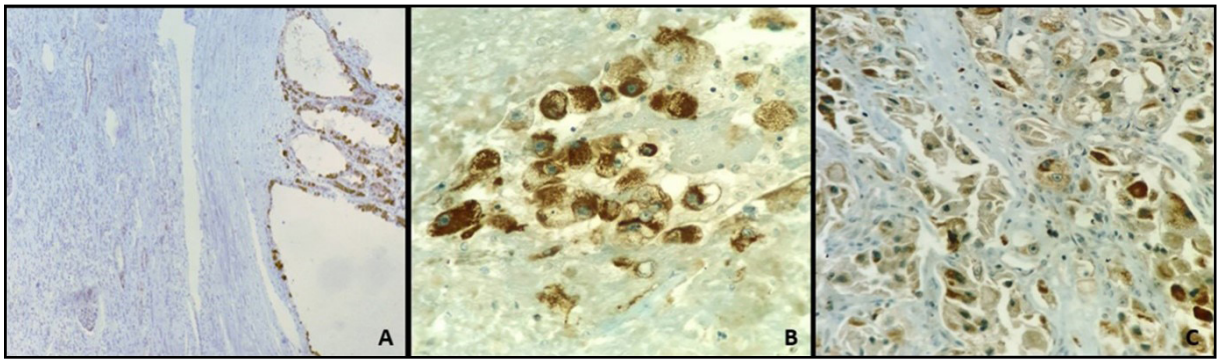

Figure 3. Histological images of MTHFD2 overexpression in different areas

A: Overexpression of MTHFD2 in tumoral areas and no staining in adjacent non-tumoral glomeruli and tubules (x200), **B:** stronger expression with MTHFD2 in areas containing rhabdoid morphology (x400), **C:** stronger expression with MTHFD2 in areas containing sarcomatoid morphology (x200)

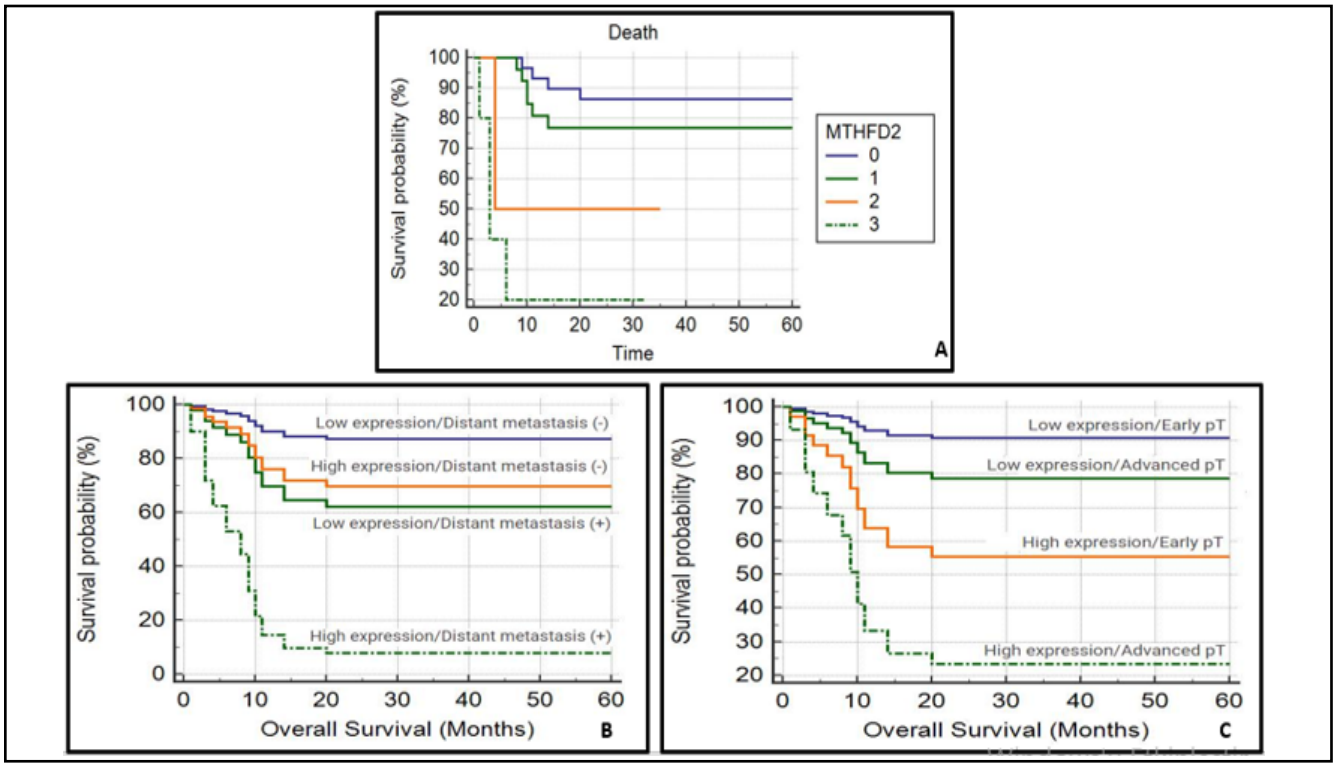

Figure 4.A: Kaplan–Meier survival curve according to MTHFD2 expression, **B:** MTHFD2 expression/distant metastasis status (Cox regression analysis), **C:** MTHFD2 expression/primary tumor (pT) status (Cox regression analysis)

DISCUSSION

In the present study, we investigated MTHFD2 expression in both tumoral and adjacent non-tumoral renal tissues to assess its prognostic value in RCC. Our results demonstrated that MTHFD2 was not expressed in normal kidney tissues but was significantly overexpressed in RCC specimens. Importantly, high MTHFD2 expression was significantly correlated with adverse prognostic factors, such as higher pT stage, distant metastasis (pM), sarcomatoid differentiation, histological grade, and reduced survival. Multivariate analysis confirmed that MTHFD2 overexpression is an independent prognostic marker in RCC. To further explore its prognostic role, subgroup survival analyses were performed based on pT stage and distant metastasis. Patients with high MTHFD2 expression combined with either distant metastasis or advanced pT stage had the shortest survival times. Remarkably, even among early-stage tumors (pT1–2), cases with MTHFD2 overexpression exhibited shorter survival compared to those with more advanced tumors (pT3–4) but low MTHFD2 expression. This finding strongly supports the role of MTHFD2 as an independent and clinically relevant prognostic biomarker.

Our findings are consistent with previous studies investigating the association of RCC and MTHFD2. In RCC, Lin et al. showed that MTHFD2 expression was significantly associated with advanced clinical stage, higher pathological grade, and reduced survival, and proposed that MTHFD2 may represent a therapeutic target (14). Silva et al. reported that MTHFD2 expression differed significantly among subtypes of RCC, and high MTHFD2 levels were associated with poor histological features and short survival (15).

In addition, recent studies in the literature have increasingly emphasized the relationship between MTHFD2 overexpression and poor prognosis in various malignancies (6, 9–12). Nilsson et al. showed that MTHFD2 is absent in normal adult tissues but is highly expressed in several cancers, particularly breast cancer, and is associated with poor prognosis, suggesting a critical role for mitochondrial one-carbon metabolism in malignancy (6). Similarly, Ju et al. reported that MTHFD2 promotes tumor growth and distant metastasis in colorectal carcinoma, and its suppression significantly reduced tumor burden (16). Miyo et al. also found that MTHFD2 overexpression correlated with lower disease-free and overall survival in colorectal cancer (17). In hepatocellular carcinoma, Liu et al. demonstrated that MTHFD2 overexpression was associated with worse outcomes, including advanced stage, recurrence, and metastasis (18).

In light of these results and the existing literature, our study further supports the hypothesis that MTHFD2 plays a pivotal role in tumor survival, progression, and metastasis. The significant associations between MTHFD2 expression and key prognostic indicators underscore its potential utility as a prognostic biomarker in RCC.

We did not find a statistically significant association between MTHFD2 expression and recurrence, which may be attributed to the low number of recurrent cases (5%) in our cohort. This limitation highlights the need for further studies with larger patient populations to explore this relationship more thoroughly.

CONCLUSION

Our findings show that MTHFD2 overexpression is associated with poor prognosis in RCC. The most notable result is that even early-stage RCC cases with high MTHFD2 expression demonstrated worse survival outcomes than those with advanced-stage disease and low expression. These results promote the potential usage of MTHFD2 as an independent prognostic biomarker in RCC. However, further validation in larger, multi-institutional cohorts is necessary to confirm its clinical utility.

Funding: Our study was supported by Atatürk University Scientific Research Project Coordination Unit (Project Code: TAB-2021-8842).

Conflict of Interest: None declared.

Ethics Approval: This study was approved by Ethics Committee of Atatürk University (Approval number: B.30.2.ATA.0.01.00/388, Date: 26.06.2020).

Author Contribution: Consept and design, data analysis, data collection, critical revision and supervision; Onur Ceylan. The draft of the manuscript, data acquisition statistical analysis; Onur Ceylan and Remzi Arslan. All

authors have read and approved the final manuscript.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin*. 2018;68(1):7–30. https://doi.org/10.3322/caac.21442
2. Ljungberg B, Bensalah K, Canfield S, Dabestani S, Hofmann F, Hora M, et al. EAU guidelines on renal cell carcinoma: 2014 update. *Eur Urol*. 2015;67(5):913–24. https://doi.org/10.1016/j.eururo.2015.01.005
3. Noone A, Howlader N, Krapcho M, Miller D, Brest A, Yu M, et al. SEER cancer statistics review, 1975–2015. Bethesda (MD): National Cancer Institute; 2018. Available from: https://seer.cancer.gov/csr/1975_2015/
4. Choueiri TK, Motzer RJ. Systemic therapy for metastatic renal-cell carcinoma. *N Engl J Med*. 2017;376(4):354–66. https://doi.org/10.1056/NEJMra1601333
5. Newman AC, Maddocks ODK. One-carbon metabolism in cancer. *Br J Cancer*. 2017;116(12):1499–504. https://doi.org/10.1038/bjc.2017.118
6. Nilsson R, Jain M, Madhusudhan N, Sheppard NG, Strittmatter L, Kampf C, et al. Metabolic enzyme expression highlights a key role for MTHFD2 and the mitochondrial folate pathway in cancer. *Nat Commun*. 2014;5:3128. https://doi.org/10.1038/ncomms4128
7. Shin M, Bryant JD, Momb J, Appling DR. Mitochondrial MTHFD2L is a dual redox cofactor-specific methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase expressed in both adult and embryonic tissues. *J Biol Chem*. 2014;289(22):15507–17. https://doi.org/10.1074/jbc.M114.555573
8. Nilsson R, Nicolaidou V, Koufaris C. Mitochondrial MTHFD isozymes display distinct expression, regulation, and association with cancer. *Gene*. 2019;716:144032. https://doi.org/10.1016/j.gene.2019.144032
9. He Z, Wang X, Zhang H, Liang B, Zhang J, Zhang Z, et al. High expression of folate cycle enzyme MTHFD1L correlates with poor prognosis and increased proliferation and migration in colorectal cancer. *J Cancer*. 2020;11(14):4213–21. https://doi.org/10.7150/jca.35014
10. Liu F, Liu Y, He C, Tao L, He X, Song H, et al. Increased MTHFD2 expression is associated with poor prognosis in breast cancer. *Tumour Biol*. 2014;35(9):8685–90. https://doi.org/10.1007/s13277-014-2144-2
11. Green NH, Galvan DL, Badal SS, Chang BH, LeBleu VS, Long J, et al. MTHFD2 links RNA methylation to metabolic reprogramming in renal cell carcinoma. *Oncogene*. 2019;38(34):6211–25. https://doi.org/10.1038/s41388-019-0869-4
12. Lee D, Xu IM, Chiu DK, Lai RK, Tse AP, Li LL, et al. Folate cycle enzyme MTHFD1L confers metabolic advantages in hepatocellular carcinoma. *J Clin Invest*. 2017;127(5):1856–72. https://doi.org/10.1172/JCI91015
13. Compérat EM, Burger M, Gontero P, Mostafid AH, Palou J, Rouprêt M, et al. Grading of urothelial carcinoma and the new WHO classification of tumours of the urinary system and male genital organs 2016. *Eur Urol Focus*. 2019;5(3):457–66. https://doi.org/10.1016/j.euf.2018.01.009
14. Lin H, Huang B, Wang H, Liu X, Hong Y, Qiu S, et al. MTHFD2 overexpression predicts poor prognosis in renal cell carcinoma and is associated with cell proliferation and vimentin-modulated migration and invasion. *Cell Physiol Biochem*. 2018;51(2):991–1000. https://doi.org/10.1159/000495380
15. Silva RV, Berzotti LA, Laia MG, Araújo LS, Silva CA, Ribeiro R, et al. Implications of MTHFD2 expression in renal cell carcinoma aggressiveness. *PLoS One*. 2024;19(2):e0299353. https://doi.org/10.1371/journal.pone.0299353
16. Ju H-Q, Lu Y-X, Chen D-L, Zuo Z-X, Liu Z-X, Wu Q-N, et al. Modulation of redox homeostasis by inhibition of MTHFD2 in colorectal cancer: mechanisms and therapeutic implications. *JNCI J Natl Cancer Inst*. 2019;111(6):584–96. https://doi.org/10.1093/jnci/djy161
17. Miyo M, Konno M, Colvin H, Nishida N, Koseki J, Kawamoto K, et al. The importance of mitochondrial folate enzymes in human colorectal cancer. *Oncol Rep*. 2017;37(1):417–25. https://doi.org/10.3892/or.2016.5289

18. Liu X, Huang Y, Jiang C, Ou H, Guo B, Liao H, et al. Methylenetetrahydrofolate dehydrogenase 2 overexpression is associated with tumor aggressiveness and poor prognosis in hepatocellular carcinoma. *Dig Liver Dis*. 2016;48(8):953–60. https://doi.org/10.1016/j.dld.2016.03.022