Cellular Physiology and Biochemistry
Cell Physiol Biochem 2018;51:991-1000
DOI: 10.1159/000495402
Published online: 22 November 2018
Accepted: 14 November 2018
© 2018 The Author(s)
Published by S. Karger AG, Basel
www.karger.com/cpb

This article is licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License (CC BY-NC-ND) (http://www.karger.com/Services/OpenAccessLicense). Usage and distribution for commercial purposes as well as any distribution of modified material requires written permission.

Original Paper

MTHFD2 Overexpression Predicts Poor Prognosis in Renal Cell Carcinoma and is Associated with Cell Proliferation and Vimentin-Modulated Migration and Invasion

Hao Lin[a] Bin Huang[b] Hua Wang[c] Xincheng Liu[a] Yuxiang Hong[a] Shaopeng Qiu[b] Junhong Zheng[a]

[a]Department of Urology, The Second Affiliated Hospital of Shantou University Medical College, Shantou, [b]Department of Urology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, [c]Department of Urology, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China

Key Words
Methylenetetrahydrofolate dehydrogenase 2 • Renal cell carcinoma • Cell proliferation • Cell migration • Cell invasion • Vimentin

Abstract
Background/Aims: To investigate the role of methylenetetrahydrofolate dehydrogenase 2 (MTHFD2) in the clinical prognosis and cell biology of renal cell carcinoma (RCC). ***Methods:*** A total of 137 RCC tissues were evaluated by immunohistochemistry. The relationship between MTHFD2 overexpression and clinical parameters and vimentin expression was assessed. Kaplan-Meier curves and the log-rank test were applied for survival analysis according to MTHFD2 and vimentin expression in RCC tissues. The expression of MTHFD2 mRNA and protein was examined by quantitative reverse transcription PCR and western blotting, respectively. To determine further the biological activity of MTHFD2 in RCC, 786-O cells were transfected with short hairpin RNA specifically targeting MTHFD2 (shMTHFD2) with or without tumor necrosis factor (TNF)-α stimulation. Cell proliferation, cell migration and invasion and drug sensitivity were subsequently assessed using Cell Counting Kit-8, wound healing, and Transwell assays. ***Results:*** Immunohistochemical analysis demonstrated that both MTHFD2 and vimentin overexpression was positively associated with clinical staging, pathological grade, and poor overall survival (all $P < 0.05$). MTHFD2 expression was closely correlated with vimentin overexpression in RCC ($r = 0.402$, $P < 0.001$). After knocking down MTHFD2 expression in 786-O cells, decreased cell proliferation, migration, and invasion were observed and accompanied by the reduced expression of vimentin. The effects of MTHFD2 down-regulation could be partially restrained by TNF-α treatment. Vimentin expression and cell migration and invasion,

H. Lin, B. Huang and H. Huang contributed equally to this work.

Hao Lin
Department of Urology, The Second Affiliated Hospital of Shantou University Medical College
Shantou, Guangdong, 515041 (China)
E-Mail linh26@mail2.sysu.edu.cn

but not cell proliferation, were reversed by TNF-α stimulation. Furthermore, treatment of 786-O cells with shMTHFD2 increased their sensitivity to chemotherapy drugs. ***Conclusion:*** The current results demonstrated that MTHFD2 was overexpressed in RCC and associated with poor clinical characteristics, vimentin expression, and cellular features connected to malignant disease, thus, implicating MTHFD2 as a potential target for RCC therapy.

© 2018 The Author(s)
Published by S. Karger AG, Basel

Introduction

Renal cell carcinoma (RCC) is the ninth most common cancer worldwide. Neoplasms of the kidney (including the renal pelvis) are estimated to occur in 65, 340 patients in the United States and result in 14, 970 deaths in 2018 [1]. Approximately 17% of patients who present with metastatic disease are diagnosed with *de novo* RCC in the United States [2]. Therapy for RCC has changed greatly over the last decade, especially for those patients with advanced disease. Therapies targeting tumor vasculature are now the mainstays for the treatment of metastatic RCC. However, most patients ultimately die due to the gradual development of resistance in the tumor to antiangiogenic therapy. Therefore, new therapeutic approaches for patients with advanced RCC are urgently needed.

Divergent metabolic remodeling is regarded as a hallmark of malignant tumors. Recently, studies have shown that metabolic enzymes may act as oncogenes [3] and can even transform cells [4]. Nilsson et al. found that methylenetetrahydrofolate dehydrogenase 2 (MTHFD2), which has a role in mitochondrial folate metabolism, is markedly elevated in many cancers, including RCC, and positively correlated with poor prognosis in patients with breast cancer [5]. Moreover, MTHFD2 expression is very low in most healthy adult tissues, even those that actively proliferate [5, 6]. These features make it an ideal diagnostic marker for cancer and even a treatment target. However, the role of MTHFD2 in RCC has not been examined.

Normally, the enzymes involved in folate metabolism are targets of chemotherapy drugs such as methotrexate and 5-fluorouracil (5-FU); however, RCC has been demonstrated to be refractory to chemotherapy. Quite recently, researchers have shown that MTHFD2 may have an impact on cell proliferation independent of its enzymatic activity [7], which may suggest that inhibiting its enzymatic activity alone may not be sufficient to stop tumor progression. Although the enzymatic activity of MTHFD2 has been well characterized, little is known about its role in RCC cell biology. Lehtinen et al. demonstrated that MTHFD2 is a novel regulator of vimentin expression by high-throughput RNA interference (RNAi) screening [8]. It has been proposed that MTHFD2 might be a novel factor in cell proliferation [9] and has been proven to be essential for tumor metastasis [10].

In this study, we found that MTHFD2 overexpression in RCC tissues was positively associated with tumor grade, tumor–node–metastasis (TNM) stage, vimentin expression, and poor survival. By knocking down MTHFD2 expression in RCC cell lines, we found that cell proliferation was significantly decreased. Additionally, we demonstrated that MTHFD2 deletion can reduce cell migration and invasion, which was possibly modulated by the reduced expression of vimentin. Furthermore, we showed that MTHFD2 down-regulation may increase the sensitivity of RCC cells to chemotherapy. These findings indicate that MTHFD2 overexpression plays a pivotal role in the progression of RCC and might be a potential target for RCC therapy.

Materials and Methods

Patients and tissue samples

This study was approved by the Medical Ethics Committee of Sun Yat-sen University, and written informed consent was obtained from each patient for surgery and research purposes. A total of 137 patients with RCC from the First Affiliated Hospital of Sun Yat-sen University were enrolled in this study

between 2010 and 2012. The patients consisted of 81 males and 56 females with a median age of 52 years (range: 12–80 years). No patient received any treatment before radical nephrectomy or partial nephrectomy. RCC was confirmed by pathology findings. The patients were followed up by clinical interview or over the telephone; the total follow-up period ranged from 1 to 60 months. Overall survival (OS) time was calculated as the duration from the date of surgery to the date of death. Of the 137 tissue samples, 23 were papillary RCC, 17 were chromophobe RCC, and 97 were clear cell RCC. Tumors were staged according to the 2009 TNM staging system [11] and graded according to the criteria of the World Health Organization [12, 13].

Table 1. Sequences of primers for qRT-PCR

Primer	Sense (5′–3′)	Antisense (5′–3′)
MTHFD2	CTGCGACTTCTCTAATGTCTGC	CTCGCCAACCAGGATCACA
β-actin	AGCGAGCATCCCCCAAAGTT	GGGCACGAAGGCTCATCATT

Cell lines

The RCC cell lines 786-O, ACHN, and 769-P were obtained from the American Type Culture Collection (Manassas, VA) and cultured in RPMI-1640 (Gibco, Gaithersburg, MD) supplemented with 10% fetal bovine serum (FBS; Gibco). OS-RC-2 cells were a kind gift from Dr. Shenjie Guo (Department of Urology, Sun Yat-sen University Cancer Center) and were also maintained in RPMI-1640 (Gibco) supplemented with 10% FBS (Gibco). All cells were kept in a 37°C humidified incubator with 95% O_2 and 5% CO_2.

Immunohistochemistry

Immunohistochemical (IHC) staining was performed as described previously [5]. Polyclonal rabbit IgG anti-MTHFD2 (1:200; Abcam, Cambridge, MA) and polyclonal rabbit IgG anti-vimentin (1:200; Cell Signaling Technology, Danvers, MA) antibodies were used for IHC. All sections were assessed and scored by two independent pathologists based on staining intensity and the percentage of positive tumor cells, as described previously [5]. Staining intensity was graded as: negative = 0; weak = 1; moderate = 2; and strong = 3. The percentage of positive-staining cells was scored as: <5% = 0; 6–25% = 1; 26–50% = 2; 51–75% = 3; and >76% = 4. The total score was obtained by multiplying intensity by proportion (intensity score × proportion score). Positive staining was defined as a staining index ≥4, whereas negative staining was defined as a staining index <4.

RNA extraction and quantitative reverse transcription polymerase chain reaction

Total cellular RNA was isolated using the PureLink®R RNA Mini Kit (Ambion, Foster City, CA), reverse transcribed to cDNA using a RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA), and analyzed by quantitative reverse transcription PCR (TaKaRa Biotechnology Co., Ltd., Shiga, Japan) according to the manufacturers' instructions. The primer sequences are listed in Table 1.

Western blot analysis

Whole cell lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes. The membranes were blocked and incubated with antibodies targeting MTHFD2 (1:1000; Abcam) or vimentin (1:1000; Cell Signaling Technology). A horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1:5000; Abcam) was used to detect the specific bands.

Cell proliferation assay

Cell proliferation was tested using a Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan). The cells were seeded in 96-well plates at 4000 cells/100 μL/well and transfected with short hairpin RNA (shRNA) targeting MTHFD2 (shMTHFD2) or negative control shRNA, followed by incubation at 37°C in an environment with 5% CO_2 for 48 h. After transfection, the cells were treated with or without human recombinant tumor necrosis factor (TNF)-α (50 ng/mL; Invitrogen) and cultured for an additional 48 h. Then, 10 μL CCK-8 solution was added to each well, followed by incubation for 2 h. Optical density was measured at 450 nm with a microplate reader at 4d, 5d, 6d, and 7dafter transfection. All experiments were carried out in triplicate.

Cell transfection and tumor necrosis factor-α stimulation

786-O cells were transfected with shMTHFD2 (sc-75937-SH; Santa Cruz Biotechnology, Inc., Dallas, TX) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) and Opti-MEM I (Gibco). At 6 h after transfection, shRNA was removed by changing the medium with RPMI-1640 with 10% FBS, and the cells were cultured for an additional 48–72 h. After transfection, the cells were treated with or without TNF-α (50 ng/mL; Invitrogen) and cultured for an additional 48 h, and then total RNA or protein was harvested for analysis.

Wound healing and invasion assays

For the wound healing assay, the cells were plated in 6-well culture plates. When the cells grew to confluence, a straight scratch was made in the center of each well using a micropipette tip, and the cells were washed with phosphate-buffered saline (PBS) and incubated in serum-free medium. The initial gap length and residual gap length at 0, 12, and 24 h after wounding were observed under an inverted microscope and photographed. Wound area was measured using ImageJ software (http://rsb.info.nih.gov/ij/). The percentage of wound closure was estimated by 1 − (wound area at Tt)/(wound area at T0) × 100, where Tt is the time after wounding and T0 is the time immediately after wounding.

For the invasion assay, 500 μL of a serum-free suspension of cells (1.0×10^5 cells/mL) was added to the interior of each Transwell insert (pore size, 8 μm); 500 μL medium containing 10% FBS was added to the lower chamber of the insert. The cells were incubated at 37°C in a 5% CO_2 atmosphere for 36–48 h. Then, the non-invading cells in the interior of the insert were removed gently with a cotton-tipped swab; the invasive cells on the lower surface of the inserts were stained with the staining solution for 20 min and counted under a microscope. Each experiment was performed in triplicate.

Drug sensitivity assay

Cells transfected with shMTHFD2#1/2 or the negative control plasmid were plated in 96-well plates (500 cells/well) and cultured with the topoisomerase II inhibitor antineoplastic agent mitoxantrone (MTX; Sigma-Aldrich, St. Louis, MO) or 5-FU (Sigma-Aldrich) diluted in PBS at different concentrations. Four parallel wells were set for each group. After incubation for 24 h with MTX or 5-FU, the absorbance of each well at a wavelength of 450 nm (*A*450) was measured. The cell survival rate (%) was calculated using the formula: (mean *A*450 of the test wells)/(mean *A*450 of the control wells) × 100. The inhibition rate (%) was calculated using the formula: 100 − survival rate.

Statistical analysis

Data were evaluated using SPSS 13.0 (SPSS, Inc., Chicago, IL) and GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA). All experiments were repeated at least three times. Continuous data are expressed as the mean ± standard deviation and analyzed by the *t*-test or one-way analysis of variance followed by Bonferroni's post-hoc test. The relationship between MTHFD2 and vimentin expression and clinical features were assessed by the chi-square test. The correlation between MTHFD2 and vimentin expression was assessed by Spearman's test. Disease-specific survival was analyzed by the Kaplan-Meier method. Differences in survival were tested by the log-rank test. Statistical significance was established at $P < 0.05$.

Results

MTHFD2 is highly expressed in RCC tissues and is positively associated with grade, stage, and vimentin overexpression

To evaluate whether MTHFD2 was overexpressed in RCC tissues, we performed IHC in 137 RCC tissues. Positive MTHFD2 staining was observed in 80 of the 137 RCC tissues (58.39%, Fig. 1A, B). Furthermore, MTHFD2 was significantly associated with tumor stage and pathological grade (both $P < 0.01$). Interestingly, MTHFD2 staining was positively associated with clear cell RCC ($P = 0.004$, Table 1).

Of the 137 specimens, 97 were positive for vimentin expression (70.8%, Fig. 1C, D). Vimentin was also significantly associated with TNM stage and pathological grade (both $P < 0.01$). Similar to the expression pattern of MTHFD2, vimentin was predominantly expressed

DOI: 10.1159/000495402
Published online: 22 November 2018
© 2018 The Author(s). Published by S. Karger AG, Basel
www.karger.com/cpb

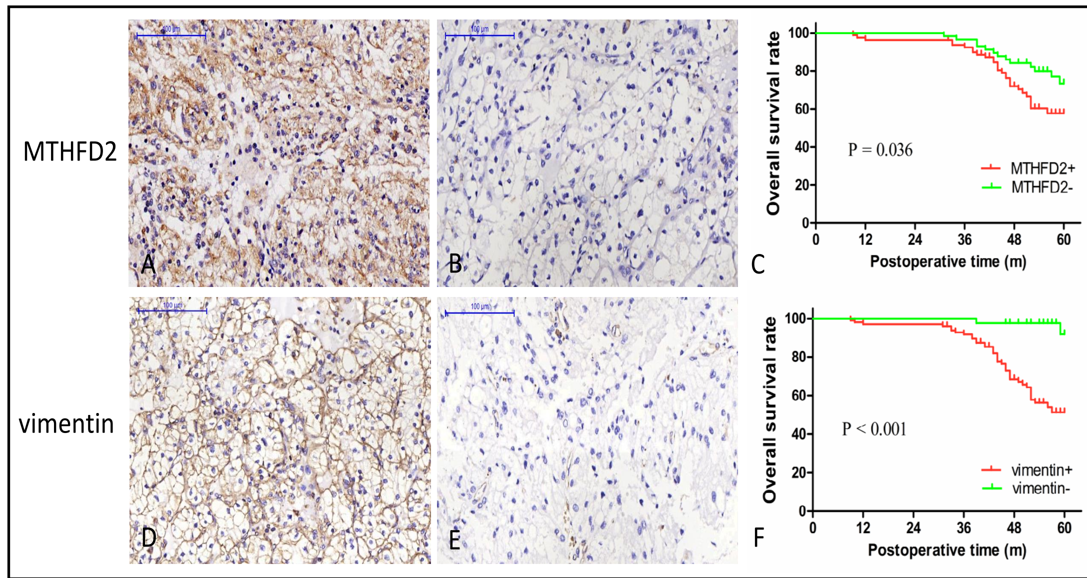

Fig. 1. MTHFD2 and vimentin protein expression in RCC tissues. (A) and (D) Representative positive staining of MTHFD2 and vimentin in RCC. MTHFD2 was observed in the cell cytoplasm, while vimentin was mainly detected in the adjacent stroma. (B) and (E) Representative images of low or negative MTHFD2 and vimentin expression in tumor tissues. (A) and (D) are samples from patients with high grade RCC and lymph node metastasis, whereas (B) and (E) are samples from patients with low grade RCC and without lymph node metastasis. Kaplan-Meier curves showing patients with different MTHFD2 (C) or vimentin (F) expression profiles. The scale bars represent 100 μM.

in clear cell RCC (P < 0.001), but not in chromophobe or papillary RCC (Table 2).

However, neither MTHFD2 nor vimentin was positively associated with sex, age, or location (all P > 0.05, Table 2). In the 137 tissues, positive vimentin expression was detected in 69 of the 80 tissues in the MTHFD2-positive group and 28 of the 57 tissues in the MTHFD2-negative group. Furthermore, correlation analysis demonstrated that MTHFD2 overexpression was significantly correlated with vimentin overexpression (r = 0.402, P < 0.001).

Table 2. Relationship between MTHFD2 and vimentin expression and clinicopathological factors in RCC

Parameter		MTHFD2			Vimentin		
	n	Negative	Positive	P-value	Negative	Positive	P-value
Age (years)				0.895			0.494
<52	61	25	36		16	45	
≥52	76	32	44		24	52	
Gender				0.805			0.606
Female	56	24	32		15	41	
Male	81	33	48		25	56	
Position				0.554			0.486
Left	69	27	42		22	47	
Right	68	30	38		18	50	
Histological type				0.004			<0.001
Clear cell	97	33	64		20	77	
Papillary	23	11	12		4	19	
Chromophobe	17	13	4		16	1	
Grade				0.001			<0.001
Well	96	49	47		37	59	
Moderate and poor	41	8	33		3	38	
TNM stage				<0.001			0.003
I+II	95	47	48		35	60	
III+IV	42	10	32		5	37	

MTHFD2 and vimentin overexpression predicts poor prognosis in RCC

Survival analysis confirmed that patients with MTHFD2-positive expression had a shorter OS time compared to those with MTHFD2-negative expression (P = 0.036). The medium OS time was 56.467 months (95% confidence interval [CI] 54.535–58.398) for the MTHFD2-

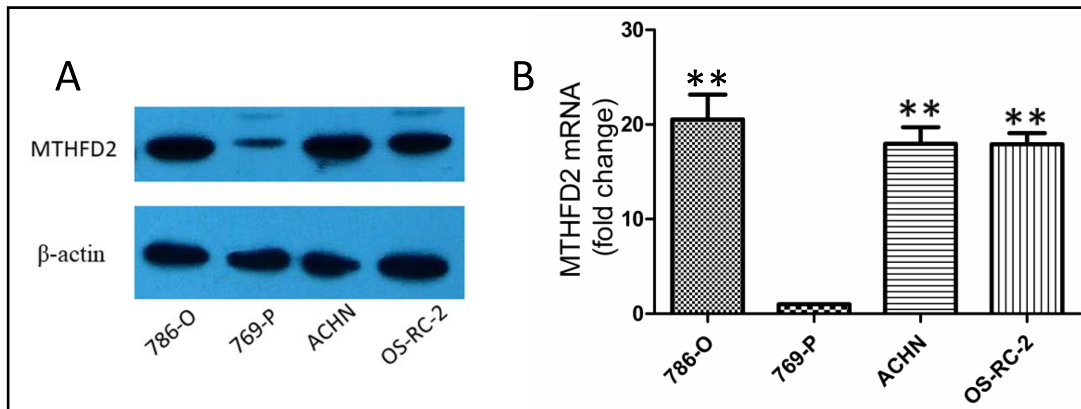

Fig. 2. Protein (A) and mRNA (B) levels of MTHFD2 were significantly higher in 786-O, ACHN, and OS-RC-2 cells than in 769-P cells (**P<0.001).

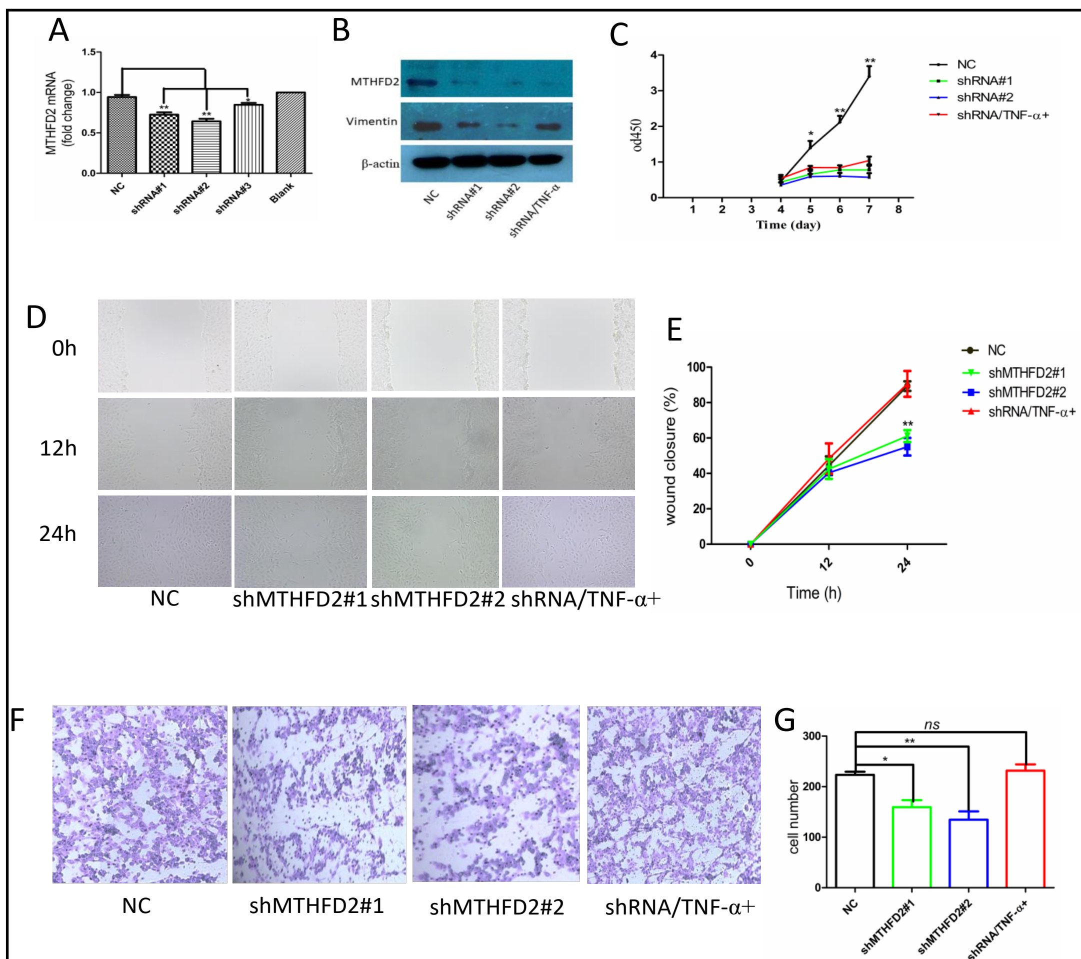

Fig. 3. (A) qRT-qPCR showing the downregulation of MTHFD2 mRNA following transfection of 786-O cells with shRNA. (B) MTHFD2 protein down-regulation by shRNA was accompanied with the reduction of vimentin expression, which was reversed by TNF-α stimulation. (C) At 5 days after MTHFD2 knockdown, proliferation of 786-O cells, even following treatment with TNF-α, was significantly inhibited.. (D, E, F, G) TNF-α stimulation reduced the inhibitory effects of shMTHFD2 transfection on the migration and invasion of 786-O cells. NC, cells transfected with negative control shRNA; Blank, cells cultured with Lipofectamine 2000, but without shRNA; ns, not significant; *P<0.05, **P<0.01.

negative group, while the corresponding medium OS time was 52.623 months (95% CI 50.023–55.223) for the MTHFD2-positive group. Consistently, patients with vimentin overexpression also showed poorer OS than those with low vimentin expression (52.011 vs. 59.418 months, 95% CI 49.717–54.305 vs. 58.399–60.437, respectively, P < 0.01, Fig. 1C, F).

MTHFD2 is highly expressed in 786-O, ACHN, and OS-RC-2 cells compared to 769-P cells at both the mRNA and protein level

To explore further the role of MTHFD2 in RCC cell biology, we used cell lines for our subsequent *in vitro* study. We found that the 786-O, ACHN, and OS-RC-2 cell lines expressed higher levels of MTHFD2 than the 769-P cell line at both the mRNA and protein level (Fig. 2).

MTHFD2 knockdown in 786-O cells decreases cell proliferation, migration, and invasion by reducing vimentin expression

To determine the role of MTHFD2 in cell biology, we down-regulated the expression of MTHFD2 in 786-O cells. As shown in Fig. 3, *MTHFD2* mRNA was significantly decreased in 786-O cells after transfection with shMTHFD2.

A CCK-8 assay was carried out to test the effects of decreased MTHFD2 expression on the proliferation of 786-O cells. The results showed that the growth of cells with lower MTHFD2 expression was significantly affected in comparison with those in the control group (Fig. 3C, P < 0.05).

Migration and invasion were tested by a wound healing experiment and a Transwell assay. Wound closure was significantly impaired in cells with MTHFD2 down-regulation (Fig. 3D, E, P < 0.05). Similarly, knocking down MTHFD2 expression led to a significant decrease in cell invasion through the Matrigel matrix (Fig. 3D, F, P < 0.05).

Furthermore, we found that vimentin expression was reduced by MTHFD2 depletion (Fig. 3B, P < 0.05). To investigate whether the reduction of migration and invasion was modulated by vimentin, cells infected with shMTHFD2 were treated with TNF-α, which enhances the expression of vimentin in RCC cells [14]. We found that TNF-α could reverse the down-regulation of vimentin following MTHFD2 knockdown (Fig. 3B). In addition, treatment with TNF-α inhibited MTHFD2-mediated cellular migration and invasion (Fig. 3D, E, F, P > 0.05). However, the reduced cell proliferation rate caused by MTHFD2 depletion was not affected by TNF-α stimulation (Fig. 3C, P < 0.05).

MTHFD2 down-regulation sensitizes 786-O cells to chemotherapy drugs

To test whether MTHFD2 sensitizes RCC cells to chemotherapy drugs, shRNA-transfected 786-O and control cells were treated with different concentrations of MTX and 5-FU. The cells transfected with shMTHFD2 showed a lower survival rate than those in the control group after treatment with MTX or 5-FU (Fig. 4, P < 0.05).

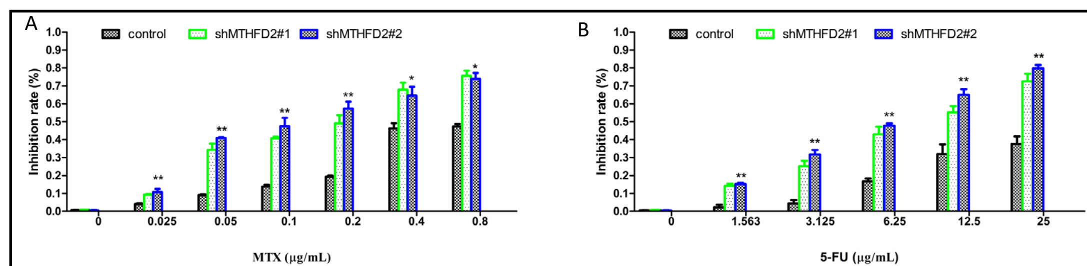

Fig. 4. At 72 h after MTHFD2 knockdown, sensitized 786-O cells were treated with MTX (A) or 5-FU (B) (*P<0.05, **P<0.01).

Discussion

MTHFD2 was first discovered in ascites cells by Scrimgeour and Huennekens in the 1960s as an NAD-dependent methylenetetrahydrofolate dehydrogenase [15]. Recently, MTHFD2 has been demonstrated to be overexpressed in rapidly growing malignant tumors and to participate in the mitochondrial folate pathway to produce additional purines due to increased growth demand [16]. Nilsson et al. identified *MTHFD2* as one of the most consistently overexpressed mRNAs across 19 different tumors; moreover, they found that MTHFD2 protein was present in a variety of different cancers, including RCC [5]. In the present study, we also demonstrated that MTHFD2 was overexpressed in RCC tissues. Furthermore, we confirmed that MTHFD2 overexpression was positively associated with pathological grade and tumor stage. These findings suggested that MTHFD2, which deserves further intensive study, may play an important role in the progression of RCC.

Recently, Lehtinen et al. identified MTHFD2 as a vimentin modulator through high-throughput RNAi screening in breast cancer cells [8]. Vimentin is a key regulator of epithelial-mesenchymal transition [17]. In RCC, vimentin has been shown to be an independent predictor of tumor recurrence and OS in patients with clear cell RCC [18]. Despite its clinical significance, the relationship between MTHFD2 and vimentin in RCC has yet to be determined. According to our study, the expression of MTHFD2 was positively associated with vimentin expression in RCC tissues. Interestingly, while MTHFD2 can hardly be detected in papillary or chromophobe RCC, vimentin expression was also significantly associated with clear cell RCC in our experiments. Actually, vimentin has been proven to be a marker that can distinguish chromophobe RCC from clear cell RCC and renal oncocytoma [19, 20]. However, due to an insufficient number of samples of the other two types of RCC in our trial, we were unable to determine definitively the expression pattern of MTHFD2 in different pathological types of RCC. Further studies in larger cohorts of papillary and chromophobe RCC would be of great important.

Nilsson et al. found that MTHFD2 overexpression was positively associated with increased mortality in patients with breast cancer [5]. Similarly, we also observed that the OS of RCC patients with positive MTHFD2 expression was significantly shorter than that of patients with negative MTHFD2 expression. These findings may suggest a potential role for MTHFD2 in the progression of RCC. According to our research, RCC patients with high vimentin expression had a worse prognosis than those with low vimentin expression. This was consistent with the findings of other studies that also validated the role of vimentin in predicting the poor outcome of patients with RCC [18, 21].

Independent of its dehydrogenase activity, MTHFD2 can drive cancer cell proliferation [7]. However, Lehtinen et al. found that inhibition of MTHFD2 did not significantly decrease cell proliferation or induce cell apoptosis in some breast cancer cells [8]. In the present study, we observed that MTHFD2 depletion significantly decreased the proliferation of RCC cells, which was in accordance with a study by Selcuklu et al. who identified MTHFD2 as a microRNA-9 target gene that affects the proliferation of breast cancer cells [22]. These findings may indicate a heterogenic property of MTHFD2 in cell biology. Moreover, we observed a reduction of the migration and invasion of 786-O cells following MTHFD2 knockdown, which was accompanied with the decreased expression of vimentin. These results were in agreement with those of Lehtinen et al. in breast cancer cells [8]. Since vimentin is a regulator of cell migration and invasion in RCC cells [23], we hypothesized that MTHFD2 may affect cell migration and invasion through the reduction of vimentin expression.

We then performed a rescue assay by TNF-α stimulation. TNF-α increases cell migration and invasion through epithelial-mesenchymal transition via the NF-κB pathway [24, 25]. We demonstrated that TNF-α could restore vimentin expression, which subsequently blocked the inhibitory influence of MTHFD2 knockdown on cell migration and invasion, in accordance with the findings of Ho et al. [14]. However, Ho et al. confirmed that TNF-α could enhance the tumorigenicity of RCC cells. In the present study, the low cell viability induced by shMTHFD2 was not significantly counteracted by TNF-α treatment. Taken together, our results indicated

that vimentin is essential for MTHFD2-mediated cell migration and invasion in 786-O cells. However, the underlying mechanisms by which MTHFD2-modulated cell proliferation and vimentin expression are regulated in RCC cells have yet to be determined.

RCC is characterized by resistance to traditional chemotherapy [26]. Although molecular-targeted therapy can offer significant clinical benefits for metastatic RCC, its effectiveness eventually declines with the development of resistance [27]. Recently, it was found that MTHFD2 suppression may increase the sensitivity of breast cancer cells to folate depletion [28]. Our study is consistent with this report, as the loss of MTHFD2 increased the sensitivity of 786-O cells to chemotherapy drugs.

Conclusion

In summary, our study indicated that MTHFD2 is an important regulator of the progression of RCC and may be a marker for poor prognosis in the clinical setting. Down-regulation of MTHFD2 suppressed cell proliferation and vimentin expression, which subsequently reduced cell migration and invasion. Therefore, targeting MTHFD2 may be a novel strategy for the treatment of RCC.

Acknowledgements

This work was supported by the Science and Technology Project of Guangdong Province (2018A030307047 and 2017A030313847) and China National Natural Science Foundation (81402116), and partially supported by the Medical Scientific Research Foundation of Guangdong Province (A2018053 and A2015210) and Shantou Science and Technology Project-Medical Treatment and Public Health Category (180709154010294).

Disclosure Statement

The authors declare that they have no conflicts of interest.

References

1 Siegel RL, Miller KD, Jemal A: Cancer statistics, 2018. CA Cancer J Clin 2018;68:7-30.
2 Siegel R, Naishadham D, Jemal A: Cancer statistics, 2013. CA Cancer J Clin 2013;63:11-30.
3 Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, Kos I, Batinic-Haberle I, Jones S, Riggins GJ, Friedman H, Friedman A, Reardon D, Herndon J, Kinzler KW, Velculescu VE, Vogelstein B, Bigner DD: IDH1 and IDH2 mutations in gliomas. N Engl J Med 2009;360:765-773.
4 Zhang WC, Shyh-Chang N, Yang H, Rai A, Umashankar S, Ma S, Soh BS, Sun LL, Tai BC, Nga ME, Bhakoo KK, Jayapal SR, Nichane M, Yu Q, Ahmed DA, Tan C, Sing WP, Tam J, Thirugananam A, Noghabi MS, Pang YH, Ang HS, Mitchell W, Robson P, Kaldis P, Soo RA, Swarup S, Lim EH, Lim B: Glycine decarboxylase activity drives non-small cell lung cancer tumor-initiating cells and tumorigenesis. Cell 2012;148:259-272.
5 Nilsson R, Jain M, Madhusudhan N, Sheppard NG, Strittmatter L, Kampf C, Huang J, Asplund A, Mootha VK: Metabolic enzyme expression highlights a key role for MTHFD2 and the mitochondrial folate pathway in cancer. Nat Commun 2014;5:3128.
6 Tedeschi PM, Vazquez A, Kerrigan JE, Bertino JR: Mitochondrial Methylenetetrahydrofolate Dehydrogenase (MTHFD2) Overexpression Is Associated with Tumor Cell Proliferation and Is a Novel Target for Drug Development. Mol Cancer Res 2015;13:1361-1366.
7 Gustafsson Sheppard N, Jarl L, Mahadessian D, Strittmatter L, Schmidt A, Madhusudan N, Tegner J, Lundberg EK, Asplund A, Jain M, Nilsson R: The folate-coupled enzyme MTHFD2 is a nuclear protein and promotes cell proliferation. Sci Rep 2015;5:15029.

8 Lehtinen L, Ketola K, Makela R, Mpindi JP, Viitala M, Kallioniemi O, Iljin K: High-throughput RNAi screening for novel modulators of vimentin expression identifies MTHFD2 as a regulator of breast cancer cell migration and invasion. Oncotarget 2013;4:48-63.
9 Tedeschi PM, Scotto KW, Kerrigan J, Bertino JR: MTHFD2--a new twist? Oncotarget 2016;7:7368-7369.
10 Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, Savagner P, Gitelman I, Richardson A, Weinberg RA: Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell 2004;117:927-939.
11 Edge SB BD, Compton CC: AJCC cancer staging manual. 7th edition. New York: Springer Verlag 2009;2009:547–560.
12 Eble JN SG, Epstein JI: Pathology and genetics of tumours of the urinary system and male genital organs. IARC 2004:12–43.
13 Störkel S EJ, Adlakha K, Amin M, Blute ML, Bostwick DG, Darson M, Delahunt B, Iczkowski K, Adlakha K: Classification of renal cell carcinoma: workgroup No.1.Union Internationale Contre Cancer (UICC) and the American Joint Committee on Cancer (AJCC). Cancer 1997:987-989.
14 Ho MY, Tang SJ, Chuang MJ, Cha TL, Li JY, Sun GH, Sun KH: TNF-alpha induces epithelial-mesenchymal transition of renal cell carcinoma cells via a GSK3beta-dependent mechanism. Mol Cancer Res 2012;10:1109-1119.
15 Scrimgeour KG, and Huennekens, F. M.: Occurrence of a DPN-linked, N5,N10-methylene tetrahydrofolic dehydrogenase in Ehrlich ascites tumor cells. Biochem. Biophys. Res. Commun. 1960;2:230–233.
16 Christensen KE, Mackenzie RE: Mitochondrial methylenetetrahydrofolate dehydrogenase, methenyltetrahydrofolate cyclohydrolase, and formyltetrahydrofolate synthetases. Vitam Horm 2008;79:393-410.
17 Thiery JP: Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2002;2:442-454.
18 Ingels A, Hew M, Algaba F, de Boer OJ, van Moorselaar RJ, Horenblas S, Zondervan P, de la Rosette JJ, Pilar Laguna Pes M: Vimentin over-expression and carbonic anhydrase IX under-expression are independent predictors of recurrence, specific and overall survival in non-metastatic clear-cell renal carcinoma: a validation study. World J Urol 2017;35:81-87.
19 Zhao W, Tian B, Wu C, Peng Y, Wang H, Gu WL, Gao FH: DOG1, cyclin D1, CK7, CD117 and vimentin are useful immunohistochemical markers in distinguishing chromophobe renal cell carcinoma from clear cell renal cell carcinoma and renal oncocytoma. Pathol Res Pract 2015;211:303-307.
20 Williams AA, Higgins JP, Zhao H, Ljunberg B, Brooks JD: CD 9 and vimentin distinguish clear cell from chromophobe renal cell carcinoma. BMC Clin Pathol 2009;9:9.
21 Sabo E, Miselevich I, Bejar J, Segenreich M, Wald M, Moskovitz B, Nativ O: The role of vimentin expression in predicting the long-term outcome of patients with localized renal cell carcinoma. Br J Urol 1997;80:864-868.
22 Selcuklu SD, Donoghue MT, Rehmet K, de Souza Gomes M, Fort A, Kovvuru P, Muniyappa MK, Kerin MJ, Enright AJ, Spillane C: MicroRNA-9 inhibition of cell proliferation and identification of novel miR-9 targets by transcriptome profiling in breast cancer cells. J Biol Chem 2012;287:29516-29528.
23 Yamasaki T, Seki N, Yamada Y, Yoshino H, Hidaka H, Chiyomaru T, Nohata N, Kinoshita T, Nakagawa M, Enokida H: Tumor suppressive microRNA138 contributes to cell migration and invasion through its targeting of vimentin in renal cell carcinoma. Int J Oncol 2012;41:805-817.
24 Wu Y, Deng J, Rychahou PG, Qiu S, Evers BM, Zhou BP: Stabilization of snail by NF-kappaB is required for inflammation-induced cell migration and invasion. Cancer Cell 2009;15:416-428.
25 Liu S, Shi L, Wang Y, Ye D, Ju H, Ma H, Yang W, Wang Y, Hu J, Deng J, Zhang Z: Stabilization of Slug by NF-kappaB is Essential for TNF-alpha -Induced Migration and Epithelial-Mesenchymal Transition in Head and Neck Squamous Cell Carcinoma Cells. Cell Physiol Biochem 2018;47:567-578.
26 Koul H, Huh JS, Rove KO, Crompton L, Koul S, Meacham RB, Kim FJ: Molecular aspects of renal cell carcinoma: a review. Am J Cancer Res 2011;1:240-254.
27 Porta C, Paglino C, Grunwald V: Sunitinib re-challenge in advanced renal-cell carcinoma. Br J Cancer 2014;111:1047-1053.
28 Koufaris C, Gallage S, Yang T, Lau CH, Valbuena GN, Keun HC: Suppression of MTHFD2 in MCF-7 Breast Cancer Cells Increases Glycolysis, Dependency on Exogenous Glycine, and Sensitivity to Folate Depletion. J Proteome Res 2016;15:2618-2625.