MTHFD2 Regulates the AKT/MYC Signaling Pathway in Bladder Cancer and Promotes Proliferation, Viability and Migration in Vitro

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Research

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Abstract

Background: Numerous studies have reported that MTHFD2 is overexpressed in several human cancers and functions as a valuable prognostic factor. However, little is known about its role in bladder cancer.

Methods: We carried out an in silicon analysis of MTHFD2 expression status in bladder cancer tissues and the impact of MTHFD2 on the overall survival of patients. Flag-MTHFD2 plasmid and MTHFD2-knockdown vector were constructed to investigate the function of MTHFD2. The role of MTHFD2 in MTHFD2-overexpressing or MTHFD2-deficient EJ cells was examined using CCK8, colony formation assays, soft agar assays and Transwell migration assays. A luciferase reporter assay was employed to test the impact of MTHFD2 expression on the transcriptional activity of AKT and MYC. The expression of CDK4 and CCND4 in MTHFD2-deficient EJ cells was detected by Western blot. To certify the AKT role in MTHFD2-modulated EJ cell behaviors, a rescue assay were carried out by re-overexpression MYC in MTHFD2-defienct EJ cells.

Results: By analyzing the GEPIA database, we found that the expression of MTHFD2 is increased in bladder cancer tissues. Patients with high MTHFD2 displayed poorer survival than patients with low MTHFD2. A series of in vitro functional assays revealed that ectopic expression of MTHFD2 enhanced cell proliferative and migratory activity while MTHFD2 deficiency had the opposite impact on the tumorigenic potential of EJ cells. Mechanistically, we found that overexpressing of MTHFD2 increased AKT and MYC transcriptional activity. Two critical downstream effectors,CDK4 and CCND2 was attenuated in MTHFD2-deficienct cells. Overexpression of MYC rescured the inhibitory effects of MTHFD2 deficiency in the CCND2 and CDK2 expression.

Conclusion: Overall, we first uncovered that MTHFD2 could play a protumor role in bladder cancer by activating the AKT/MYC signaling pathway, which may highlight its prognostic potential in bladder cancer and support the rationale for MTHFD2-targeted drug intervention.

Introduction

Bladder cancer is the most frequent neoplasm in the urinary system, accounting for 4.5% of newly diagnosed cases and 3.0% of cancer-associated deaths[1]. Despite the improved treatment of bladder cancer, its clinical outcome remains discouraging, especially for advanced bladder cancer [2, 3]. Currently, it is recognized that many risk factors, including genetic variation, aggravate the burden of this malignant disease[4]. Elucidating the underlying mechanism of these genes will be conducive to disentangling the complex etiologic scenario of bladder cancer and guiding therapeutic decisions based on molecular pathway alterations.

Metabolic reprogramming has been implicated in tumor occurrence, progression and metastasis through alternation of the folate metabolism pathway. MTHFD2 is a critical C1-folate-utilizing metabolic enzymes related to rapidly proliferating malignant tumors [5]. This mitochondrial enzyme can supply format for purine or thymidine biosynthesis to support cell proliferation [6]. It can also regenerate NADH/NADPH to

eliminate ROS and maintain redox balance, thus facilitating macromolecular synthesis[7]. Additionally, MTHFD2 was identified to be a universal methyl donors [8]. Due to its involvement in these critical steps, MTHFD2 has attracted substantial clinical and research interest. Overexpression of MTHFD2 has been found in human solid cancers, including breast [9], renal[10], and pancreatic cancers[11], and is correlated with unfavorable clinical outcomes[6]. The oncogenic potential of MTHFD2 in vitro or in vivo has been validated in various cancers [12, 13].

Regarding the underlying mechanism of MTHFD2 in cell proliferation and tumorigenesis, most studies have concentrated on metabolic phonotype alteration of its enzymatic function. However, its enzymatic function has little impact on cell proliferative capacity [14]. A nonmetabolic role of MTHFD2 was also reported to be involved in the progression of cancer. For instance, overexpression of MTHFD2 enhanced the oncogenic activity of vimentin and lncRNA taurine-upregulated gene 1[10, 15, 16]. In non-small-cell lung cancer, MTHFD2 knockdown attenuated tumor cell behaviors via regulating cell cycle-associated genes[17]. These findings suggest that MTHFD2 has broader roles in neoplastic transformation and tumorigenesis.

One paper demonstrated that the MTHFD2 SNP can increase bladder cancer risk [18]. However, no studies have elucidated the biological function and pathological mechanism of MTHFD2 in bladder cancer. In our study, we revealed an oncogenic role for MTHFD2 in bladder cancer. We also found that MTHFD2 enhanced viability and migration via the AKT/MYC signaling pathway, which supports the rationale of MTHFD2-targeted therapeutic intervention.

Methods

Bioinformation data mining and analysis

The MTHFD2 mRNA status in Bladder Urothelial Carcinoma (BLCA) was analyzed via the Gene Expression Profiling Interactive Analysis (GEPIA) online database (http://gepia.cancer-pku.cn/) according to the creator of this website. |Log2FC| Cutoff and p-value Cutoff were set as 1 and 0.01, respectively. For the prognostic analysis of MTHFD2 in bladder cancer, the patients with BLCA was median dichotomized into high MTHFD2 group and low MTHFD2 group (cutoff=50%).

Cell culture, plasmids and transfection

Bladder cancer cell line EJ and the HEK239T cell line were purchased from the Cell Bank Wuhan University (China), and maintained in DMEM medium supplemented with 5% FBS and penicillin-streptomycin 100 U/mL in a standard cell culture incubator (37°C, 5% CO_2). The AKT and MYC luciferase reporter gene vectors and Flag-MYC were gifted by school of life sciences, Wuhan University, Wuhan, China. Cell transfection was achieved with the aid of Lipofectamine 2000.

Overexpression of MTHFD2 in EJ cells

For construction of MTHFD2-flag plasmids, RT-PCRs were carried out to clone and amplify the human MTHFD2 and MYC cDNA from EJ cells. The PCR-amplified products were cleaved with BamHI and SalI and recovered by DNA gel recovery kit. The recovered SalI-BamH I sequences were inserted into pHAGE puro with 6tag, named Flag-MTHFD2 vectors and were subsequently introduced into $1x10^5$ HEK293T cells for 2 days. The obtained retrovirus particles was filtered and infected EJ cells, following additional two-day selection with puromycin at 1 μg/ml. The positive cells was further validated by western blot analysis. The normal EJ cells served as the control.

Targeted depletion of MTHFD2 in EJ cells

MTHFD2 targeted depletion were produced in EJ cells via CRISPR/Cas9 system. The guide RNAs (gRNAs) targeted exon 1 of MTHFD2 were designed via the CRISPR online software (http://crispr.dfci.harvard.edu/SSC/) and inserted into pSpCas9 (BB)-2A-Puro plasmid. The recombinant vectors were introduced into EJ cells for 48h and the cells was subjected to 1 μg/ml Puromycin screening for 2 days. The MTHFD2 deficient EJ cells (KO-1, KO-2) were utilized for further cell functional assays after validation by western blot. The normal EJ cells served as the control.

CCK8 assays

Cell proliferation rate was examined with CCK8 approach.5.0×10^3 cells /well EJ cells were plated in 96-well plates. After 1, 3, and 5 days, 10 μl CCK8 solution was supplemented and cells were subjected to another 2h incubation. The OD value was monitor at 450 nm with a microplate reader.

Colony formation assay

After trypsinization, the logarithmic growth phase EJ cells were made into single cell suspensions by mechanical dissociation. A total of 2×10^2 EJ cells were seeded on dishes and incubated for 3 weeks. After fixation with 4% paraformaldehyde, colonies of EJ cells were visualized using 0.5% crystal violet and the crystal violet-stained colonies were counted under a microscope.

Soft agar colony formation assay

A 1×10^3 EJ cell-agarose suspension was pipetted onto a solidified bottom layer made of 0.5% agar in a 6-well plate. After 2-week growth of EJ cells, visible cell colonies were counted, and images were captured at ×20 magnification.

Transwell cell migration assay

Migration media containing 5×10^5 EJ cells/100 μl were added to each Transwell insert onto the receiving 12-well plate with medium containing 20% fetal bovine. After incubation for 2.5 h, the cell debris on the upper side of the insert was removed. The cells that migrated across the membrane were fixed with methanol for 10min, visualized with 1% crystal violet and counted using an inverted microscope.

Luciferase reporter gene assay

Considering AKT activation in cell metabolism[19], we examined the change in transcriptional activity of AKT upon MTHFD2 stimulation using dual-luciferase reporter gene assays. 10 ng AKT Luc vectors and 5 ng prl-tk empty control plasmid were cotransfected into HEK293T cells with 0,50,100, and 200ng MTHFD2-flag plasmids. After cotransfection for 2 days, the transcriptional activity was measured and standardized by Renilla control luciferase activity.

Besides AKT transcriptional activity in MTHFD2 EJ cells, further luciferase reporter assays were performed to examine the transcriptional activity of MYC which is one of active downstream molecules of AKT signaling pathway in multiple types of cancers[20, 21]. In brief, 10 ng AKT Luc vectors or 10ng MYC Luc vectors were separately introduced into 1.5 × 105 cells/well wide-type (WT) or both MTHFD2-deficient EJ cells with 5 ng prl-tk empty control plasmid. AKT and MYC luciferase activities were assessed again using the Renilla Luciferase assay kit.

Quantitative RT-PCR

Total RNA was extracted with Trizol agent and subjected for DNase I treatment. Subsequently, Denatured RNA was reverse transcribed into CDNA with random primer and an oligo(dT) primer. mRNA expression level was determined by qPCR with 2x SYBR Green qPCR SuperMix. The primers as follows:

CDK4:Forward primer GGATGACTGGCCTCGAGATG, Reverse primer: CAAGAAAGTTGGGCACTCCG;
CCND2: Forward primer CCAACACAGACGTGGATTGT, Reverse primer:CAACTGGCATCCTCACAGGT.
GAPDH: Forward primer CATGGCACCGTCAAGGCTGA, Reverse primer: ACGTACTCAGCGCCAGCATC.

Western blot analysis

Lysis of the indicated cells was performed by using RIPA buffer with cocktail inhibitor, and the supernatants containing proteins were collected after centrifuging lysates. Next, the protein concentration was determined by Bradford Assay, and equal amounts of proteins (20μg) were separated by 10% SDS-PAGE. The separated proteins were transferred to PVDF membranes. The membranes were blocked to eliminate nonspecific binding, and the primary and secondary antibodies were successively applied to detect the signals on the membranes. The following primary antibodies were used: anti-flag,anti-MTHFD2,anti-CCND2,anti-CDK4,anti-GAPDA

Rescue of AKT activity by Overexpression of MYC

To verify if AKT/MYC is essential for MTHFD2-induced bladder cancer genesis and development, we transfected the 1.5×10^5 MTHFD2-deficient EJ cells with Flag-MYC vectors. The CCND2 and CDK4 expression was visualized by westernblot in widetype (WT), KO EJ cells and MYC-reexpressing KO EJ cells.

Statistical Analysis

All data were analyzed by Prism 8 statistical software. A difference was considered significant if p was less than 0.05. All data from experiments are presented as the mean ± SEM. Comparisons of two variables were examined with unpaired two-tailed Student's t test. Comparisons of multiple variables were examined by ANOVA with Tukey's post hoc test.

Results

MTHFD2 upregulation exists in bladder cancer tissues and predicts an unfavorable clinical outcome.

First, we examined MTHFD2 expression in 404 bladder cancer tissues and 28 normal paratumor tissues from the TCGA database, and the elevation of MTHFD2 expression was observed in bladder cancer tissues (Figure 1A). Moreover, survival analysis between MTHFD2-high and MTHFD2-low bladder cancer patients demonstrated that MTHFD2 functioned as a poor prognostic indicator in bladder cancer (Figure 1B).

Upregulation of MTHFD2 influences the cell viability and migration of EJ cells.

Given the abovementioned role of MTHFD2, we overexpressed this protein in EJ cells, as verified by western blotting (Figure 2A). Subsequently, we quantified the effect of MTHFD2 on the viability and tumorigenicity of EJ cells using CCK8, colony formation, soft agar and Transwell assays. As seen in Figure 2B-2H, overexpression of MTHFD2 obviously enhanced viability, proliferation and migration, indicating that MTHFD2 plays a critical role in the malignancy of bladder cancer.

Depletion of MTHFD2 lessens the tumor formation capacity of EJ cells.

To further explore the pathological role of MTHFD2 in bladder cancer, we next constructed MTHFD2-deficient cell lines via the CRISPR/Cas9-mediated knockout system in EJ cells (Figure 3A). Next, we performed a panel of quantitative assays to evaluate the effect of MTHFD2 deficiency on malignant cell behaviors. Figure 3B, 3C and 3D showed that MTHFD2-deficient EJ cells showed lower colony formation and cell proliferation rates than those in the normal cells. The obviously deceased tendency in the invasive rate was also displayed in MTHFD2-deficient cells when compared with the untranfected cells (Figure 3), indicating the oncogenic role of MTHFD2 in bladder cancer.

MTHFD2 triggersAKT signaling cascades

On account of the involvement of AKT in cellular metabolism reprogramming [19], we cotransfected MTHFD2 overexpression plasmids and AKT-Luc reporter plasmids into HEK239T cells. As shown in Figure 4A, MTHFD2 dose-dependently drove the transcriptional activation of AKT. The increased transcriptional activities of AKT and MYC were also observed in wild-type (WT) EJ cells compared with that in two MTHFD2-deficients EJ cells (Figure 4B). Subsequently, we tested the two critical downstream effectors of AKT: CCND2 and CDK4 in two KO cell clones and the wild-type EJ cells. Unsurprisingly, targeted depletion of MTHFD2 resulted in the reduced expression of CCND2 and CDK4 (Figure 4C and

4D).These findings indicate that MTHFD2 modulated malignant cell behaviors via the AKT signaling pathway.

MYC overexpression rescues the AKT activity in MTHFD2-deficient EJ cells

Subsequently, we performed an AKT function rescue assay to validate the MYC critical role of MTHFD2/AKT-induced bladder cancer progression by re-exrepssing MYC in MTHFD2-deficient EJ cells. The result manifested that re-expression of MYC in KO EJ cells led the promotion of CCND2 and CDK2 expression compared with WT and KO EJ cells (Figure 4E).Collectively, MTHFD2 enhanced the AKT-induced EJ cell proliferation and migration by increasing MYC expression

Discussion

In our study, we identified that MTHFD2 was accumulated in bladder cancer tissues and that high MTHFD2 expression was an independent prognostic factor for bladder cancer patients. More importantly, we found that MTHFD2 suppressed cell viability, proliferation and migration in EJ cells by positively modulating of the AKT/MYC signaling pathway, which provided a novel theoretical basis for MTHFD2-targeted therapy.

MTHFD2 overexpression has been examined in various solid cancers and likely represents a reliable prognostic indicator for cancers [6]. Deregulation of MTHFD2 significantly affects the tumor formation capacity of tumor cancer cells, including breast cancer[6], non-small-cell lung cancer[17], etc. Thus, MTHFD2 could serve as a valid target for cancer treatment. Based on these results, we investigated the impact of MTHFD2 on bladder cancer. Clinically, we compared MTHFD2 mRNA levels in 404 bladder cancer tissues and 28 tumor-adjacent samples, and the results revealed that MTHFD2 was considerably increased in bladder cancer specimens and associated with unfavorable clinical outcomes. *In vitro*, MTHFD2 influenced the viability, proliferation and migration of EJ cells. These findings suggest that MTHFD2 plays a putative role in the malignancy of bladder cancer.

MTHFD2 is reported as an established cancer-related metabolic enzyme that can help cancer cells overcome growth restrictions and sustain chronic proliferation. A recent metabolic profile of ShMTHFD2 breast cancer cells demonstrated that MTHFD2 depletion contributed to increased glycolysis and glycine auxotrophy as well as increased exogenous folate dependency, highlighting the enzymatic function of MTHFD2 in metabolic alteration[22]. However, the abovementioned cell phenotype changes have no evident mechanistic connection with MTHFD2-mediated one-carbon metabolism since rapid tumor cell growth showed no absolute reliance on the reported enzymatic functions of MTHFD2[14]. In other words, MTHFD2 may be involved in regulating some signaling to direct cell-autonomous nutrient uptake and metabolism beyond its enzymatic role in metabolism. This finding was supported by a documented viewpoint that signaling mechanisms played a critical role in physiologic cell proliferation and tumorigenesis by regulating metabolic activity in proliferating cells[19]. Additionally, other mitochondrial enzymes, such as PYCR1, were verified to influence tumor formation by deregulating signaling pathways [23]. Subsequently, we carried out a dual-luciferase reporter gene assay to determine whether MTHFD2

expression impacts the transcriptional activity of AKT, which is reported as the key signaling effector in cell metabolism[19]. The results showed that overexpression of MTHFD2 significantly activated the transcriptional activity of AKT. AKT is a critical regulator of AKT signaling cascade, and its activation could trigger the accumulation of AKT which increases some transcriptional cell cycle regulators, such as CDK4 and CCND2. Consistently, we also observed a considerably decreased mRNA level of CCND2 and CKD4 in MTHFD2-defient EJ cells Therefore, MTHFD2 might cause AKT activation to increase tumor cell malignant phenotypes and, thereby led to tumorigenesis in bladder cancer. In addition, our findings offer a good explanation for why mutant MTHFD2, which has depleted enzymatic activity, can still increase tumor cell proliferation and growth. Our findings are also supported by the nuclear localization of MTHFD2 [14].

AKT functions as a critical component of the signal transduction of many cell-extrinsic signals and affects various physiological and pathological processes. Notably, aberrant activation of AKT actively regulates cellular metabolism in cancer cells to support macromolecular synthesis and cell survival in the absence of growth factors[19]. First, activation of AKT can stimulate intracellular trafficking of facilitative nutrient transporters to ensure various nutrient availability and guarantee cell survival and growth [24]. Second, AKT can regulate glucose homeostasis and strengthen aerobic glycolysis [25]. This switch is one of the typical characteristics of metabolic reprogramming and is beneficial for both bioenergetics and biosynthesis in cancer cells. Third, the AKT functional repertoire can be expanded by downstream effectors. For example, AKT can promote protein biosynthesis by activating mTORC1[26],which is reported to participate in regulation of the cyclin-dependent kinase activity and D-type cyclins stability[27]. As another signaling pathway of regulate metabolism, the oncogenic Myc pathway is reported to activate and reinforce metabolic pathways in transformed cells[28]. On the other hand, MYC is a well-established downstream effector molecule of the AKT through which AKT governs multifaceted procedure of various malignancies[29, 30]. To further detail the MTHFD2-medidated BC malignancy, luciferase and rescue assays validated that the regulatory association between AKT and MTHFD2 through activation of MYC. Thereofore,MTHFD2 partly regulates metabolic alternation by activating the AKT/MYC signaling pathway, which can maintain sufficient cell-autonomous nutrient uptake and enable tumor cells to overcome normal physiologic constraints for cell doubling. However, aberrance of Akt and Myc pathways are examined in various types of cancer [29, 30, 31], which may support the universality of metabolic remodeling in tumor cells. Therefore, MTHFD2 may have a more complex regulatory network in tumor cell proliferation and tumorigenesis. Next, work is required to clarify the complex regulatory role of MTHFD2 in bladder cancer.

In short, we found that MTHFD2 is overexpressed in bladder cancer and confirmed its tumor-promoting role in partly activating the AKT/MYC signaling pathway. Our findings broaden the knowledge about MTHFD2-mediated metabolic reprogramming in cancer progression and enrich the theoretical basis for the use of MTHFD2-targeted therapy in bladder cancer.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this mauscript

Competing interests

All authors declared there were no conflict of interests involved.

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Authors' contributions

LH and HYS made substantial contributions to the conception and design of the work; CDH and LHH are responsible for the acquisition, analysis,and drafted the work or revised it;

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020 Jan 8. doi: 10.3322/caac.21590. PubMed PMID: 31912902.
2. Chang SS, Bochner BH, Chou R, et al. Treatment of Non-Metastatic Muscle-Invasive Bladder Cancer: AUA/ASCO/ASTRO/SUO Guideline. J Urol. 2017 Sep;198(3):552-559. doi: 10.1016/j.juro.2017.04.086. PubMed PMID: 28456635; PubMed Central PMCID: PMC5626446.
3. Mattiuzzi C, Lippi G. Current Cancer Epidemiology. J Epidemiol Glob Health. 2019 Dec;9(4):217-222. doi: 10.2991/jegh.k.191008.001. PubMed PMID: 31854162.
4. Malats N, Real FX. Epidemiology of bladder cancer. Hematol Oncol Clin North Am. 2015 Apr;29(2):177-89, vii. doi: 10.1016/j.hoc.2014.10.001. PubMed PMID: 25836927.
5. Jain M, Nilsson R, Sharma S, et al. Metabolite profiling identifies a key role for glycine in rapid cancer cell proliferation. Science. 2012 May 25;336(6084):1040-4. doi: 10.1126/science.1218595. PubMed PMID: 22628656; PubMed Central PMCID: PMC3526189.
6. Nilsson R, Jain M, Madhusudhan N, et al. Metabolic enzyme expression highlights a key role for MTHFD2 and the mitochondrial folate pathway in cancer. Nat Commun. 2014;5:3128. doi: 10.1038/ncomms4128. PubMed PMID: 24451681; PubMed Central PMCID: PMC4106362.

7. Shin M, Momb J, Appling DR. Human mitochondrial MTHFD2 is a dual redox cofactor-specific methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase. Cancer Metab. 2017;5:11. doi: 10.1186/s40170-017-0173-0. PubMed PMID: 29225823; PubMed Central PMCID: PMC5718140.
8. Green NH, Galvan DL, Badal SS, et al. MTHFD2 links RNA methylation to metabolic reprogramming in renal cell carcinoma. Oncogene. 2019 Aug;38(34):6211-6225. doi: 10.1038/s41388-019-0869-4. PubMed PMID: 31289360.
9. Liu F, Liu Y, He C, et al. Increased MTHFD2 expression is associated with poor prognosis in breast cancer. Tumour Biol. 2014 Sep;35(9):8685-90. doi: 10.1007/s13277-014-2111-x. PubMed PMID: 24870594.
10. Lin H, Huang B, Wang H, 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. doi: 10.1159/000495402. PubMed PMID: 30466107.
11. Noguchi K, Konno M, Koseki J, et al. The mitochondrial one-carbon metabolic pathway is associated with patient survival in pancreatic cancer. Oncol Lett. 2018 Aug;16(2):1827-1834. doi: 10.3892/ol.2018.8795. PubMed PMID: 30008872; PubMed Central PMCID: PMC6036444.
12. Wei Y, Liu P, Li Q, et al. The effect of MTHFD2 on the proliferation and migration of colorectal cancer cell lines. Onco Targets Ther. 2019;12:6361-6370. doi: 10.2147/OTT.S210800. PubMed PMID: 31496738; PubMed Central PMCID: PMC6697661.
13. Ju HQ, Lu YX, Chen DL, et al. Modulation of Redox Homeostasis by Inhibition of MTHFD2 in Colorectal Cancer: Mechanisms and Therapeutic Implications. J Natl Cancer Inst. 2018 Dec 8. doi: 10.1093/jnci/djy160. PubMed PMID: 30534944; PubMed Central PMCID: PMC6579745.
14. Gustafsson Sheppard N, Jarl L, Mahadessian D, et al. The folate-coupled enzyme MTHFD2 is a nuclear protein and promotes cell proliferation. Sci Rep. 2015 Oct 13;5:15029. doi: 10.1038/srep15029. PubMed PMID: 26461067; PubMed Central PMCID: PMC4602236.
15. Lehtinen L, Ketola K, Makela R, et al. High-throughput RNAi screening for novel modulators of vimentin expression identifies MTHFD2 as a regulator of breast cancer cell migration and invasion. Oncotarget. 2013 Jan;4(1):48-63. doi: 10.18632/oncotarget.756. PubMed PMID: 23295955; PubMed Central PMCID: PMC3702207.
16. Zhao XB, Ren GS. LncRNA Taurine-Upregulated Gene 1 Promotes Cell Proliferation by Inhibiting MicroRNA-9 in MCF-7 Cells. J Breast Cancer. 2016 Dec;19(4):349-357. doi: 10.4048/jbc.2016.19.4.349. PubMed PMID: 28053623; PubMed Central PMCID: PMC5204041.
17. Yu C, Yang L, Cai M, et al. Down-regulation of MTHFD2 inhibits NSCLC progression by suppressing cycle-related genes. J Cell Mol Med. 2019 Nov 28. doi: 10.1111/jcmm.14844. PubMed PMID: 31778025.
18. Andrew AS, Gui J, Sanderson AC, et al. Bladder cancer SNP panel predicts susceptibility and survival. Hum Genet. 2009 Jun;125(5-6):527-39. doi: 10.1007/s00439-009-0645-6. PubMed PMID: 19252927; PubMed Central PMCID: PMC2763504.

19. DeBerardinis RJ, Lum JJ, Hatzivassiliou G, et al. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. Cell Metab. 2008 Jan;7(1):11-20. doi: 10.1016/j.cmet.2007.10.002. PubMed PMID: 18177721.
20. Li L, Jiang S, Li K, et al. Assessment of tris (1, 3-dichloro-2-propyl) phosphate toxicology in PC12 cells by using digital gene expression profiling. Chemosphere. 2017 Sep;183:353-360. doi: 10.1016/j.chemosphere.2017.05.108. PubMed PMID: 28554019.
21. Yamamoto M, Xin B, Watanabe K, et al. Oncogenic Determination of a Broad Spectrum of Phenotypes of Hepatocyte-Derived Mouse Liver Tumors. Am J Pathol. 2017 Dec;187(12):2711-2725. doi: 10.1016/j.ajpath.2017.07.022. PubMed PMID: 28964793.
22. Koufaris C, Gallage S, Yang T, et al. Suppression of MTHFD2 in MCF-7 Breast Cancer Cells Increases Glycolysis, Dependency on Exogenous Glycine, and Sensitivity to Folate Depletion. J Proteome Res. 2016 Aug 5;15(8):2618-25. doi: 10.1021/acs.jproteome.6b00188. PubMed PMID: 27315223.
23. Zhuang J, Song Y, Ye Y, et al. PYCR1 interference inhibits cell growth and survival via c-Jun N-terminal kinase/insulin receptor substrate 1 (JNK/IRS1) pathway in hepatocellular cancer. J Transl Med. 2019 Oct 16;17(1):343. doi: 10.1186/s12967-019-2091-0. PubMed PMID: 31619254; PubMed Central PMCID: PMC6796468.
24. Wieman HL, Wofford JA, Rathmell JC. Cytokine stimulation promotes glucose uptake via phosphatidylinositol-3 kinase/Akt regulation of Glut1 activity and trafficking. Mol Biol Cell. 2007 Apr;18(4):1437-46. doi: 10.1091/mbc.e06-07-0593. PubMed PMID: 17301289; PubMed Central PMCID: PMC1838986.
25. Elstrom RL, Bauer DE, Buzzai M, et al. Akt stimulates aerobic glycolysis in cancer cells. Cancer Res. 2004 Jun 1;64(11):3892-9. doi: 10.1158/0008-5472.CAN-03-2904. PubMed PMID: 15172999.
26. Ersahin T, Tuncbag N, Cetin-Atalay R. The PI3K/AKT/mTOR interactive pathway. Mol Biosyst. 2015 Jul;11(7):1946-54. doi: 10.1039/c5mb00101c. PubMed PMID: 25924008.
27. Balcazar N, Sathyamurthy A, Elghazi L, et al. mTORC1 activation regulates beta-cell mass and proliferation by modulation of cyclin D2 synthesis and stability. J Biol Chem. 2009 Mar 20;284(12):7832-42. doi: 10.1074/jbc.M807458200. PubMed PMID: 19144649; PubMed Central PMCID: PMC2658077.
28. Tong X, Zhao F, Thompson CB. The molecular determinants of de novo nucleotide biosynthesis in cancer cells. Curr Opin Genet Dev. 2009 Feb;19(1):32-7. doi: 10.1016/j.gde.2009.01.002. PubMed PMID: 19201187; PubMed Central PMCID: PMC2707261.
29. Wei C, Dong X, Lu H, et al. LPCAT1 promotes brain metastasis of lung adenocarcinoma by up-regulating PI3K/AKT/MYC pathway. J Exp Clin Cancer Res. 2019 Feb 21;38(1):95. doi: 10.1186/s13046-019-1092-4. PubMed PMID: 30791942; PubMed Central PMCID: PMC6385475.
30. Zhang F, Li K, Yao X, et al. A miR-567-PIK3AP1-PI3K/AKT-c-Myc feedback loop regulates tumour growth and chemoresistance in gastric cancer. EBioMedicine. 2019 Jun;44:311-321. doi: 10.1016/j.ebiom.2019.05.003. PubMed PMID: 31078520; PubMed Central PMCID: PMC6603849.

31. Liang Z, Liu Z, Cheng C, et al. VPS33B interacts with NESG1 to modulate EGFR/PI3K/AKT/c-Myc/P53/miR-133a-3p signaling and induce 5-fluorouracil sensitivity in nasopharyngeal carcinoma. Cell Death Dis. 2019 Apr 3;10(4):305. doi: 10.1038/s41419-019-1457-9. PubMed PMID: 30944308; PubMed Central PMCID: PMC6447525.

Figures

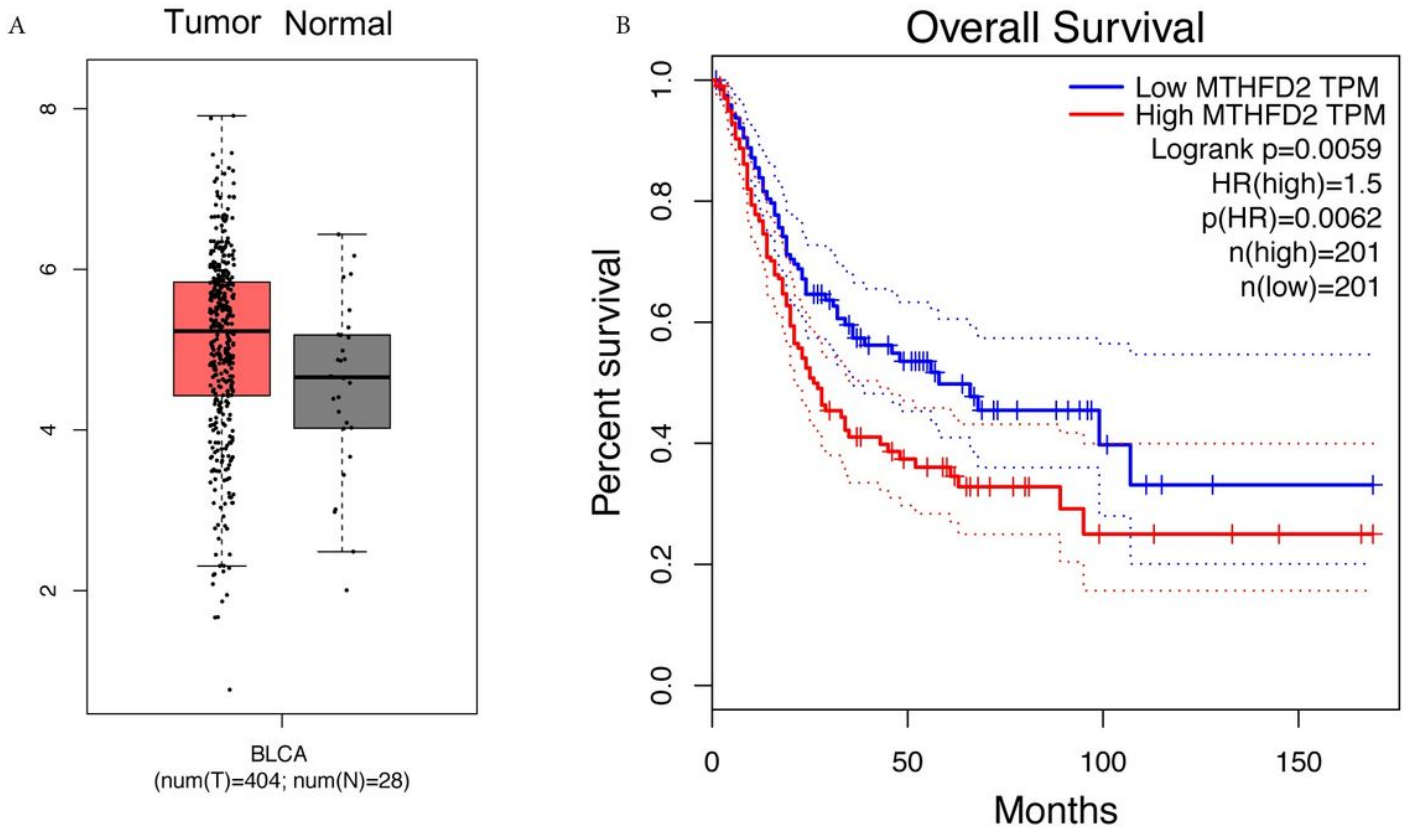

Figure 1

MTHFD2 mRNA was enhanced in bladder cancer tissues and predicted prognosis in bladder cancer. A. MTHFD2 mRNA expression was increased in bladder cancer tissues according to analysis of GEPIA data containing 404 cancer tissues and 28 normal tissues. B. The prognostic value of MTHFD2 expression in GEPIA-derived bladder cancer patients. MTHFD2-high patients displayed an unfavorable clinical outcome.

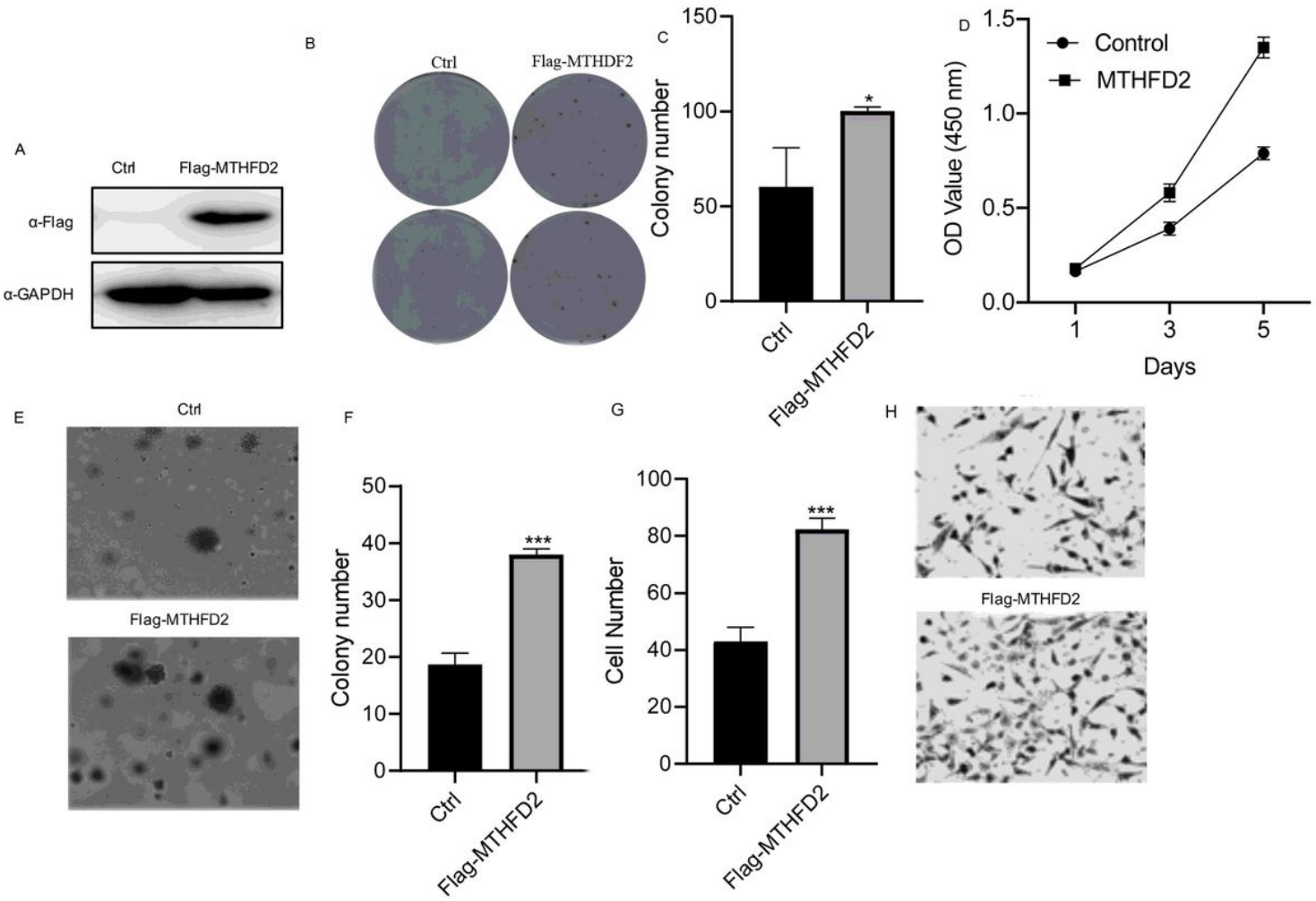

Figure 2

Overexpression of MTHFD2 promotes cell proliferation, viability and migration in EJ cells. A. Overexpression of MTHFD2 was verified by western blot analysis. B and C. The viability of MTHFD2-overexpressng and normal EJ cells was examined by colony formation assay. D. Cell proliferative rate of MTHFD2-overexpressing and normal EJ cells was assessed by CCK8 assays. E and F. A soft agar colony formation assay was performed to examine the impact of MTHFD2 overexpression on the anchorage-independent tumor growth of bladder cancer cells. G and H. Transwell assays were implemented to assess the migration rate of MTHFD2-overexpressing and normal EJ cells. *p < 0.05, ***p < 0.001

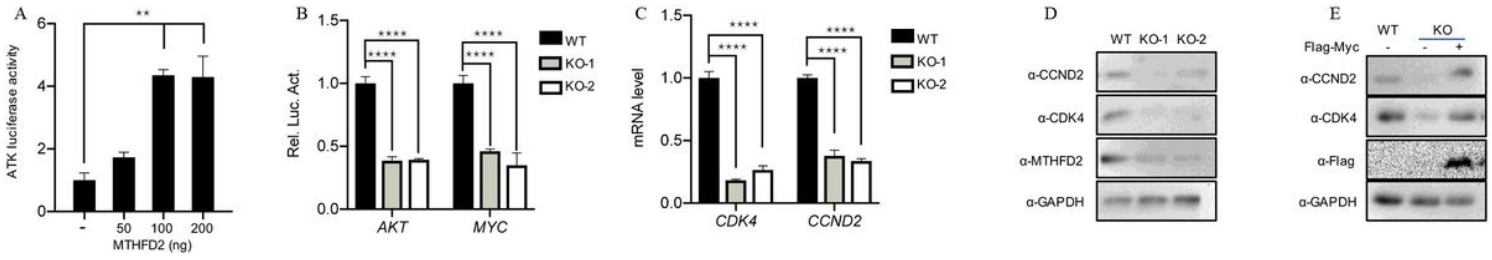

Figure 3

MTHFD2 deficiency suppresses cell proliferation, viability and migration in EJ cells. A. Western blot analysis confirmed MTHFD2 expression in MTHFD2-deficient and normal EJ cells. B. Cell proliferative rate of MTHFD2-deficient and normal EJ cells was assessed by CCK8 assays. C and D. Colony formation assays demonstrated the viability of MTHFD2-deficient and normal EJ cells. E and F. A soft agar assay was executed to detect the anchorage-independent growth of MTHFD2-deficient and normal EJ cells. G and H. Transwell experiments were utilized to test the influence of MTHFD2 depletion on the migration rate of EJ cells. ****p < 0.0001

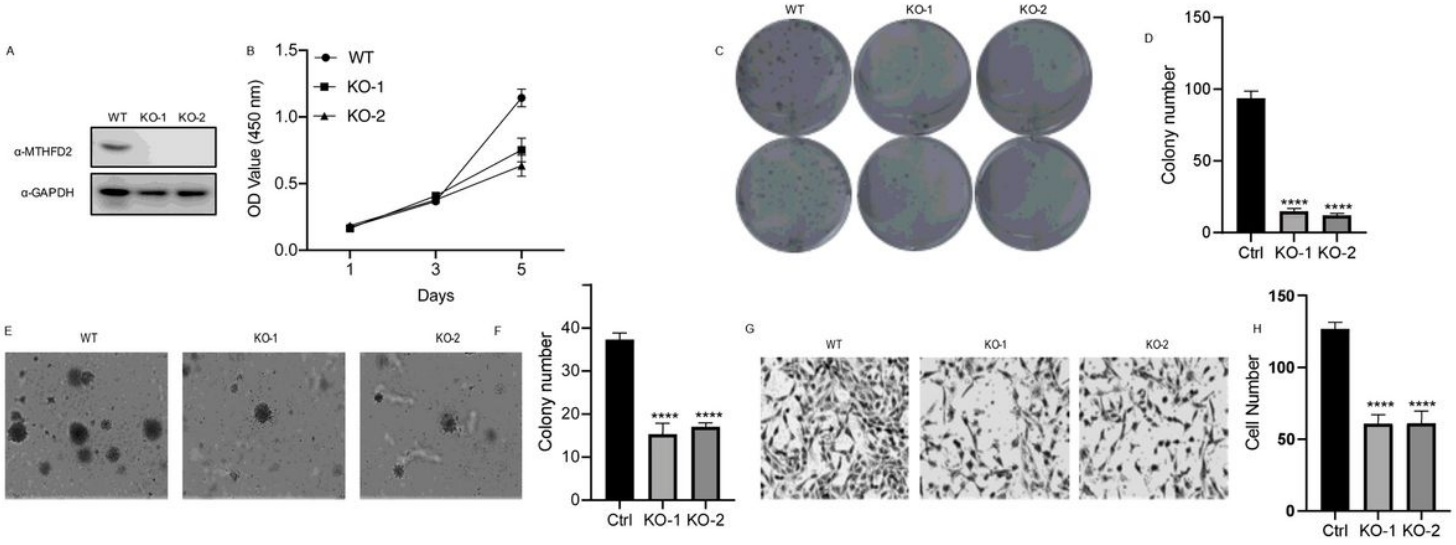

Figure 4

Overexpression of MTHFD2 activates the AKT/MYC signaling pathway. A, Dual-luciferase reporter gene assays demonstrated that AKT transcriptional activity in the increased MTHFD2 ectopic expression vectors in EJ cells. B. Dual-luciferase reporter gene assays manifested AKT and MYC activity in MTHFD2-deficient EJ cells.C and D. MTHFD2 targeted depletion impaired the CDK4 and CCND2 expression level by RT-qPCR and western blot. **p < 0.01,****p < 0.0001. E. reexpressing MYC rescued the expression of CCND2 and CKD4 in MTHFD2-deficient EJ cells