Cai *et al. Journal of Cardiothoracic Surgery* (2025) 20:244
https://doi.org/10.1186/s13019-025-03481-z

Journal of Cardiothoracic Surgery

RESEARCH Open Access

Transcription factor E2F1 promotes non-small cell lung cancer progression by activating the PI3K/AKT pathway through MCM4

Yuyin Cai[1], Xinyan Lu[2], Tingting Li[2], Jia Liu[1] and Lifeng Jiang[2*]

Abstract

Purpose Among malignant tumors, non-small cell lung cancer (NSCLC) remains a major threat to human life and health. Studies have illustrated that minichromosome maintenance protein 4 (MCM4) has complex interactions with the progression of many cancers, yet the role and mechanism of MCM4 in NSCLC remain to be elucidated.

Methods MCM4 expression in NSCLC tissues was assessed using the TCGA database. MCM4 levels in NSCLC cells and tissues was validated utilizing qRT-PCR and western blot. Cell proliferation, metastasis and EMT were measured by CCK-8, transwell, and western blot assays. Subsequently, E2F1 bound with the promoter of MCM4 was predicted via JASPAR database. Luciferase assay and chromatin immunoprecipitation (ChIP) were utilized to evaluate the binding relationship between the two. Finally, rescue experiments were performed to demonstrate the mechanism of MCM4 regulating NSCLC progression. Xenograft model was utilized to prove the role of MCM4 and E2F1 in NSCLC in vivo.

Results MCM4 was markedly elevated in NSCLC tumor samples and intimately linked to poor patient prognosis. Silencing of MCM4 repressed growth, migration, invasion, and EMT of cells. In vivo test findings displayed that knockdown of MCM4 suppressed changes in tumor volume and weight in mice. Moreover, E2F1 bound with the promoter of MCM4 was predicted by JASPAR database. E2F1 was heightened in NSCLC tissues and cells. Then, the outcome of rescue assays confirmed that E2F1 introduction attenuated the depressing influence of MCM4 knockdown on NSCLC. Moreover, E2F1/MCM4 promoted the progression of NSCLC by activating the PI3K/AKT signaling pathway.

Conclusions E2F1 accelerated NSCLC progression by activating the PI3K/AKT pathway through MCM4. Our outcomes confirmed that MCM4 is a potential target for the treatment of NSCLC patients.

Keywords NSCLC, MCM4, E2F1, Progression, Xenograft model

*Correspondence:
Lifeng Jiang
jlfandcjx@163.com
[1]Thoracic Surgery, The Second Affiliated Hospital of Kunming Medical University, Kunming, Yunnan 650101, China
[2]Oncology Department, The Second Affiliated Hospital of Kunming Medical University, No. 374 Dianmian Avenue, Wuhua District, Kunming, Yunnan 650101, China

Introduction

Until now, lung cancer, a malignant tumor, is still a severe danger to the life and health of people. The results of the Global Cancer Survey shown that in 2020, there was about 1.8 million deaths of lung cancer, ranking the first in mortality [1]. Non small cell lung cancer (NSCLC) accounts for approximately 85% of all cases [2]. With the popularization of lung cancer screening and people's precautions against lung cancer, the incidence of cancer has decreased. Besides, with the improvement of medical care, molecular targeted therapy and immunotherapy have led to an increase in the survival rate of patients. However, the relatively insidious onset of lung cancer and the less obvious early symptoms have led to the majority of patients tending to be in an advanced stage when their first diagnosis, and the overall survival rate of patients in the advanced stage is less than 20% [3]. Therefore, searching new molecular markers in NSCLC are crucial for improving patient outcomes and prolonging patient survival.

The distinguishing feature of cancer cells is that they can replicate indefinitely and the cell cycle is not regulated by the organism. Minichromosome maintenance proteins have deconjugating enzyme activity during replication priming and play a crucial role in regulating the timing of replication during the cell cycle [4]. Minichromosome maintenance proteins (MCMs) are a family of 10 proteins with highly conserved sequences of each member, mostly consisting of 776–1017 amino acid residues [5]. In addition to this, MCM proteins are involved in the prolongation of DNA replication and other chromosomal reactions, including damage response, transcription, and also constitute the structure of chromatin [6]. Unlimited replication of DNA is part of the hallmark of heterotrophic proliferative and malignant cells. Many DNA replication proteins are considered to be promising cancer biomarkers [7]. MCM proteins have also been proved to be strongly related to the occurrence of cancer. MCM is aberrantly expressed in kinds of cancer tissues and cancer cells and is considered to be a diagnostic marker for cancer [8].

Within the MCM family, minichromosome maintenance proteins 4 (MCM4) is recognized as the most conserved protein throughout evolution. It has been shown that MCM4 expression is enhanced in various malignant tumor tissues, such as hepatocellular carcinoma, ovarian carcinoma, and glioma, which may promote the development of malignant tumors, thus MCM4 is considered a prospective target for the treatment of malignant tumors [9]. In hepatocellular carcinoma, MCM4 mRNA and protein were obviously magnified in hepatocellular carcinoma tissues, and high MCM4 was markedly and inversely related to the survival of hepatocellular carcinoma patients [10]. Yang et al. confirmed MCM4 was enhanced in gliomas and had a significant effect on the proliferation and cell cycle of glioma cells, and could promote the growth of cancer cells in gliomas [11]. MCM4 has been reported as a biomarker for lung adenocarcinoma, and high expression of MCM4 is associated with poor prognosis in lung adenocarcinoma patients [12–15]. Although previous studies have shown that MCM4 has important biological functions in lung cancer and serves as a potential prognostic biomarker, its specific molecular mechanisms in NSCLC progression still need further investigation.

The work aimed to reveal the potential mechanisms by which MCM4 regulates the development of NSCLC. Our study found that MCM4 was highly expressed in NSCLC tissues and cell lines. Knockdown of MCM4 inhibited proliferation, migration, invasion, and EMT of NSCLC cells. In addition, we discovered that E2F1 bound to the MCM4 promoter by database prediction, and E2F1 was strengthened in NSCLC tissues and cells. Mechanistic studies revealed that E2F1 regulated NSCLC progression through MCM4. This study may provide new insights into the treatment of NSCLC patients.

Materials and methods

Bioinformatics analysis

The MCM4 and E2F1 levels in NSCLC tissues were analyzed via TCGA database. E2F1 binding with the promoter of MCM4 was predicted through JASPAR database.

Cell culture

All human lung cancer cell lines (H1299, A549, H1975), and human bronchial epithelial cell line (BEAS-2B) were purchased from American Type Culture Collection (ATCC). Cells were cultured in RPMI-1640 medium (Gibco) containing 10% FBS. All cells were cultured in an incubator at 37 °C with 5% CO_2.

Cell transfection

Si-MCM4, si-NC targeting MCM4 and oe-E2F1, oe-NC targeting E2F1 were purchased from GenePharma. Subsequently, the above oligosynucleotide solutions or plasmids were transfected into cells via Lipofectamine 3000 reagent. After 24 h of transfection, the medium was changed to continue incubation for 24 h. Cells were collected, and the transfection efficiency was detected by qRT-PCR and western blot.

Lentiviral vectors sh-MCM4 and sh-NC were purchased from GenePharma. Cells were infected with sh-MCM4 and sh-NC according to the manufacturer's guidelines. After 48 h, the stably transfected cells were screened with 2 μg/ml puromycin (sigama). Finally, qRT-PCR and western blot were used to measure the

transfection efficiency. The sequence of si-MCM4, si-NC, sh-NC and sh-MCM4 was shown in supplementary files.

Quantitative real time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from the cells using TRIzol reagent (Solarbio, China) and RNA concentration was determined. Total RNA was reverse transcribed into complementary DNA (cDNA) using a reverse transcription kit (Takara, China). Subsequently, qRT-PCR was performed on a 7500 ABI Biosystem using gene-specific primers. GAPDH was used as an internal control. Primer sequences were as follows (5'→3'):

MCM4 F: GACGTAGAGGCGAGGATTCC.
MCM4 R: AGAGCAGTTTGACGTGCTTCC.
E2F1 F: ACGTGACGTGTCAGGACCT.
E2F1 R: GATCGGGGCCTTGTTTGCTCTT.
GAPDH F: CGCTGAGTACGTCGTGGAGTC.
GAPDH R: GCTGATGATCTTGAGGCTGTTGTC.

Western blot (WB)

Total protein was extracted by lysing cells with RIPA lysate (Boster, China) and centrifuged. The density was estimated via BCA kit (Beyotime). Subsequently, proteins were isolated on 10% SDS-PAGE gel, after that the proteins were moved to a PVDF membrane. The membranes were blocked with 5% skimmed milk for 2 h. After that, incubated with antibodies against MCM4 (ab124836, 1:1000; Abcam), E-cadherin (ab231303, 1:1000; Abcam), N-cadherin (ab280375, 1:1000; Abcam), Vimentin (ab92547, 1:1000; Abcam), E2F1 (ab288369, 1:1000; Abcam), PI3K (#4292, 1:1000; Cell Signaling Technology), p-PI3K (#4228, 1:1000; Cell Signaling Technology), p-AKT (#9272, 1:1000; Cell Signaling Technology), p-AKT (#9271, 1:1000; Cell Signaling Technology), and GAPDH (#2118, 1:1000; Cell Signaling Technology) at 4 °C overnight. Next day, the membranes were incubated with HRP-conjugated secondary antibodies. Finally, specific protein signals were measured through an ECL kit, and analyzed in grayscale via Image J.

Cell counting kit-8 (CCK-8) assay

The assay was performed by Cell Counting Kit-8 (CCK-8). Transfected lung cancer cells (24 h post-transfection) were inoculated into 96-well plates, 100 μl complete medium was added and incubated at 37 °C. At the end of each experiment, 10 μl CCK-8 reagent was added, then the cells were continued to be incubated at 37 °C for 2 h. The OD450 was then determined through an enzyme marker (Thermo Fisher Scientific, USA).

Transwell assay

Transwell assay was utilized to detect the migration and invasion ability of the cells. In the migration assay, cells were inoculated into a 24-well Transwell at a density of 4×10^4 cells, 600 μl of medium containing 10% FBS was added to the lower chamber. 24 h later, cells remaining in the upper chamber were discarded. Subsequently, migrated cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet for 10 min. In the invasion assay, cells were kept in matrigel-coated (BD, USA) chambers for 48 h. Other steps were the same as for the migration assay.

Dual luciferase assay

Wild-type (WT) and mutant (MUT) plasmids containing MCM4 were constructed by inserting sequences into the pmirGLO plasmid. Cells (5×10^4 cells) were placed in 24-well plates and co-transfected with the E2F1 mimic using either the WT or MUT luciferase reporter plasmid. 48 h later, the relative luciferase activity in the cells was measured via a luciferase reporter gene kit (Promega, USA). The sequences of MCM4 wildtype and mutant promoters were shown in supplementary files.

Chromatin Immunoprecipitation (ChIP)

ChIP assays were performed using a ChIP kit (Millipore, USA). Cells were crosslinked with 1% formaldehyde, and the crosslinked chromatin was isolated and sonicated to fragment the DNA. Next, the sonicated chromatin was incubated overnight with corresponding antibodies at 4 °C, with IgG serving as the negative control. Then, the immunoprecipitated complexes were washed, the precipitated DNA was extracted and analyzed by qRT-PCR.

Xenograft tumor model

NSCLC cells were taken for experiments to construct the xenograft model. 4-week-old BALB/c nude mice were randomly classified into three groups, the control group, the MCM4 knockdown (sh-MCM4) group, the MCM4 knockdown and E2F1 overexpression (sh-MCM4 + oe-E2F1) group. The corresponding cells were implanted subcutaneously into the nude mice. The physical condition and tumor formation of the nude mice were observed at any time.After 4 weeks, the nude mice were sacrificed by de-necking, the transplanted tumors were removed, and the volume was measured and weighed.

Statistical analysis

Data were expressed as mean ± standard deviation and plotted using GraphPad Prism 8.0 software. Analysis between two groups was performed using Student's t-test. Analysis between multiple groups was performed by one-way analysis of variance (ANOVA). The relationship between MCM4 expression and patient prognosis was analyzed using Kaplan-Meier survival curves. $P < 0.05$ was considered statistically significant difference.

Results

MCM4 is Raised in NSCLC tissues and cell lines

To investigate the effect of MCM4 on the development of NSCLC, our study first evaluated the levels of MCM4 in various types of cancers by the TCGA database, which showed that MCM4 was highly expressed in a variety of cancers including NSCLC (Fig. 1A). To further explore the expression of MCM4 in NSCLC, MCM4 expression was analyzed in 59 normal tissue samples and 515 tumor samples from the TCGA database, which also revealed that MCM4 was significantly highly expressed (Fig. 1B). The different stages of tumors in the TCGA database and the immunohistochemical analysis results in the Human Protein Atlas also confirmed the high expression of MCM4 (Fig. 1C, D). Next, we validated the expression of MCM4 in NSCLC using qRT-PCR and WB experiments. Elevated MCM4 mRNA and protein levels were found in NSCLC tissues (Fig. 1E, F). In addition, MCM4 was markedly heightened in NSCLC cell lines (H1299, A549, H1975) contrasted to BEAS-2B (Fig. 1G). Moreover, this was confirmed by the experimental results of WB (Fig. 1H). There was a obvious difference between the survival rate of patients with high and low MCM4 expression, and patients with high MCM4 had notably

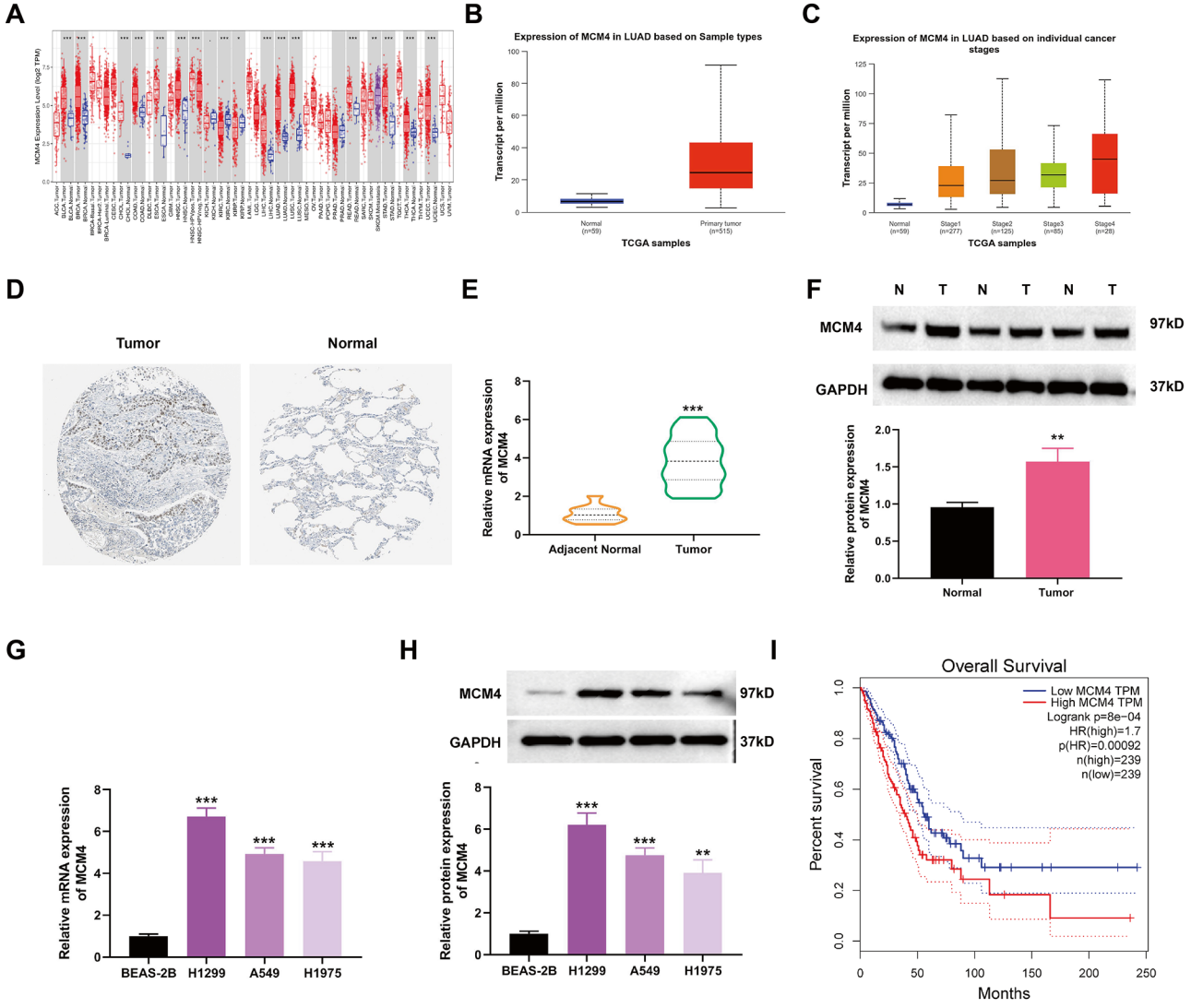

Fig. 1 MCM4 was highly expressed in NSCLC tissues and cell lines. **A**. The expression level of MCM4 in various types of cancers tissues and normal tissues was assessed using TCGA database. **B**. The expression level of MCM4 in cancer tissues and normal tissues was assessed using TCGA database. **C**. The expression of MCM4 in different stages of tumors was analyzed by TCGA database. **D**. The expression of MCM4 was analyzed by immunohistochemical in the Human Protein Atlas. **E**. MCM4 mRNA expression in NSCLC tissues and normal tissues was measured by qRT-PCR. **F**. MCM4 protein level in NSCLC tissues and normal tissues was measured by western blot. **G**. MCM4 mRNA expression in different NSCLC cell lines compared with human normal lung epithelial cell line was measured by qRT-PCR. **H**. MCM4 protein level in NSCLC cell lines was measured by western blot. I. The relationship between MCM4 expression and patient prognosis was analyzed using Kaplan-Meier survival curves. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

lower survival than patients with low MCM4 expression and had a poorer prognosis (Fig. 1I). These findings implied MCM4 was enhanced in NSCLC, its high expression was strongly associated with low survival.

Silencing of MCM4 weakens proliferation, migration, invasion and EMT of NSCLC cells

To examine the biological function of MCM4 in NSCLC, we transfected NSCLC cells with si-MCM4 and detected the transfection efficiency of cells via qRT-PCR. The findings displayed MCM4 was dramatically reduced in cells after transfection with si-MCM4 (Fig. 2A). To further verify the transfection efficiency of the cells, we performed WB experiments. Similar results were obtained, and the MCM4 protein expression was obviously reduced in cells after transfection with si-MCM4 (Fig. 2B). The above findings indicated that the cell transfection was successful. Subsequently, we utilized CCK-8 test to measure the cell growth ability. The outcomes exhibited that the cells growth ability was markedly declined after MCM4 silencing compared with the control (Fig. 2C). Next, we also used transwell experiment to test the metastatic ability of NSCLC cells after MCM4 knockdown. The experimental results demonstrated that the number of migrating and invading cells was notably diminished after MCM4 silencing, implying a clear degraded in cell migration (Fig. 2D) and invasion (Fig. 2E) abilities. In addition, we assayed EMT-related markers level using WB assay. The findings disclosed that the E-cadherin protein content of the cells was enlarged after knockdown of MCM4, while the N-cadherin and Vimetin protein content was reduced, indicating that MCM4 silencing remarkably attenuated the EMT process (Fig. 2F). The above outcomes displayed MCM4 inhibition hampered the growth, migration, invasion and EMT of NSCLC cells.

E2F1 acts as a transcription factor to activate MCM4 expression in NSCLC cells

To characterize the molecular mechanism by which MCM4 facilitates the progression of NSCLC cells, the binding factors of MCM4 were predicted via JASPAR database. The results demonstrated that E2F1 bound with the MCM4 promoter (Fig. 3A). Subsequently, we analyzed the expression of E2F1 in NSCLC tissues by database. The findings suggested that E2F1 was notably enhanced in NSCLC tumor samples (Fig. 3B). Next, the E2F1 mRNA level in different NSCLC cells was detected through qRT-PCR. We disclosed that E2F1 mRNA expression was raised in NSCLC cells compared with BEAS-2B (Fig. 3C). Moreover, the experimental results of WB presented similar results. E2F1 protein expression was also heightened in NSCLC cells (Fig. 3D). In addition, to research the biological function of E2F1 in NSCLC, cells were transfected with oe-E2F1 overexpression vector. The overexpression efficiency of E2F1 was measured via qRT-PCR. The findings indicated that E2F1 mRNA in cells was notably strengthened after transfection of oe-E2F1 overexpression vector (Fig. 3E). Then the overexpression efficiency of E2F1 was verified by WB assay, and it was found the E2F1 protein expression was clearly enlarged after transfection with oe-E2F1 insertion vector (Fig. 3F). The above outcomes indicated that the cell transfection was successful. After that, we used luciferase assay to validate the binding relationship between E2F1 and MCM4. It was discovered that after overexpression of E2F1, luciferase activity of MCM4-WT was heightened, whereas there was no obvious change in the luciferase activity of MCM4-MUT, suggesting that there was a binding relationship between E2F1 and MCM4 (Fig. 3G). The ChIP experiment results also confirmed the binding relationship between E2F1 and MCM4 (Fig. 3H). Subsequently, we determined the effect of E2F1 overexpression on MCM4 mRNA and protein expression via qRT-PCR and WB. The levels of MCM4 mRNA and protein were found to be considerably reduced after inhibition of MCM4, whereas MCM4 levels were increased after cotransfection of si-MCM4 and oe-E2F1 (Fig. 3I, J). The above outcomes displayed that E2F1 acted as a transcription factor to activate MCM4 levels in NSCLC cells.

E2F1 overexpression alleviates the inhibitory effect of MCM4 Silencing on NSCLC

To probe the specific functions of E2F1 and MCM4 in NSCLC development, we performed rescue experiments. First, cell viability was examined using CCK-8 experiment. We identified that MCM4 knockdown diminished the cell viability, which was reversed by the addition of oe-E2F1 (Fig. 4A). Subsequently, the metastatic ability of the cells was assessed transwell experiment. Cell migration and invasion abilities were dramatically depressed after transfection with si-MCM4, whereas co-transfection with si-MCM4 and oe-E2F1 alleviated the repressive action of transfection with si-MCM4 on cell migration and invasion abilities (Fig. 4B, C). Next, EMT-related proteins level was determined using the WB. It was noticed that overexpression of E2F1 returned the promoting effect on E-cadherin proteins and the inhibitory effect on N-cadherin and Vimetin proteins after MCM4 silencing (Fig. 4D, E). The above outcomes suggested that E2F1 introduction attenuated the suppressive effect of MCM4 inhibition on NSCLC.

E2F1/MCM4 plays a role in NSCLC by activating the PI3K/AKT signaling pathway

Next, we further evaluated the role of the E2F1/MCM4 axis in PI3K/AKT signaling transduction. WB results showed that MCM4 knockdown inhibited the activation

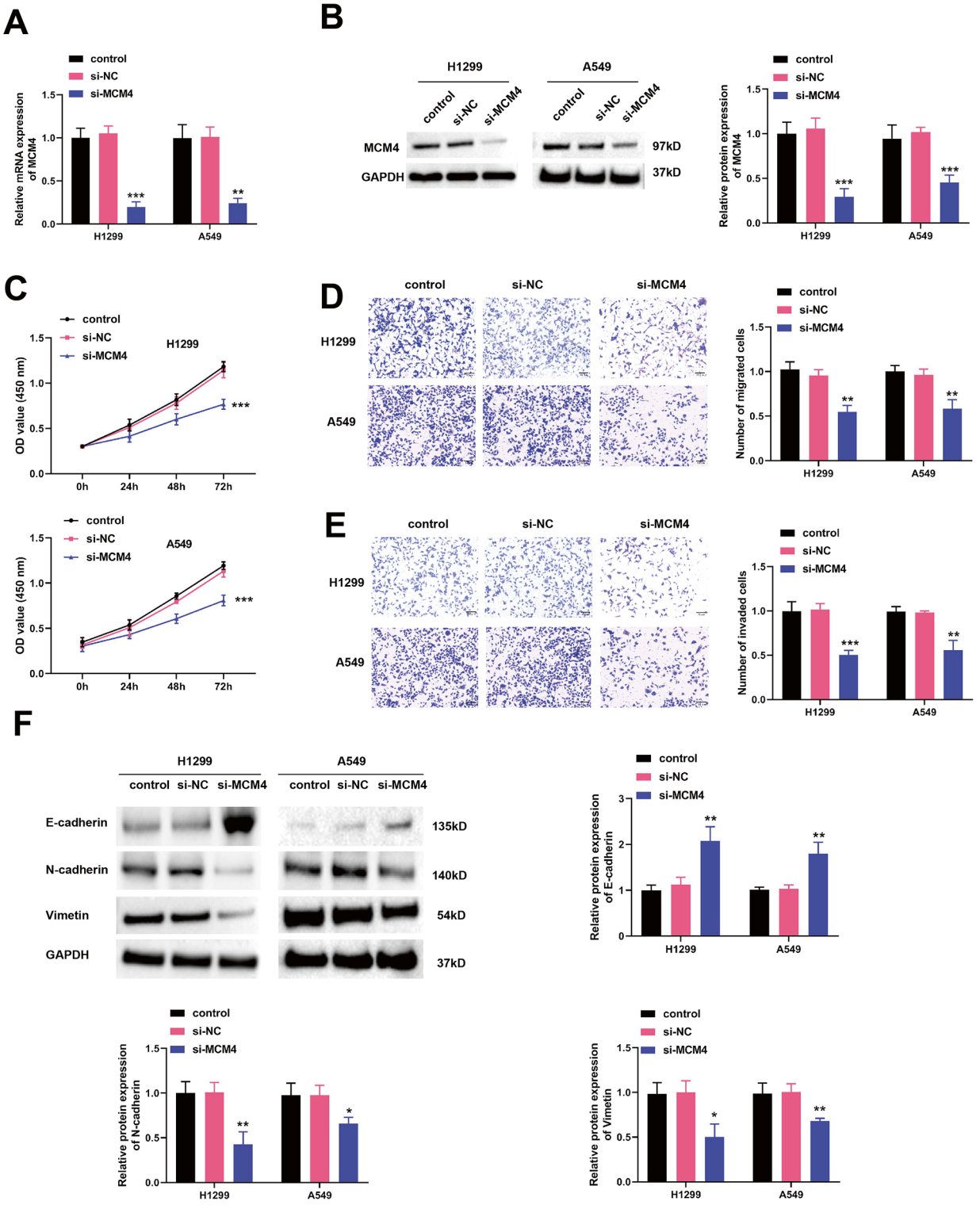

Fig. 2 MCM4 silencing suppressed proliferation, migration, invasion and EMT of NSCLC cells. **A**. qRT-PCR was used to detect the knockdown efficiency of siRNA. **B**. Western blot was used to confirm the knockdown efficiency of siRNA. **C**. Cell viability of NSCLC cell after MCM4 knockdown was measured with CCK-8 assay. **D**. Cell migration of NSCLC cell after MCM4 knockdown was tested by transwell assay. **E**. Invasive ability of NSCLC cell after MCM4 knockdown was examined with transwell assay. **F**. EMT-related markers was detected by western blot. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

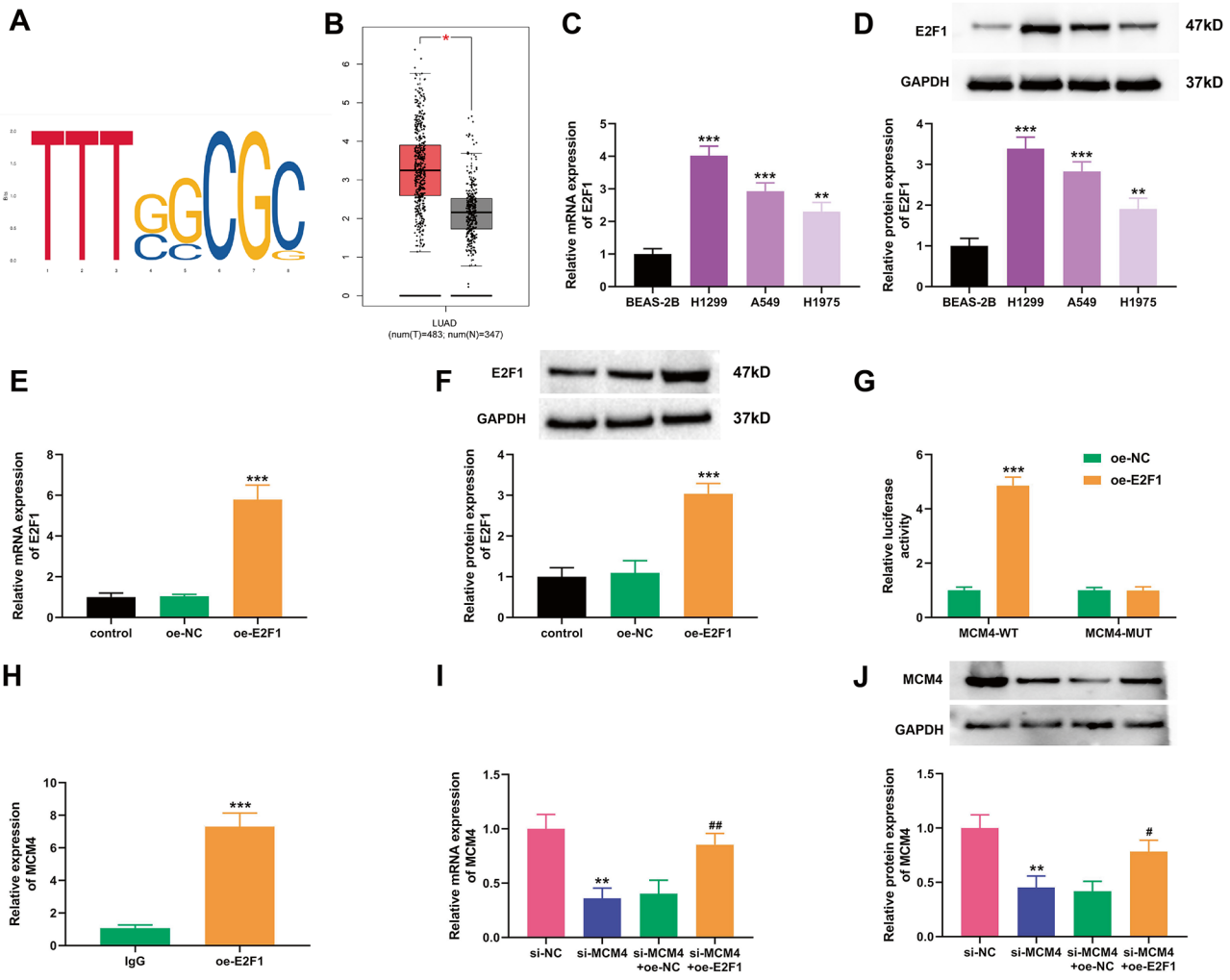

Fig. 3 E2F1 acted as transcription factor to activate MCM4 expression in NSCLC cells. **A**. E2F1 binding with the promoter of MCM4 was predicated by JASPAR databases. **B**. E2F1 expression in NSCLC tissues was tested by database. **C**. E2F1 mRNA expression in different NSCLC cell lines compared with human normal lung epithelial cell line was measured by qRT-PCR. **D**. E2F1 protein level in NSCLC cell lines was measured by western blot. **E**. qRT-PCR was used to detect the overexpression efficiency of E2F1. **F**. Western blot was used to confirm the overexpression efficiency of E2F1. **G**. The binding relationship between E2F1 and MCM4 was confirmed by luciferase assay. **H**. The binding relationship between E2F1 and MCM4 was confirmed by ChIP experiment. **I**. MCM4 mRNA level were assessed by qRT-PCR after E2F1 overexpression. **J**. MCM4 protein level were assessed by western blot after E2F1 overexpression. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{\#}P<0.05$, $^{\#\#}P<0.01$

of PI3K/Akt signaling, and overexpression of E2F1 reversed this inhibitory effect (Fig. 5A, B). These findings suggested that E2F1/MCM4 promoted NSCLC progression by activating the PI3K/AKT signaling pathway.

E2F1 promotes the development of NSCLC tumors through MCM4 in vivo

To study the functions of MCM4 and E2F1 in vivo, we established an animal xenograft model. The sh-MCM4 or oe-E2F1 was transfected into NSCLC cells. The representative image of the tumor was shown in Fig. 6A. Compared with sh-NC, the tumor volume in the sh-MCM4 group was dramatically reduced, while oe-E2F1 reversed this phenomenon (Fig. 6B). Similarly, oe-E2F1 returned the inhibitory effect of sh-MCM4 on tumor weight (Fig. 6C). In addition, qRT-PCR experiments disclosed that oe-E2F1 reversed the effect of knockdown MCM4 on MCM4 expression (Fig. 6D). The above results indicated that E2F1 accelerated the development of NSCLC through MCM4 in vivo.

Discussion

For decades, lung cancer has been a tumor with high malignancy and mortality, NSCLC is the major case type of lung cancer, even with chemotherapy and surgery, most lung cancer patients still have a short survival period, which is mainly due to the metastasis of the cancer and rapid tumor progression [16]. Therefore, research of the targeted therapeutic mechanism of NSCLC and

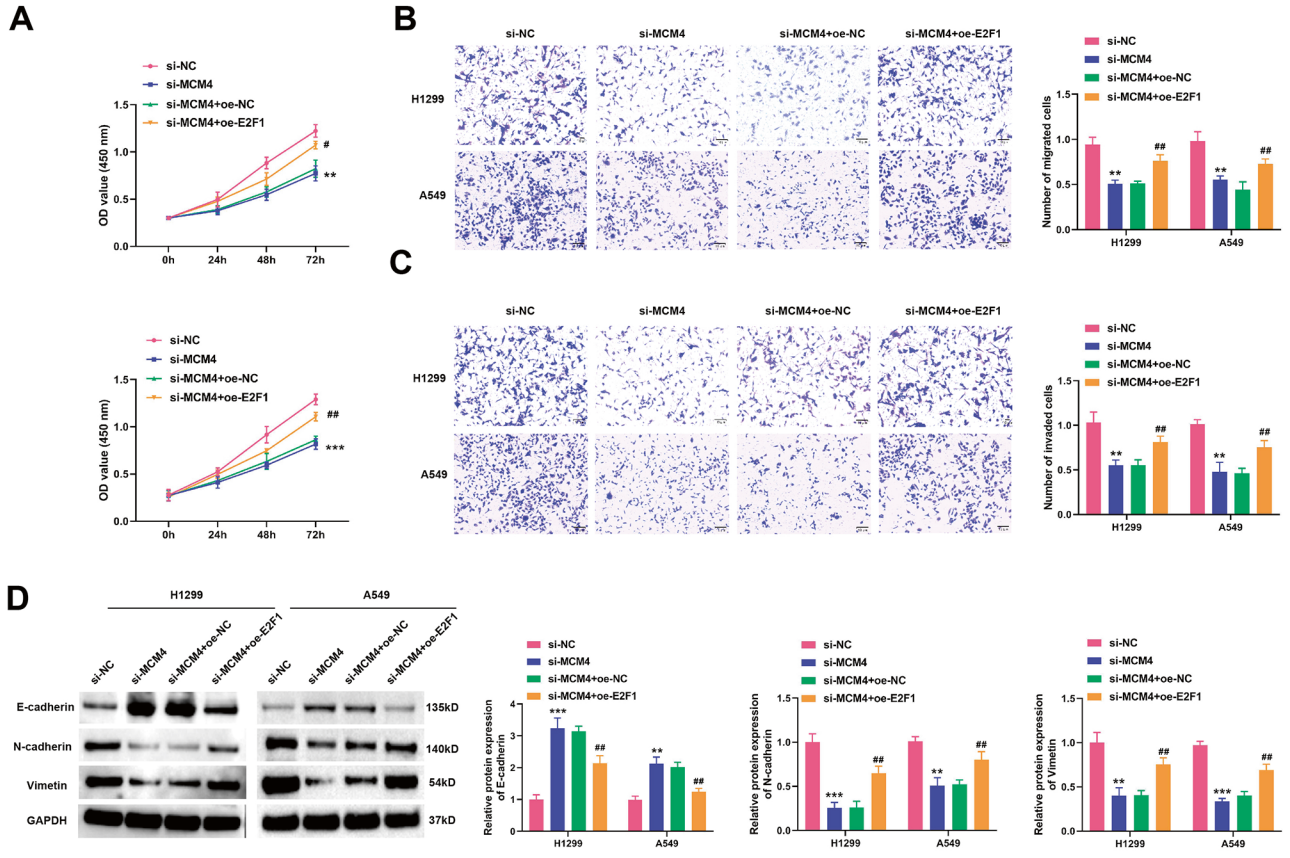

Fig. 4 E2F1 overexpression alleviated the inhibitory effects of MCM4 knockdown in NSCLC. **A**. Cell viability was tested using CCK-8 assay. **B**. Cell migration was assessed by transwell assay. **C**. Cell invasion was evaluated by means of transwell assay. **D**-**E**. The expression of EMT-related proteins was determined using western blot. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$

searching for effective targets for NSCLC patients are essential.

Current studies have well established that epigenetic and genetic alterations such as transcription factors, chemokines, growth factors, proteases, etc. can be involved in cancer progression in specific microenvironments [17]. One of the transcription factors is the microchromosomal protein (MCM) family. It has been pointed out that the association between MCM5 and HDAC1 exacerbates the malignant progression of lung cancer [18]. Lei et al. displayed high MCM6 was closely associated with tumor size and lymph node metastasis in patients with breast cancer, and that MCM6 was a danger element affecting the prognosis of breast cancer patients [19]. Furthermore, the expression of MCM2 is linked to poor prognosis in pancreatic cancer and has been identified as a potential biomarker for the diagnosis and prognosis of pancreatic cancer [20]. MCM4 is a core component of the MCM family, and multiple studies have demonstrated that MCM4 serves a pivotal part in DNA replication and tumorigenesis [21]. Kobayashi et al. demonstrated that the MCM4 was related to poor prognosis and tumor progression in uroepithelial carcinoma [22]. Choy et al. manifested that MCM4 could be used as a biomarker for precancerous lesions of esophageal cancer, and was significantly correlated with the Ki-67, Bmi1, and cytokinin E levels [23]. Xiao et al. revealed that MCM4 was associated with tumorigenesis and triple-negative breast cancer progression, and could be used as a triple-negative breast cancer prognostic biomarker and therapeutic target [24]. However, a comprehensive analysis of the diagnostic value of MCM4 in lung cancer and its mechanism affecting the development of lung cancer remain to be elucidated. Tan et al. found that MCM4 was strengthened in lung adenocarcinoma tumors, MCM4 overexpression promoted the growth and inhibited the apoptosis of lung adenocarcinoma cells [25]. Our results have similarities with the previous findings, in our study, by database analysis, we disclosed MCM4 was raised in NSCLC, the same results were obtained in cell lines. Survival curve analysis displayed patients with high MCM4 had a worse prognosis. To study the function of MCM4 in NSCLC cells, we constructed NSCLC cell lines with knockdown MCM4 expression. We discovered that MCM4 inhibition clearly weakened cell growth, metastasis, and suppressed the EMT process. The outcomes of the xenograft model also

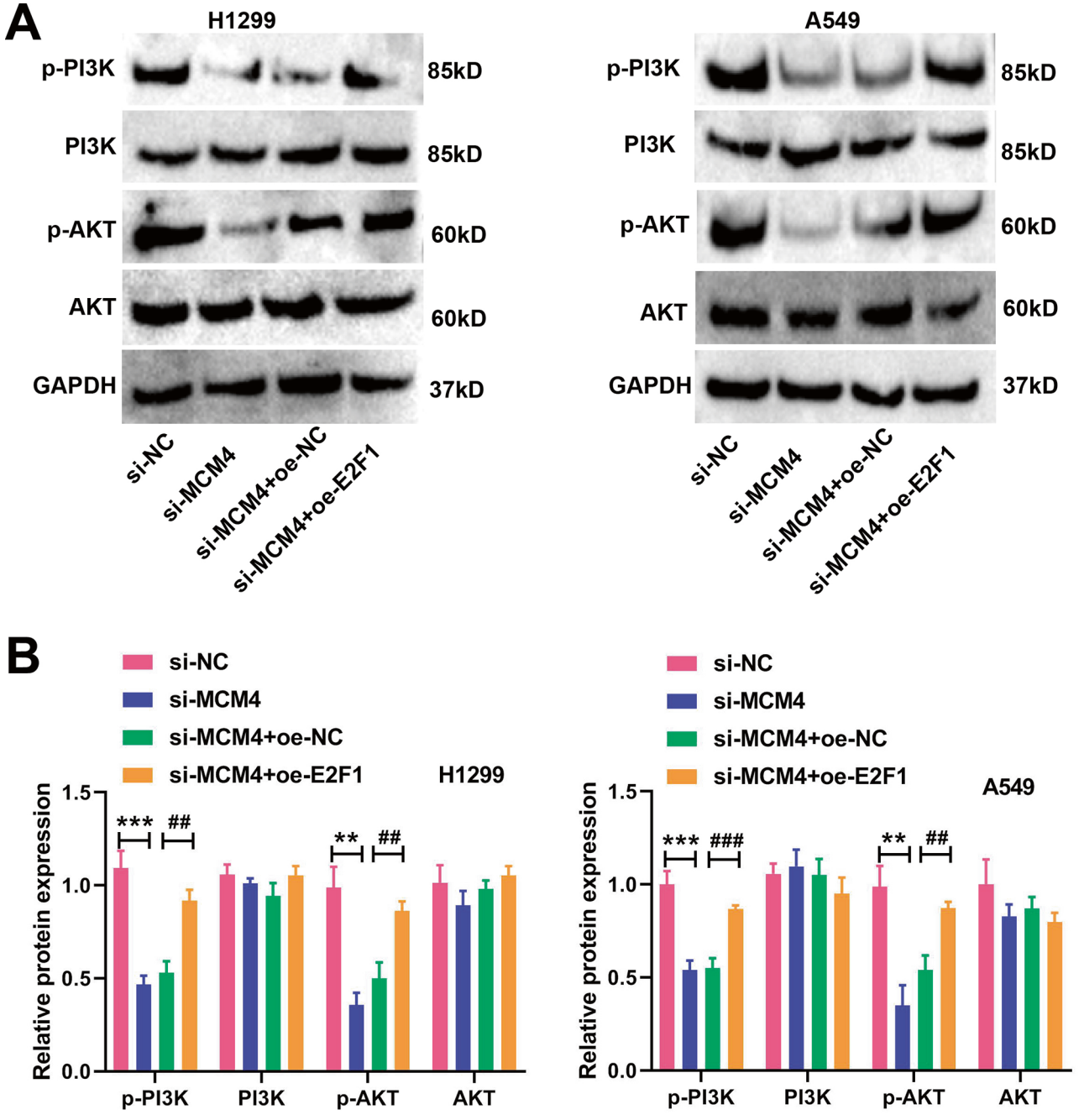

Fig. 5 E2F1/MCM4 plays a role in NSCLC by activating the PI3K/AKT signaling pathway. **A**. The levels of phosphorylated PI3K and AKT in NSCLC cells after silencing MCM4 or overexpressing E2F1 was analyzed by western blot. **B**. Quantitative analysis of phosphorylated PI3K and AKT levels. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$

declared that knockdown of MCM4 hampered changes in tumor volume and weight and restrained growth in mice.

On this basis, to research the mechanism by which MCM4 promotes cancer development, we predicted the binding sites of MCM4 through the JASPAR database and discovered E2F1 bound to the MCM4 promoter. This relationship was also confirmed by dual luciferase experiments. E2F1 is an important member of the E2F transcription factor family, which consists of eight members (E2F1-E2F8) that can be categorized into two groups in terms of their protein structures, collaborating factors and transcriptional features. E2F1-E2F3 are transcription promoters and E2F4-E2F8 are transcription repressors [26]. E2F1 is located on human chromosome 20q11, with a fragment size of approximately 11 kb [27]. E2F1

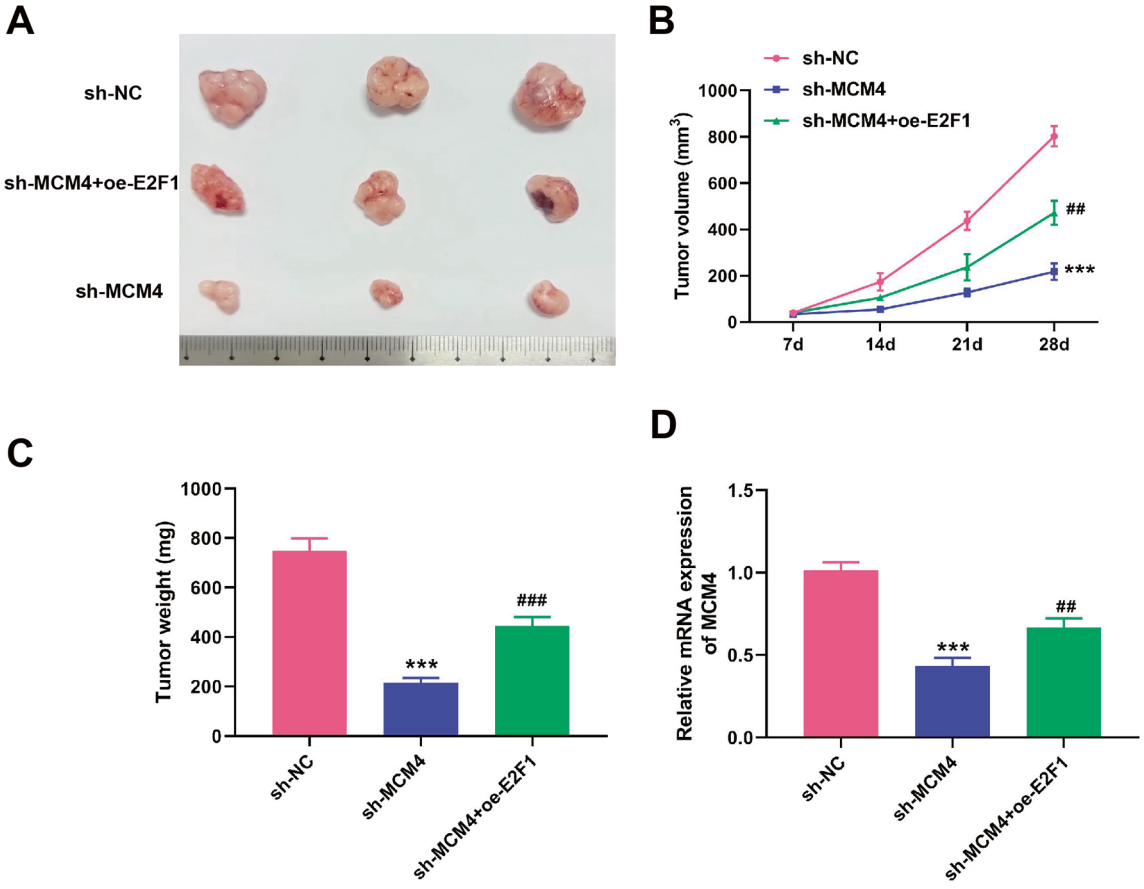

Fig. 6 E2F1 promotes the development of NSCLC tumors through MCM4 in vivo. **A**. Representative images of tumors. **B**. The changes in tumor volume. **C**. The changes in tumor weight. **D**. The MCM4 expression was detected by qRT-PCR. $^{***}P < 0.001$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$

is involved in the regulation of various biological activities, ranging from apoptosis, angiogenesis, and invasive metastasis, etc. Song et al. confirmed that E2F1 initiated PRSS22 transcription accelerates breast cancer metastasis through cleavage of ANXA1 and activation of FPR2/ERK pathway [28]. Lv et al. declared that E2F1-activated SPIN1 facilitates cell growth in gastric cancer through the MDM2-p21-E2F1 feedback loop [29]. The results of this study revealed that E2F1 was obviously enhanced in NSCLC tissues and cell lines. Subsequently, we performed rescue experiments and disclosed E2F1 insertion attenuated the effect of MCM4 knockdown on NSCLC. In vivo experiments also confirmed that overexpression of E2F1 reversed the inhibitory effect of MCM4 knockdown on tumor growth in mice. In addition, PI3K/AKT has been reported to regulate cell proliferation and metastasis in a variety of cancers [30, 31]. This study found that knockdown of MCM4 inhibited the activation of PI3K/AKT signaling, and overexpression of E2F1 reversed this inhibitory effect. These findings suggested that E2F1/MCM4 promoted NSCLC progression by activating the PI3K/AKT signaling pathway.

Conclusions

MCM4 is enhanced in NSCLC, and high MCM4 is closely correlated to worse prognosis of NSCLC patients. In addition, MCM4 facilitates NSCLC cell proliferation, metastasis, and EMT, promotes tumor growth. Our findings suggest E2F1 accelerated tumor progression by activating the PI3K/AKT signaling pathway through MCM4, providing a probable new therapy for the future treatment of NSCLC.

Abbreviations

NSCLC	Non-Small Cell Lung Cancer
MCMs	Minichromosome Maintenance Proteins
MCM4	Minichromosome Maintenance Proteins 4
ATCC	American Type Culture Collection
qRT-PCR	Quantitative Real Time Polymerase Chain Reaction
cDNA	Complementary DNA
WB	Western Blot
CCK-8	Cell Counting Kit-8
WT	Wild-Type
MUT	Mutant
ANOVA	Analysis of Variance

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13019-025-03481-z.

Supplementary Material 1

Acknowledgements
Not applicable.

Author contributions
YYC designed the study and writing original draft. XYL collected data, processed statistical data, and performed the experiments. TTL and JL collected the data and partly contributed to the experiments. LFJ designed, supervised and revised the study. All authors reviewed the results and approved the final version of the manuscript.

Funding
Not applicable.

Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee of the Second Affiliated Hospital of Kunming Medical University. All participants were provided with written informed consent at the time of recruitment, and all experiments involving human tissue specimens comply with the Declaration of Helsinki. Animal studies were performed in compliance with the ARRIVE guidelines.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 20 September 2024 / Accepted: 18 May 2025
Published online: 28 May 2025

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209–49.
2. Chen J, Rao B, Huang Z, Xie C, Yu Y, Yang B, et al. Circular RNA Hsa_circ_0050386 suppresses non-small cell lung cancer progression via regulating the SRSF3/FN1 axis. J Transl Med. 2024;22(1):47.
3. Tanigawa K, Tomioka Y, Misono S, Asai S, Kikkawa N, Hagihara Y, et al. Minichromosome maintenance proteins in lung adenocarcinoma: clinical significance and therapeutic targets. FEBS Open Bio. 2023;13(9):1737–55.
4. Kwok HF, Zhang SD, McCrudden CM, Yuen HF, Ting KP, Wen Q, et al. Prognostic significance of minichromosome maintenance proteins in breast cancer. Am J Cancer Res. 2015;5(1):52–71.
5. Ishimi Y. Regulation of MCM2-7 function. Genes Genet Syst. 2018;93(4):125–33.
6. Li Z, Xu X. Post-Translational modifications of the Mini-Chromosome maintenance proteins in DNA replication. Genes (Basel). 2019;10(5).
7. Semple JW, Duncker BP. ORC-associated replication factors as biomarkers for cancer. Biotechnol Adv. 2004;22(8):621–31.
8. Yu S, Wang G, Shi Y, Xu H, Zheng Y, Chen Y. MCMs in cancer: prognostic potential and mechanisms. Anal Cell Pathol (Amst). 2020;2020:3750294.
9. Xie L, Li T, Yang LH. E2F2 induces MCM4, CCNE2 and WHSC1 upregulation in ovarian cancer and predicts poor overall survival. Eur Rev Med Pharmacol Sci. 2017;21(9):2150–6.
10. Zheng R, Lai G, Li R, Hao Y, Cai L, Jia J. Increased expression of MCM4 is associated with poor prognosis in patients with hepatocellular carcinoma. J Gastrointest Oncol. 2021;12(1):153–73.
11. Yang S, Yuan Y, Ren W, Wang H, Zhao Z, Zhao H, et al. MCM4 is a novel prognostic biomarker and promotes cancer cell growth in glioma. Front Oncol. 2022;12:1004324.
12. Jia M, Feng S, Cao F, Deng J, Li W, Zhou W et al. Identification of EGFR-Related LINC00460/mir-338-3p/MCM4 regulatory Axis as diagnostic and prognostic biomarker of lung adenocarcinoma based on comprehensive bioinformatics analysis and experimental validation. Cancers (Basel). 2022;14(20).
13. Li S, Jiang Z, Li Y, Xu Y. Prognostic significance of minichromosome maintenance mRNA expression in human lung adenocarcinoma. Oncol Rep. 2019;42(6):2279–92.
14. Liu K, Kang M, Liao X, Wang R. Genome-wide investigation of the clinical significance and prospective molecular mechanism of minichromosome maintenance protein family genes in patients with lung adenocarcinoma. PLoS ONE. 2019;14(7):e0219467.
15. Sanada H, Seki N, Mizuno K, Misono S, Uchida A, Yamada Y et al. Involvement of dual strands of miR-143 (miR-143-5p and miR-143-3p) and their target oncogenes in the molecular pathogenesis of lung adenocarcinoma. Int J Mol Sci. 2019;20(18).
16. Vasiukov G, Zou Y, Senosain MF, Rahman JSM, Antic S, Young KM, et al. Cancer-associated fibroblasts in early-stage lung adenocarcinoma correlate with tumor aggressiveness. Sci Rep. 2023;13(1):17604.
17. Nowińska K, Dzięgiel P. [The role of MCM proteins in cell proliferation and tumorigenesis]. Postepy Hig Med Dosw (Online). 2010;64:627–35.
18. Zhang LL, Li Q, Zhong DS, Zhang WJ, Sun XJ, Zhu Y. MCM5 aggravates the HDAC1-Mediated malignant progression of lung Cancer. Front Cell Dev Biol. 2021;9:669132.
19. Lei Z, Wang P, Jia DQ, Li LL, Wu YP, Yang Y, et al. MCM6 is a poor prognostic biomarker and promotes progression in breast Cancer. Front Biosci (Landmark Ed). 2023;28(8):128.
20. Deng Y, Ma H, Hao J, Xie Q, Zhao R. MCM2 and NUSAP1 are potential biomarkers for the diagnosis and prognosis of pancreatic Cancer. Biomed Res Int. 2020;2020:8604340.
21. Champasa K, Blank C, Friedman LJ, Gelles J, Bell SP. A conserved Mcm4 motif is required for Mcm2-7 double-hexamer formation and origin DNA unwinding. Elife. 2019;8.
22. Kobayashi G, Hayashi T, Sentani K, Uraoka N, Fukui T, Kido A, et al. MCM4 expression is associated with high-grade histology, tumor progression and poor prognosis in urothelial carcinoma. Diagn Pathol. 2023;18(1):106.
23. Choy B, LaLonde A, Que J, Wu T, Zhou Z. MCM4 and MCM7, potential novel proliferation markers, significantly correlated with Ki-67, Bmi1, and Cyclin E expression in esophageal adenocarcinoma, squamous cell carcinoma, and precancerous lesions. Hum Pathol. 2016;57:126–35.
24. Xiao X, Zhang Z, Luo R, Peng R, Sun Y, Wang J, et al. Identification of potential oncogenes in triple-negative breast cancer based on bioinformatics analyses. Oncol Lett. 2021;21(5):363.
25. Tan Y, Ding L, Li G. MCM4 acts as a biomarker for LUAD prognosis. J Cell Mol Med. 2023;27(21):3354–62.
26. Black EP, Hallstrom T, Dressman HK, West M, Nevins JR. Distinctions in the specificity of E2F function revealed by gene expression signatures. Proc Natl Acad Sci U S A. 2005;102(44):15948–53.
27. Helin K, Lees JA, Vidal M, Dyson N, Harlow E, Fattaey A. A cDNA encoding a pRB-binding protein with properties of the transcription factor E2F. Cell. 1992;70(2):337–50.
28. Song L, Li H, Ma RR, Liu S, Zhang GH, Guo XY, et al. E2F1-initiated transcription of PRSS22 promotes breast cancer metastasis by cleaving ANXA1 and activating FPR2/ERK signaling pathway. Cell Death Dis. 2022;13(11):982.
29. Lv BB, Ma RR, Chen X, Zhang GH, Song L, Wang SX, et al. E2F1-activated SPIN1 promotes tumor growth via a MDM2-p21-E2F1 feedback loop in gastric cancer. Mol Oncol. 2020;14(10):2629–45.
30. Zhang M, Wang X, Zhao J, Yan J, He X, Qin D, et al. CircRNA-CIRH1A promotes the development of osteosarcoma by regulating PI3K/AKT and JAK2/STAT3 signaling pathways. Mol Biotechnol. 2024;66(9):2241–53.
31. Liu W, Zhang J, Zhang J, Ye Y, Zhu J, Yu Q, et al. EIF4A3-induced circ_0022382 promotes breast cancer cell progression through the let-7a-5p/PI3K/AKT/mTOR signaling pathway and SLC7A11 axis. Front Oncol. 2024;14:1476731.

Publisher's note