

Minichromosome maintenance protein 2 correlates with the malignant status and regulates proliferation and cell cycle in lung squamous cell carcinoma

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Background: Minichromosome maintenance protein 2 (MCM2), which is a member of MCM family, has been found to be a relevant marker for progression and prognosis in a variety of human cancers. Due to lack of effective therapeutic target in lung squamous cell carcinoma (LUSC) patients, the aim of our study was to screen and identify biomarkers which are associated to clinicopathological characteristics including prognosis in LUSC patients.

Methods: The expression status of MCM2 in lung cancer was analyzed using the publicly available Gene Expression Omnibus databases (GSE3268 and GSE10245). The mRNA and protein expression of MCM2 in lung cancer tissues and cell lines was detected by quantitative real-time PCR and Western blot, and the association between MCM2 expression and clinicopathological factors was analyzed by immunohistochemistry. The loss-of-function study of MCM2 was conducted in LUSC cell lines.

Results: In our study, we found MCM2 expression was increased in LUSC tissues compared with paired adjacent normal lung tissues or lung adenocarcinoma tissues through analyzing microarray data sets (GSE3268 and GSE10245), which confirmed that MCM2 mRNA and protein were overexpressed in LUSC tissues and cell lines. Meanwhile, we analyzed the association between MCM2 protein expression and clinicopathological characteristics of LUSC patients, and found high expression of MCM2 protein was obviously associated with malign differentiated degree, advanced clinical stage, large tumor size, more lymph node metastasis and present distant metastasis. The survival analysis showed MCM2 overexpression was an independent unfavorable prognostic factor for LUSC patients.

Conclusion: MCM2 is involved in the development and progression of LUSC as an oncogene, and thereby may act as a potential therapeutic target for LUSC patients.

Keywords: MCM2, lung cancer, lung squamous cell carcinoma, biomarker, proliferation, cell cycle

Introduction

Lung cancer ranks first among all cancers in morbidity and mortality worldwide, and the incidence and mortality of lung cancer are increasing every year.¹ According to the GLOBOCAN 2012 data, lung cancer is the most frequently diagnosed cancer worldwide with an estimated 1,824,700 cases and 1,589,900 deaths in 2012.¹ In People's Republic of China, about 733,300 newly diagnosed lung cancer cases and 610,200 lung cancer deaths were reported in 2015.² In the USA, it is estimated that 234,030 new lung cancer cases and 154,050 lung cancer deaths will be recorded in 2018.³

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Based on histological classification, lung cancer can be divided into small-cell lung cancer, lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), large-cell lung cancer and other rare types.^{4,5} LUAD and LUSC are the two major histological types of lung cancer, accounting for 80% of lung cancer cases.⁶ In recent decades, molecular targeted therapy has seen a rapid growth including the development of epidermal growth factor (EGF) receptor tyrosine kinase inhibitors and anaplastic lymphoma tyrosine kinase inhibitors, which are effective for the treatment of LUAD carrying activating mutations.^{7,8} Due to lack of effective therapeutic target in LUSC patients, the overall survival of LUSC patients is significantly shorter compared with LUAD patients.^{9,10} Hence, it is urgently necessary to identify novel and credible biomarkers for developing molecular targeted therapy for LUSC patients.

Minichromosome maintenance protein (MCM) family is composed of six related proteins (MCM2–MCM7), which were originally identified in yeast to activate DNA replication.^{11,12} Closure of the MCM2/5 gate is required to activate DNA unwinding and elongation.¹³ Upon S-phase entry, several regulatory cyclin-dependent kinases and DBF4-dependent kinase activate MCM2–MCM7 by enabling the loading of the key accessory factors CDC45 and GINS that in combination with MCM2–MCM7 form the CMG complex.¹³ MCM2 has been found to be a relevant marker for progression and prognosis in a variety of human cancers.¹³ The aim of our study was to screen and identify biomarkers which are associated to clinicopathological characteristics including prognosis in LUSC patients. In the early stage of our study, we analyzed microarray data sets (GSE3268 and GSE10245), and found MCM2 expression was increased in LUSC tissues compared with paired adjacent normal lung tissues from microarray data (GSE3268) and MCM2 was overexpressed in LUSC tissue samples compared with LUAD tissue samples based on microarray data sets (GSE10245). Thus, we further explored the clinical value and biological function of MCM2 in LUSC. We measured MCM2 expression in LUSC tissues to observe the correlation between MCM2 expression and clinicopathological characteristics, and performed loss-of-function study to explore the effect of MCM2 on LUSC cell lines.

Methods

Ethics statement

The Research Ethics Committees of Affiliated Hospital of Jining Medical University and Jining No 1 People's Hospital approved this protocol, and written informed consent was

obtained from each patient. The entire study was performed based on the Declaration of Helsinki.

Database analysis

Microarray data sets were retrieved from the Gene Expression Omnibus database. The microarray data set GSE3268 comprised five pairs of LUSC tissues and adjacent normal lung tissues, and was submitted by Shinichiro Wachi et al (email: mogushi-k@umin.ac.jp). The microarray data set GSE10245 included LUSC tissue samples and LUAD tissue samples, and was submitted by Ruprecht Kuner et al (email: r.kuner@dkfz.de). Raw data were processed, and differentially expressed genes were screened.

Patients and samples

Fresh and paraffin-embedded clinical samples (including LUAD tissue, LUSC tissue and normal lung tissue) were collected, and the pathological information (age, gender, histological type, clinical stage, tumor depth, lymph node metastasis, distant metastasis, differentiation and family history) was retrieved from the archives of the Department of Pathology of Affiliated Hospital of Jining Medical University and Jining No 1 People's Hospital. None of the patients had received antitumor therapy before diagnosis. Clinical staging and system treatment were performed according to the 7th edition of the American Joint Committee on Cancer (AJCC) Cancer Staging Manual and National Comprehensive Cancer Network (NCCN) guideline, respectively.

RNA extraction and quantitative real-time PCR (qRT-PCR)

The total RNA from cell or tissue was extracted with RNAiso Plus (TaKaRa, Otsu, Japan). Total RNA was reverse-transcribed using PrimeScript[®] RT reagent kit (TaKaRa). The qRT-PCR was performed with SYBR[®] Premix Ex Taq[™] II (TaKaRa) on a LightCycler[®] (Roche, Basel, Switzerland). The following primers were used for MCM2 and GAPDH: MCM2 forward, 5'-TGTCACCTGCTCTGCCACTAA-3' and reverse, 5'-GCAGCATGCGCAAGACTTT-3'; GAPDH forward, 5'-CCATCAATGACCCCTTCATTG-3' and reverse, 5'-CATGGGTGGAATCATATTGGAAC-3'. Relative expression was calculated via the comparative cycle threshold method and was normalized to the expression of GAPDH.

Immunohistochemistry

Immunohistochemical analysis was performed to detect MCM2 protein expression in LUAD tissues, LUSC tissues and normal lung tissues. In brief, paraffin-embedded sections

were deparaffinized in xylene for 20 min and rehydrated in graded concentrations of ethanol (100%, 95%, 90%, 80% and 70%). The sections were submerged in ethylene diamine tetraacetic acid antigenic retrieval buffer and microwaved for antigen retrieval. They were then treated with 3% hydrogen peroxide in methanol to quench endogenous peroxidase activity, followed by incubation with 5% bovine serum albumin to block nonspecific binding. Sections were incubated with anti-MCM2 (Abcam, Cambridge, UK) overnight at 4°C. After washing, tissue sections were treated with secondary antibody, followed by incubation with conjugated horseradish peroxidase–streptavidin. Tissue sections were then counterstained with hematoxylin, dehydrated and mounted.

Evaluation of staining

The tissue sections stained immunohistochemically for MCM2 were reviewed, and scored separately by two pathologists blinded to the clinical parameters. Any disagreements were arbitrated by a third pathologist. For MCM2 assessment, staining intensity was scored as 0=negative, 1=weak, 2=moderate or 3=strong, and staining extent was scored as 0=0%, 1=1%–25%, 2=26%–50%, 3=51%–75% or 4=70%–100%, depending on the percentage of positively stained cells.¹⁴ The sum of staining intensity and staining extent scores was used as final staining score. Low expression of MCM2 was defined as scores 0–4, and high expression was defined as scores >4.

Western blot

Total protein was extracted using radioimmunoprecipitation assay buffer (Thermo Fisher Scientific, Waltham, MA, USA) for Western blot. Equal amounts of protein were denatured and then separated by 10% SDS-PAGE. The target proteins were incubated with the following primary antibodies: MCM2 antibody (Abcam) or GAPDH antibody (CWBIO, Beijing, People's Republic of China). Then, the proteins were incubated with homologous secondary antibodies (CWBIO). For horseradish peroxidase detection, an enhanced chemiluminescent kit (CWBIO) was used. Intensity of blots was determined by Quantity One Software (Bio-Rad, Hercules, CA, USA).

Cell lines and cell cultures

Two human LUAD cell lines (A549 and H1299), two human LUSC cell lines (SK-MES-1 and H2170) and a human bronchial epithelial cell line (HBEC) were obtained from American Type Culture Collection and Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. A549,

H1299, SK-MES-1 and H2170 were cultured in Roswell Park Memorial Institute 1640 medium (Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific). HBEC was cultured in complete Keratinocyte-SFM (Thermo Fisher Scientific) supplemented with 5 mg/L EGF and 50 mg/L bovine pituitary extract. All cells were maintained at 37°C in a humidified atmosphere with 5% CO₂.

Cell transfection

The siRNA for downregulation of MCM2 was purchased from Guangzhou RiboBio Co., Ltd. (Guangzhou, People's Republic of China). Cells were transfected using Lipofectamine™ 3000 reagent (Thermo Fisher Scientific) in Opti-MEM (Gibco), according to the manufacturer's instructions. The transfection efficiency was tested by qRT-PCR and Western blot.

Cell proliferation and colony formation assays

Cell proliferation was analyzed using MTT assay and colony formation assay according to previous description.¹⁵ After transfected with plasmids and cultured in normal medium, cells were grown in a 96-well plate for 24, 48, 72, 96 and 120 h.

Cell cycle analysis

LUSC cells transfected with siRNA-MCM2 or siRNA-negative control (NC) were harvested after 48 h, rinsed with cold phosphate buffer saline and fixed with 70% ice-cold ethanol for 48 h at 4°C overnight. After incubation with PBS containing 10 mg/mL propidium iodide and 0.5 mg/mL RNase A for 15 min at 37°C, fixed cells were washed with cold PBS three times. FACSCalibur™ flow cytometry (BD Biosciences, Franklin Lakes, NJ, USA) was used to determine the DNA content of labeled cells.

Statistical analysis

All data were analyzed for statistical significance using SPSS 17.0 software and GraphPad Prism 5.0 software. The chi-square test was used to analyze the correlation between MCM2 expression and clinicopathological characteristics of lung cancer patients. Survival times were detected using the Kaplan–Meier survival curves, and compared by the log-rank test. The prognostic significance of various variables was analyzed by univariate and multivariate survival analysis using Cox's regression model. Two-tailed Student's *t*-test was used for comparisons of two independent groups. A *P*-value of <0.05 was considered statistically significant.

Results

Expression of MCM2 in LUSC tissue

We analyzed the microarray data (GSE3268) to screen and identify valuable biomarkers for LUSC, and found MCM2 expression was increased in LUSC tissues compared with paired adjacent normal lung tissues (Figure 1A). Meanwhile, MCM2 was overexpressed in LUSC tissue samples compared with LUAD tissue samples based on microarray data sets (GSE10245; Figure 1B). Furthermore, qRT-PCR and immunohistochemistry were performed to detect mRNA and protein expressions of MCM2 in LUAD tissues, LUSC tissues and normal lung tissues. Compared with normal lung tissues or LUAD tissues, MCM2 mRNA expression was significantly elevated in LUSC tissues (both $P < 0.001$; Figure 1C). Meanwhile, immunohistochemical analysis

(Table 1 and Figure 2A–L) showed MCM2 protein expression was increased in LUSC tissues (52.4%, 66/126) in comparison to normal lung tissues (16.1%, 5/31, $P < 0.001$) and LUAD tissues (38.1%, 32/84, $P = 0.042$).

Expression of MCM2 in LUSC cells

MCM2 mRNA and protein levels were detected in two human LUAD cell lines (A549 and H1299), two human LUSC cell lines (SK-MES-1 and H2170) and a HBEC through qRT-PCR and Western blot. Levels of MCM2 mRNA were increased in LUSC cells compared with bronchial epithelial cells or LUAD cells (both $P < 0.001$; Figure 1D). Meanwhile, we observed that MCM2 protein level was increased in LUSC cells compared with bronchial epithelial cells or LUAD cells (Figure 1E).

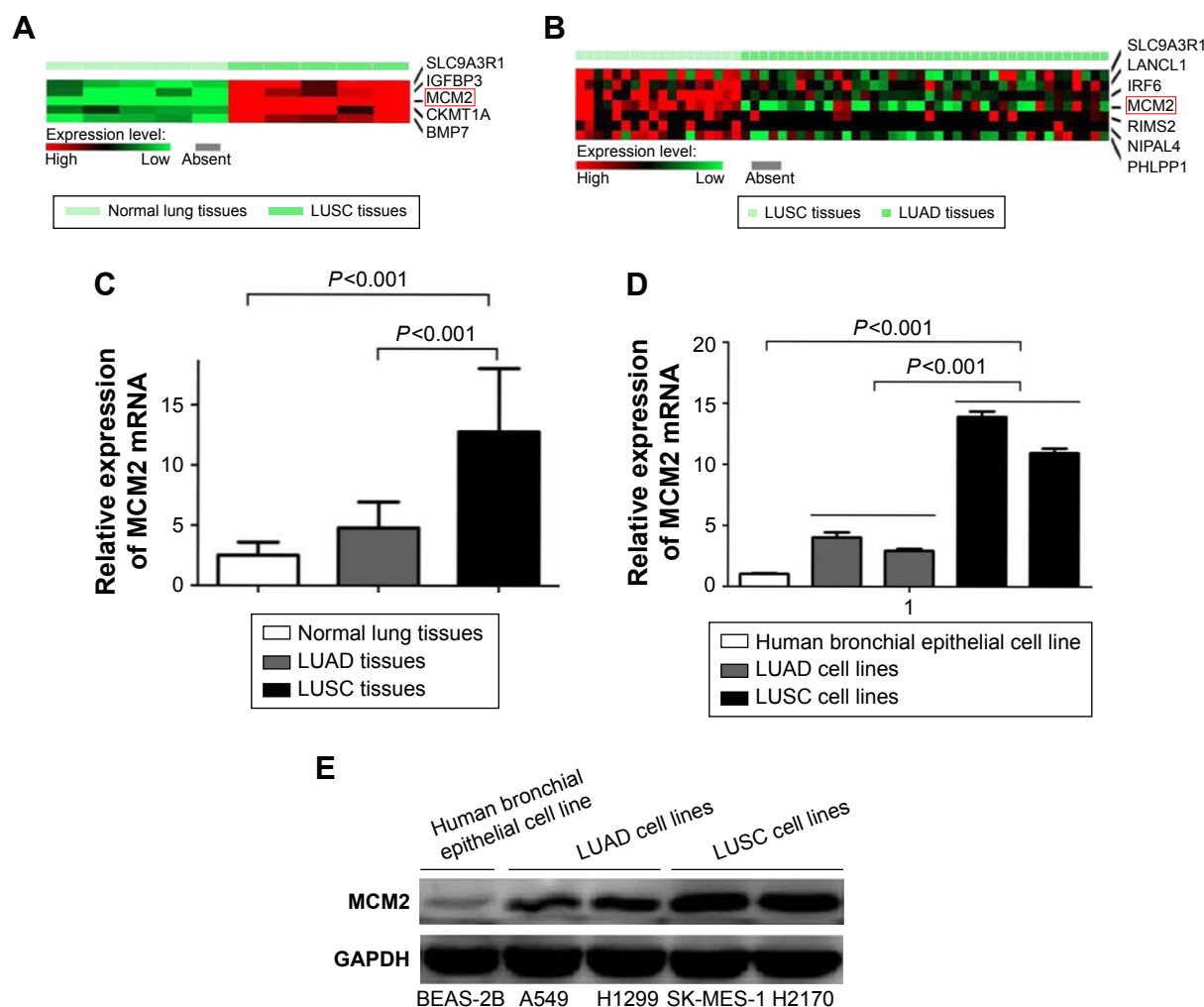


Figure 1 MCM2 is overexpressed in LUSC.

Notes: (A) MCM2 overexpression is observed in LUSC tissues compared with normal lung tissues (GEO accession number: GSE3268). (B) MCM2 expression is elevated in LUSC tissues than those in LUAD tissues (GEO accession number: GSE10245). (C) High expression of MCM2 mRNA is observed in LUSC tissues compared with normal lung tissues or LUAD tissues. (D) MCM2 mRNA expression is increased in LUSC cells than those in bronchial epithelial cells or LUAD cells. (E) MCM2 protein is overexpressed in LUSC cells compared with bronchial epithelial cells or LUAD cells.

Abbreviations: GEO, Gene Expression Omnibus; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MCM2, minichromosome maintenance protein 2.

Table 1 Expression of MCM2 protein between lung cancer tissues and normal lung tissues

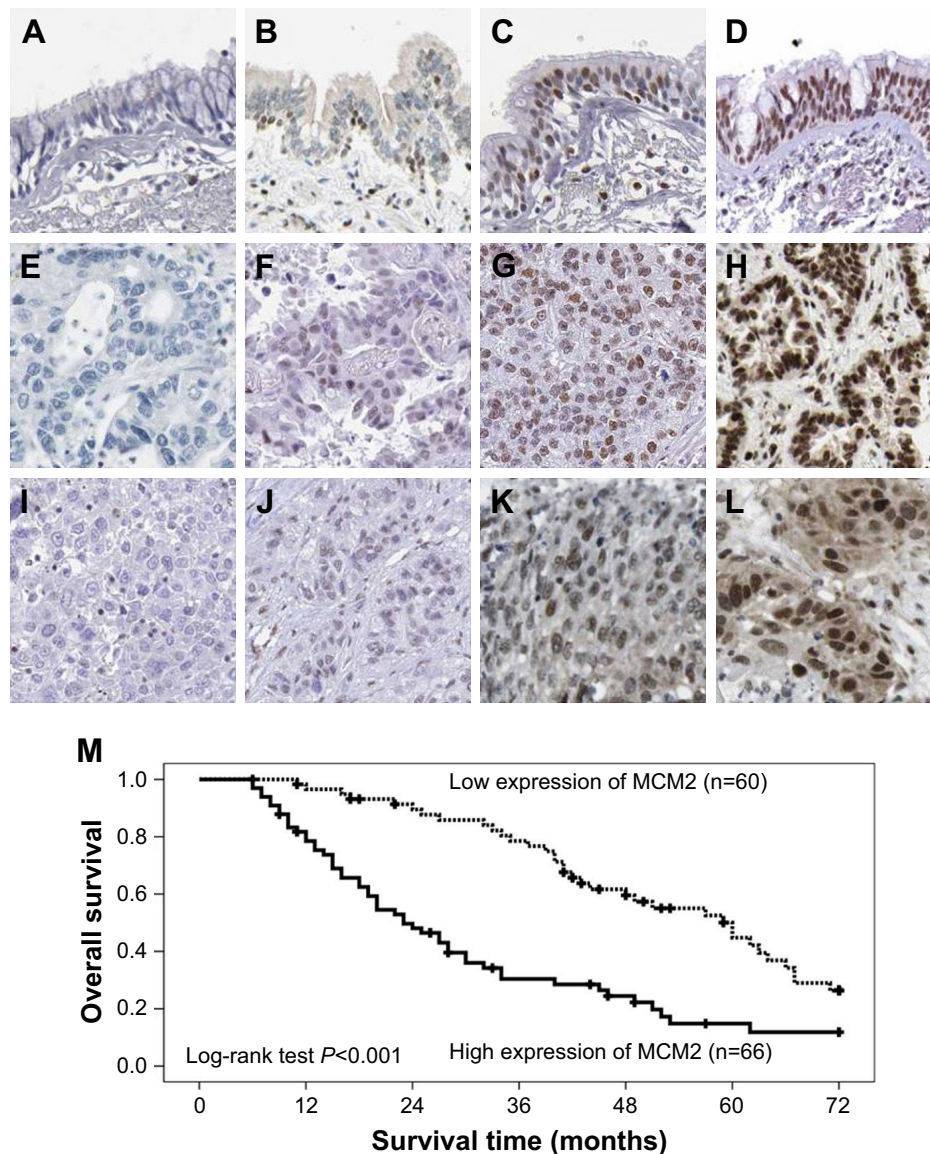
| Group | Cases | MCM2 | | P-value |
|---------------------|-------|---------------------|--------------------|---------|
| | | High expression (%) | Low expression (%) | |
| Normal lung tissues | 31 | 5 (16.1) | 26 (83.9) | <0.001* |
| LUAD tissues | 84 | 32 (38.1) | 52 (61.9) | 0.042* |
| LUSC tissues | 126 | 66 (52.4) | 60 (47.6) | |

Note: *Compared with LUSC tissues.

Abbreviations: LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MCM2, minichromosome maintenance protein 2.

MCM2 overexpression correlates with the malignant status of LUSC patients

We measured MCM2 protein expression in LUAD tissue, LUSC tissue and normal lung tissue by using immuno-histochemical staining (Figure 2A–L), and explored the association between MCM2 protein expression and clinico-pathological characteristics of LUSC patients. As summarized in Table 2, MCM2 protein expression was obviously associated with differentiated degree (high and middle vs low; $P=0.003$), clinical stage (I–II vs III–IV; $P<0.001$), tumor

**Figure 2** Immunohistochemical staining of MCM2 in lung cancer clinical samples.

Notes: (A) Negative expression of MCM2 in normal bronchial epithelial cells. (B) Weak expression of MCM2 in normal bronchial epithelial cells. (C) Moderate expression of MCM2 in normal bronchial epithelial cells. (D) Strong expression of MCM2 in normal bronchial epithelial cells. (E) Negative expression of MCM2 in LUAD cells. (F) Weak expression of MCM2 in LUAD cells. (G) Moderate expression of MCM2 in LUAD cells. (H) Strong expression of MCM2 in LUAD cells. (I) Negative expression of MCM2 in LUSC cells. (J) Weak expression of MCM2 in LUSC cells. (K) Moderate expression of MCM2 in LUSC cells. (L) Strong expression of MCM2 in LUSC cells. (M) High expression of MCM2 is negatively correlated with overall survival in LUSC patients from our study.

Abbreviations: LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MCM2, minichromosome maintenance protein 2.

Table 2 Correlation between clinicopathological characteristics and expression of MCM2 protein in LUSC patients

| Characteristics | n | MCM2 | | P-value |
|-----------------------|-----|---------------------|--------------------|---------|
| | | High expression (%) | Low expression (%) | |
| Gender | | | | |
| Female | 43 | 18 (41.9) | 25 (58.1) | 0.089 |
| Male | 83 | 48 (57.8) | 35 (42.2) | |
| Age (years) | | | | |
| ≤50 | 51 | 26 (51.0) | 25 (49.0) | 0.795 |
| >50 | 75 | 40 (53.3) | 35 (46.7) | |
| Smoking | | | | |
| No | 67 | 36 (53.7) | 31 (46.3) | 0.746 |
| Yes | 59 | 30 (50.8) | 29 (49.2) | |
| Differentiated degree | | | | |
| High and middle | 73 | 30 (41.1) | 43 (58.9) | 0.003 |
| Low | 53 | 36 (67.9) | 17 (32.1) | |
| Clinical stage | | | | |
| I–II | 54 | 14 (25.9) | 40 (74.1) | <0.001 |
| III–IV | 72 | 52 (72.2) | 20 (27.8) | |
| Tumor size | | | | |
| ≤5 cm | 76 | 34 (44.7) | 42 (55.3) | 0.034 |
| >5 cm | 50 | 32 (64.0) | 18 (36.0) | |
| Lymph node metastasis | | | | |
| N0–N1 | 58 | 17 (29.3) | 41 (70.7) | <0.001 |
| N2–N3 | 68 | 49 (72.1) | 19 (27.9) | |
| Distant metastasis | | | | |
| No | 111 | 52 (46.8) | 59 (53.2) | 0.001 |
| Yes | 15 | 14 (93.3) | 1 (6.7) | |

Abbreviations: LUSC, lung squamous cell carcinoma; MCM2, minichromosome maintenance protein 2.

size (≤5 cm vs >5 cm; $P=0.034$), lymph node metastasis (N0–N1 vs N2–N3; $P<0.001$) and distant metastasis (No vs Yes; $P=0.001$). However, MCM2 protein expression was not associated with gender, age and smoking.

MCM2 overexpression is an independent poor predictor for LUSC patients

According to LUSC patients' prognosis information, we analyzed the relationship between MCM2 expression and patients' survival. We observed that patients with higher levels of MCM2 expression had poorer survival than those with lower levels of MCM2 expression ($P<0.001$; Figure 2M), as MCM2 overexpression level predicted overall survival. Univariate and multivariate analyses suggested that MCM2 overexpression was an independent unfavorable prognostic factor for LUSC patients (HR=1.847, 95% CI: 1.080–3.158, $P=0.025$; Table 3).

Downregulation of MCM2 suppresses LUSC cell proliferation

To explore the biological function of MCM2 in LUSC cells, we induced downregulation of MCM2 in SK-MES-1 and H2170 cells by transfecting siRNA-MCM2, and the results were confirmed by qRT-PCR and Western blot (Figure 3A and B). The results of MTT assay showed that downregulation of MCM2 obviously decreased SK-MES-1 and H2170

Table 3 Summary of univariate and multivariate Cox regression analyses of overall survival duration

| Parameters | Univariate analysis | | | Multivariate analysis | | |
|---|---------------------|-------|-------------|-----------------------|-------|-------------|
| | P-value | HR | 95% CI | P-value | HR | 95% CI |
| Gender | | | | | | |
| Female vs male | 0.194 | 1.363 | 0.855–2.173 | | | |
| Age (years) | | | | | | |
| ≤50 vs >50 | 0.579 | 0.886 | 0.577–1.360 | | | |
| Smoking | | | | | | |
| No vs yes | 0.833 | 1.947 | 0.684–1.602 | | | |
| Differentiated degree | | | | | | |
| High and middle vs low and undifferentiated | 0.894 | 0.971 | 0.629–1.498 | | | |
| Clinical stage | | | | | | |
| I–II vs III–IV | <0.001 | 2.573 | 1.622–4.081 | 0.809 | 0.834 | 0.191–3.632 |
| Tumor size | | | | | | |
| ≤5 cm vs >5 cm | 0.185 | 1.348 | 0.867–2.096 | | | |
| Lymph node metastasis | | | | | | |
| N0–N1 vs N2–N3 | <0.001 | 2.675 | 1.699–4.213 | 0.325 | 2.046 | 0.491–8.524 |
| Distant metastasis | | | | | | |
| No vs yes | <0.001 | 4.702 | 2.463–8.976 | 0.004 | 2.046 | 0.491–8.524 |
| MCM2 expression | | | | | | |
| Low vs high | <0.001 | 2.706 | 1.743–4.202 | 0.025 | 1.847 | 1.080–3.158 |

Abbreviation: MCM2, minichromosome maintenance protein 2.

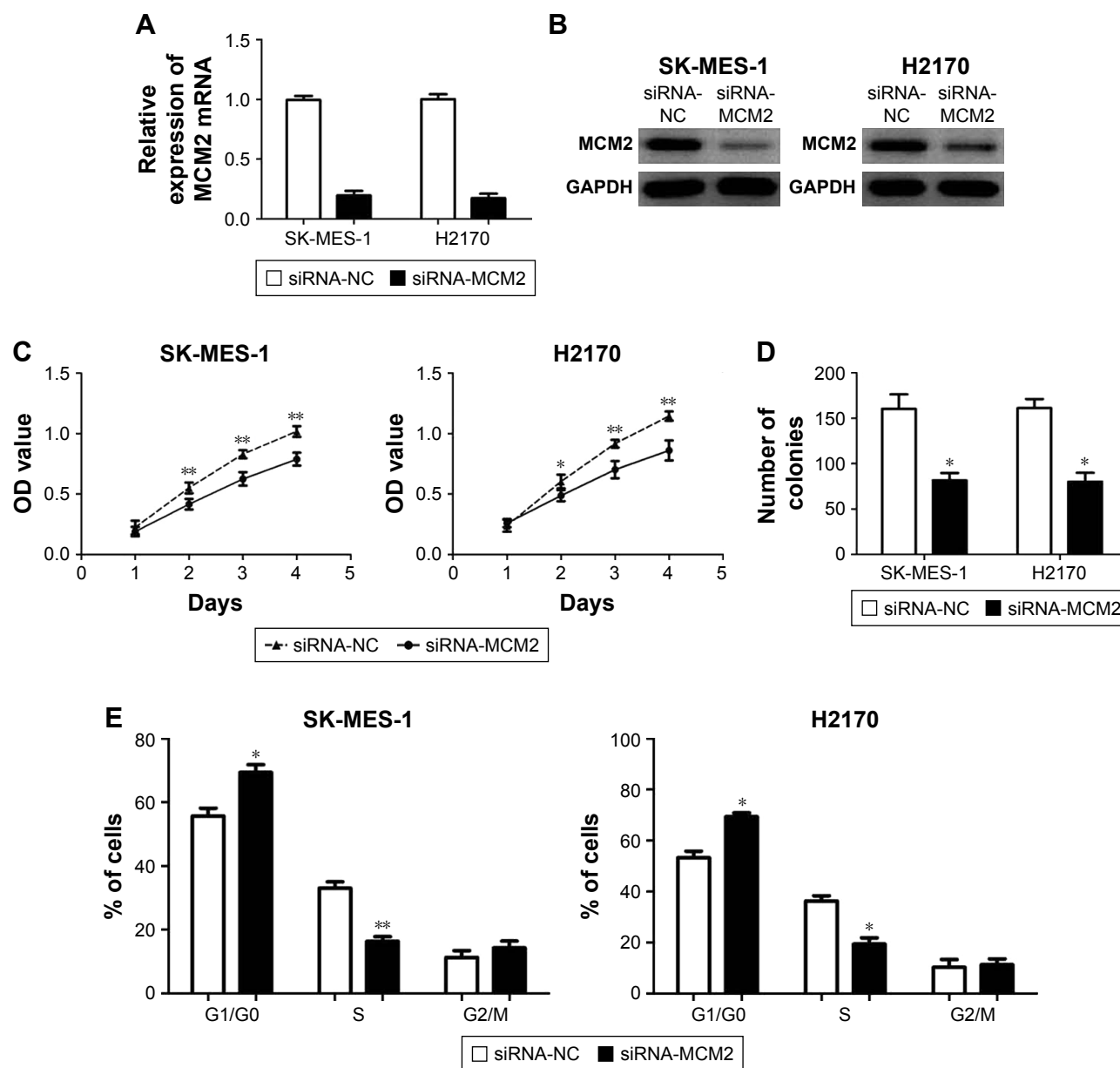


Figure 3 The biological function of MCM2 in LUSC cells.

Notes: (A) The efficiency of siRNA-MCM2 is confirmed by qRT-PCR in LUSC cells. (B) The efficiency of siRNA-MCM2 is confirmed by Western blot in LUSC cells. (C) Downregulation of MCM2 decreases SK-MES-1 and H2170 viability in comparison to control cells after transfection for 48, 72 and 96 h. (D) Downregulation of MCM2 reduces the number of colonies in SK-MES-1 and H2170 cells. (E) Downregulation of MCM2 decreases the percentage of S-phase cells and increases the percentage of G0/G1-phase cells. Each experiment was performed in triplicate (* $P < 0.05$; ** $P < 0.001$).

Abbreviations: LUSC, lung squamous cell carcinoma; MCM2, minichromosome maintenance protein 2; qRT-PCR, quantitative real-time PCR; NC, negative control.

viability in comparison to control cells after transfection for 48, 72 and 96 h (all $P < 0.05$; Figure 3C). The colony formation assay suggested that downregulation of MCM2 markedly reduced the number of colonies in SK-MES-1 and H2170 cells over a 12-day period (both $P < 0.05$; Figure 3D). The analysis of cell cycle distribution showed that downregulation of MCM2 obviously decreased the percentage of S-phase cells and increased the percentage of G0/G1-phase

cells, suggesting that downregulation of MCM2 might cause a G0/G1- to S-phase arrest in SK-MES-1 and H2170 cells (all $P < 0.05$; Figure 3E).

Discussion

MCM2, which is a member of the MCM family, has been found to be a relevant marker for progression and prognosis in a variety of human cancers.¹³ Primarily, MCM2 has

been considered as a sensitive marker for early detection of pulmonary malignant lesions. Tan et al reported that MCM2 was detectable in two to three times more proliferating premalignant lung cells than Ki-67 index, and acted as an easy-to-use marker for the early detection of lung cancer which may significantly enhance lung cancer survival.¹⁶ Furthermore, Muñoz-Antonia et al showed progression of premalignant lesions toward carcinoma in situ was accompanied by an increase in the expression of MCM2.¹⁷ Subsequently, Dehan et al and Kadara et al congruently suggested that MCM2 was significantly upregulated in non-small-cell lung cancer through microarrays.^{18,19} In our study, we analyzed microarray data sets (GSE3268 and GSE10245), and found MCM2 expression was increased in LUSC tissues compared with paired adjacent normal lung tissues and LUAD tissues. Furthermore, we confirmed that MCM2 mRNA and protein expressions were increased in LUSC tissues and cell lines compared with normal lung tissues and LUAD tissues, or bronchial epithelial cell line or LUAD cell lines. In addition, high expression of MCM2 was observed in several types of human cancers such as breast cancer,^{20,21} gastric cancer,^{22,23} colorectal cancer,^{24,25} esophageal cancer,²⁶ liver cancer,^{27,28} renal cell carcinoma²⁹ and so on.

Due to lack of effective therapeutic target in LUSC patients, we supposed MCM2 might act as an important biomarker for LUSC patients based on the expression of MCM2 in LUSC tissues. We analyzed the association between MCM2 protein expression and clinicopathological characteristics of LUSC patients, and found high expression of MCM2 protein was obviously associated with malign differentiated degree, advanced clinical stage, large tumor size, more lymph node metastasis and present distant metastasis. Similarly, Veena et al found MCM2 proteins have significant association with tumor stage in lung cancer patients,³⁰ and Liu et al reported that high expression of MCM2 in non-small cell lung cancer was observed more frequently in aged patients and in patients at later stage.³¹ Moreover, MCM2 expression positivity significantly increased with increasing tumor grade, the presence of residual disease and advancing tumor stage in ovarian cancer patients.³² In esophageal squamous cell carcinoma patients, Kato et al demonstrated that there were significant associations between MCM2 expression and tumor status, lymph node status, metastatic status, pathological stage and histological grade.³³ On the whole, high expression of MCM2 is associated with aggressive progression in most types of human cancers.

In the last 10 years, the prognostic significance of MCM2 has been explored in various types of human cancers. Generally, high expression of MCM2 predicts a

poor prognosis in most types of human cancers such as gastric cancer,²² osteosarcoma,³⁴ oral cavity squamocellular carcinoma,³⁵ bladder cancer,³⁶ prostate cancer,³⁷ breast cancer,³⁸ renal cell cancer^{39,40} and penile cancer.⁴¹ In non-small-cell lung cancer, Liu et al,³¹ Ramnath et al,⁴² Hashimoto et al⁴³ and Cheung et al⁴⁴ consistently revealed MCM2 overexpression was associated with unfavorable prognosis. However, Werynska et al reported no significant differences in overall survival related to the expression of MCM-2 in non-small-cell lung cancer patients.⁴⁵ In our study, we presented more evidence suggesting that MCM2 expression was significantly associated with poor overall survival of LUSC patients, and MCM2 overexpression was an independent unfavorable prognostic factor for LUSC patients. However, Nishihara et al showed that no correlation was observed between the MCM2 expression and cumulative survival in colorectal cancer patients.⁴⁶ Similarly, MCM2 expression has been indicated to lack prognostic significance in medulloblastomas and myxofibrosarcomas.^{47,48}

In order to explore the biological function of MCM2 in LUSC cells, we performed loss-of-function study to explore the effect of MCM2 on LUSC cell line. In our study, we found downregulation of MCM2 obviously decreased LUSC cell proliferation, and also decreased the percentage of S-phase cells and increased the percentage of G0/G1-phase cells. Similarly, Cheung et al showed MCM2 promoted lung cancer cell proliferation via the regulation of HMGA1 phosphorylation.⁴⁴ Moreover, Zhang et al confirmed that MCM2 was a therapeutic target of lovastatin in suppressing lung cancer cell viability.⁴⁹ Meanwhile, Zhang et al found that downregulation of MCM2 expression increased Rb, cyclin D1 and CDK4 expression, and decreased p53 and p21 expression, which suggested that MCM2 triggered the arrest of G1/S or G2/M through the induction of cyclin-dependent kinase inhibitor p21cip14 and cyclin D/CDK4 cyclin D/CDK4.⁴⁹ Besides, MCM2 has been found to act as a functional target for lncRNA and microRNA in regulating tumor cell proliferation, migration and apoptosis.⁵⁰⁻⁵²

Conclusion

In summary, MCM2 is significantly increased in LUSC tissues and cell lines, and correlates with the malignant status and prognosis of LUSC patients. MCM2 functions as an oncogene in regulating LUSC cell proliferation and cell cycle distribution.

Disclosure

The authors report no conflicts of interest in this work.

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