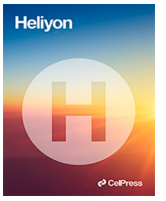
Heliyon 10 (2024) e29933

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

FAM83B promotes cell proliferation via regulating the expression of CDK4/CDK6/CCND1 complex in laryngeal squamous cell carcinoma

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ARTICLE INFO

Keywords:
Laryngeal squamous cell carcinoma
FAM83B
Cell proliferation
Cell cycle
CDK4/CDK6/CCND1

ABSTRACT

FAM83B, as one of the FAM83 family members, has been closely involved in cell transformation, and a growing number of scholars have been studied its role in tumours over the years. Whereas the effect and potential mechanism of FAM83B in laryngeal squamous cell carcinoma (LSCC) have not been investigated. In this research, we discovered that the expression quantity of FAM83B was remarkably higher in LSCC tissues (79.65 ± 35.98) than in matched adjacent tissues (59.34 ± 32.59) by tissue microarrays and immunohistochemistry. Furthermore, expression of FAM83B was knocked down in HEP-2 and TU177 cell lines via lentivirus, and in the course of intracorporal and extracorporeal experiments, FAM83B knockdown showed the inhibition of tumour growth, migration, and invasion ability. Moreover, cell cycle assay showed that FAM83B knockdown leads to an apparent accumulation of cells in the G1 phase, indicating that FAM83B knockdown can inhibit cell proliferation. Meanwhile, western blotting (WB) demonstrated that FAM83B knockdown led to a significant reduction in CDK4/CDK6/CCND1 protein expression, which may have decelerated cell cycle progression. Collectively, this study demonstrates that FAM83B serves as an oncogene in LSCC, promoting cell proliferation by controlling the protein expression of CDK4, CDK6, and CCND1, thus inducing a transference of the G1 stage to S stage in cell-cycle of LSCC cells. These results provide an academic foundation for elucidating the mechanism of LSCC occurrence and evolution and for developing treatment strategies for LSCC.

1. Introduction

Cancer is one of the primary reasons of global death rate. Laryngeal cancer is the sixth most frequent cancer on earth, in oto-rhinolaryngeal area, laryngeal cancer accounts for 5.7–7.6 % of systemic malignant tumours and 7.9–35 % of malignant tumours [1]. It is a malignant tumor originating in the mucous epidermic texture of the larynx, and accounts for 2.4 % of the annual increase in

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https://doi.org/10.1016/j.heliyon.2024.e29933
Received 27 December 2023; Received in revised form 17 April 2024; Accepted 17 April 2024
Available online 23 April 2024

malignant tumours in the world. Laryngeal squamous cell carcinoma (LSCC) is the most frequent type of laryngocarcinoma, which accounts for 93–99 % in all forms [2]. At present, employing combined therapy to cure LSCC, surgical operation is the prime intervening measure and radiation treatment, chemotherapeutics and immunization therapy are auxiliary interventions [3,4]. Currently, LSCC lacks specific therapeutic targets; thus identifying alterant pathways and new remedial target spots are vital to enhance the management of a good chunk of the population with LSCC.

Family with sequence similarity 83, member B (FAM83B) has been implicated in tumour development [5]. Located at 6p12.1, the FAM83B gene is 3035 bp, and the protein comprises 1011 amino acids and its formula weight is 111 KD. It is a signal protein belonging to the FAM83 family, which has eight members [6]. All eight members contain the highly conserved DUF1669 domain, which plays an important role in mediating tumourigenic transformation [7–10]. According to multiple literatures reported, the DUF1669 domain directly bind to and promote the localisation of CRAF and enhance the downstream mitogen activated protein kinase (MAPK) signal [6, 11]. However, there are only a few studies on the expression of FAM83B in tumours, and the role of this gene in LSCC remains unexplored.

Therefore, in this study, we established LSCC stable cell strains with knockdown of FAM83B and utilized them to investigate the function of FAM83B in the multiplication, invasiveness, and migration ability of LSCC cells using MTT, colony formation, and Transwell assays. The impact of FAM83B knockdown on the cell cycle about LSCC cells was examined by using flow cytometry. Changes in the cell proliferation marker CDK4/CDK6/CCND1 after FAM83B knockdown were examined using RT-PCR and WB. In this study, we were designed to study the expression of FAM83B in LSCC, and the effect on cell proliferation, invasion and migration, elucidating its mechanism for the first time. The study is expected to supply discernments of the mechanisms governing the emergence and progression of LSCC and thereby aid in developing clinical strategies and potential therapeutic targets for the preclusion and therapy of LSCC.

2. Materials and methods

2.1. Cell culture

LSCC cell strains were acquired from the Medicine Research Center, Shanxi Provincial People's Hospital Affiliated to Shanxi Medical University (Taiyuan, China) and cultivated in nutrient medium, which included 10 % fetal calf serum (FCS; bought from HyClone, Logan, UT, USA), the temperature condition is 37 °C and the gas condition is 5 % CO_2. The culture solution generally replaced every 2–3 days; when the cells showed 80–90 % confluency that performed cell subculture, and in the subsequent experiment phase, we used cell in the logarithmic growing period.

2.2. Ethics statement

All assays were authorized by the ethics committee of Shanxi Provincial People's Hospital Affiliated to Shanxi Medical University (2019-164). All samples were collected before treatment according to the guidelines of the native ethics board, and also gained the informed and consented forms of each participator.

2.3. Tissue microarrays (TMAs) and immunohistochemistry (IHC)

TMAs were prepared using 30 LSCC tumour textures and 30 paired nontumorous textures received from Shanxi Provincial People's Hospital that it from 2020 to 2021. The FAM83B protein expression was detected by IHC. The TMA slices were firstly deparaffinised with xylene, then used a range of grades of alcohol solution to rehydrated and put them in 3 % H_2O_2 solution, the time is 15 min, the slices were put in sodium citrate solution (pH 6.0) in an auto rice cooker for 2 min for antigen retrieval. After let them cool for half-hour and then harnessed phosphate-buffered saline (PBS) to purge slices, in the next moment, we added monoclonal antibody on the slice tissues for incubation, the temperature condition is 37 °C and the time is 6o minutes. Next up the TMA slices were needed to soak in the secondary antibody. Sections were dyed with DAB and haematoxylin successively. The expression quantity and localisation of FAM83B were analysed using the software. The FAM83B protein expression was reckoned in line with the entire dyeing percentage and membrane strength of the target antibody.

2.4. Plasmid constructs and transfection

Plasmid constructs and transfection methods were used to achieve the knockdown of FAM83B expression in LSCC cells. For knocking down endogenous FAM83B, vectors containing two different shRNAs targeting FAM83B (named FAM83B shRNA1 and FAM83B shRNA2) and scramble control sequence as negative control (named NC) were used. These shRNAs were cloned into the vector pLKO.1-puro-FAM83B–shRNA and co-transfected into 293T cells with packaging plasmids. Used lentivirus supernatant to infect HEP-2 and TU177 cell strains. The negative control was corresponding empty vectors. Stable cell strains were coped with 2 μg/ml puromycin (Invitrogen) for two weeks to screen efficient cell. The knockdown efficiency of gene was examined employing RT-qPCR and WB tests.

2.5. MTT assay

MTT assay was utilized to assess cell growth. LSCC cells (the quantity is five thousand) were placed in 96-pore boards in a final culture medium volume of 200 μl/well and incubated under normal conditions for 1, 2, 3, 4 and 5 days. Thereafter, MTT (5 μg/μl, Invitrogen) was put in every hole and the volume is 20 μl. Then put boards into the shaker for 4 h, abandoned MTT liquid and placed DMSO liquid in every hole, the later volume is 200 μl and the plate was swung to dissolve the bottom precipitate in DMSO liquid. We made use of a tecan sunrise to gauge Absorbance at 490 nm using (BioTek Instruments, Inc, Winooski, VT, USA). We carried out at least three independent tests, with five duplicates for each group in each individual experiment.

2.6. Colony-formation assay

The cell colony-forming capacity was assessed using clone formation assays. Cells were placed into 6- pore boards and its density is 1500 cells/hole with 2.5 ml culture solution. After 14–20 days, 1 ml of 4 % formaldehyde was added to each well to immobilize the cell clones. These boards were then dyed harnessing crystal violet (the concentration is 0.1 %). Clones containing approximately 50 cells, observed under a microscope, were considered as meeting the minimum clone standard. Subsequently, we utilized Image J software to count all clones.

2.7. Transwell experiments

Migration and invasion experiments were executed by using 24-pore boards and cabinets (8 μm; Corning, Inc). Briefly, 5×10^4 cells were placed into every hole with basal culture medium inside the chambers, the difference is invasion test possessed Matrigel, but migration test did not have. And the ratio of Matrigel and serum-free medium was 1:6. Medium with 10 % FBS was put into the plates, which located the below the chamber. After 24 h of culture, cells in the chamber passed through the membrane into the lower chamber were immobilized with 4 % paraformaldehyde and dyed employing crystal violet (the concentration is 0.1 %), finally we tallied cells by employing a microscope (Olympus Corporation, Tokyo, Japan).

2.8. Live cell imaging

We monitored cell division cycle by using viable cell formation of image technology. Viable cell formation of image technology was carried out as anteriorly described by Hu et al. [11]. Briefly, LSCC cells (HEP-2NC, HEP-2FAM83BshRNA, TU177NC, and TU177FAM83BshRNA) were seeded in chamber overnight at a density of 4×10^3 cells/hole. Acquired pictures automatically at many positions on the covering glass utilizing a microscope, it contains a linearly-encoded stage and camera. A mercury spectrum-lamp with two neutral thickness rejectors was served for using fluorescence to illuminate. Fluorescence and differential interference contrast pictures were received every 5 min and lasted 60 h totally.

2.9. Cell cycle assay by flow cytometry

Cells were gained and the density is a million cells of 1 ml, then we cleaned the cell pellet twice with PBS solution. During cell-cycle experiment, the sediment was cleaned with PBS solution and immobilized in microthermal 70 % grain alcohol for 4 h, the temperature condition is 4 °C. The sediment was centrifuged and discarded the liquid, then disposed cells though using nuclease-free water, the time is 10 min and the temperature is 37 °C. All DNA in cells was dyed with 0.05 % propidium iodide (PI), the time of duration is 20 min and put it on ice in the darkness. We next put the cells in the machine and the cells were analysed utilizing a flow cytometry with an application program. Three independent experiments were carried out.

2.10. Mouse xenograft assay

Mouse xenograft assay was employed to detect the influence of FAM83B on tumours in vivo. The influence of FAM83B on tumourigenesis in vivo was assessed through mouse xenograft experiment. Briefly, we injected five million cells into subdermal axilla of female BALB/c nude mice aged two months (sourced from Vital River Laboratory Animal Technology Co, Ltd, Beijing, China). The dimension of the tumour was measured using calipers omnibus diebus quaternis. Following a period of five weeks, these mice models were sacrificed under anaesthesia, and the tumours were extracted for measurement.

2.11. Statistical analyses

Data analysis was conducted with the SPSS 21.0 program. trials were replicated three times, and results are shown as the mean ± standard deviations (SD). The statistical analysis took into account the normal distribution of the data. Data from carcinoma and adjacent tissue, which did not exhibit normal distribution, was subjected to statistical analysis using a two-tailed rank sum test. Data from two or more groups that followed a normal distribution, which were analysed utilizing Student's *t*-test and analysis of variance (ANOVA), with the Student–Newman–Keuls method as applied a post-hoc test. A P value of <0.05 was identified as statistically significant.

3. Results

3.1. FAM83B expression is up-regulated in LSCC tissues

FAM83B protein expression quantity was tested in 30 pairs of laryngeal carcinoma textures and paired adjacent nontumorous textures via TMA-immunohistochemistry approach. FAM83B dyeing was viewed in the protoplasm and karyon of tumour and non-tumour textures. Notably, the expression of FAM83B in nontumorous textures was remarkably lower than the gene in tumour textures in the cytoplasm and nucleus ($P < 0.01$) (Fig. 1A and B and table S1). The demographic information of patients with LSCC from whom samples were collected is presented in table S2.

3.2. Knockdown of FAM83B in LSCC cells

The knockdown efficiency of FAM83B was verified by performing qPCR and WB assays. HEP-2 and TU177 cell lines were used for the knockdown experiment. These two cell lines have been widely utilized in research related to LSCC. The transfection efficiency of HEP-2 and TU177 cells was more than 90 %. qPCR was used to assess FAM83B mRNA expression quantity and WB was applied to detect the protein expression quantity of FAM83B in LSCC stable cell strains. The experiment data indicated that the knockdown efficiency of FAM83B was more than 70 % (Fig. 2 and fig. S1A).

3.3. Decreased FAM83B expression inhibits HEP-2 and TU177 cell proliferation

FAM83B knockdown dramatically restrained cell growth and colony-formation capacity in both HEP-2 and TU177 cell lines (Fig. 3A and B and table S3). Furthermore, live cell imagingassays manifested that FAM83B knockdown inhibited the rate of cell growth and lengthened the cell karyokinesis cycle (Fig. 3C). Live cell imaging enabled the study of real-time dynamic processes within cells, offering spatial and temporal information on molecular events that occur during cell growth [12,13]. In this study, we utilized live cell imaging technology to calculate the time required for one complete cell division, known as the cell mitotic cycle. Any extension of the cell mitotic cycle duration indicates a reduction in the rate of cell proliferation. The results demonstrated that knockdown FAM83B restrained cell proliferation, suggesting that FAM83B probably is a carcinogenic gene in tumourigenesis of LSCC.

3.4. FAM83B knockdown inhibits cell migration and invasion in LSCC

Transwell assays confirmed that FAM83B had an influence on migration ability and invasiveness in HEP-2 and TU177 cell strains. The outcomes indicated that FAM83B knockdown brought about a noteworthy decrease in terms of cell migration and invasion capacities in the HEP-2 and TU177 cell strains (Fig. 4A and B and table S3). The above discoveries show that FAM83B can promote cell migration ability and invasiveness in LSCC.

3.5. FAM83B knockdown deregulates the cell cycle

Flow cytometry was utilized to analyse the cell-cycle distribution. Compared with the NC group, FAM83B knockdown of HEP-2 and TU177 cell strains showed that the number of cells increased in G1 phase and the number of cells decreased in S phase. This result demonstrates that FAM83B knockdown results in cells in the G1 phase had a notable accumulation, indicating cell proliferation inhibition, which is consistent with the above results. This suggests that FAM83B promotes cell growth through promoting the

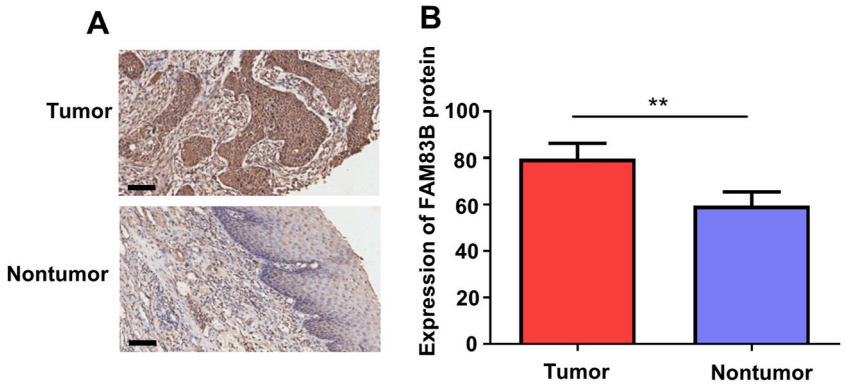

Fig. 1. Expression of FAM83B in tumour textures were in comparison with that in nontumorous textures. **(A)** Representative immunohistochemistry images of FAM83B staining in tumour and nontumorous textures. **(B)** Analysis of FAM83B protein expression quantity on account of texture microarray data. FAM83B staining in the protoplasm and karyon of tumour and nontumorous textures. FAM83B expression in the cytoplasm and nucleus in non-tumour tissues is remarkably lower than the gene in tumour textures (**$p < 0.01$).

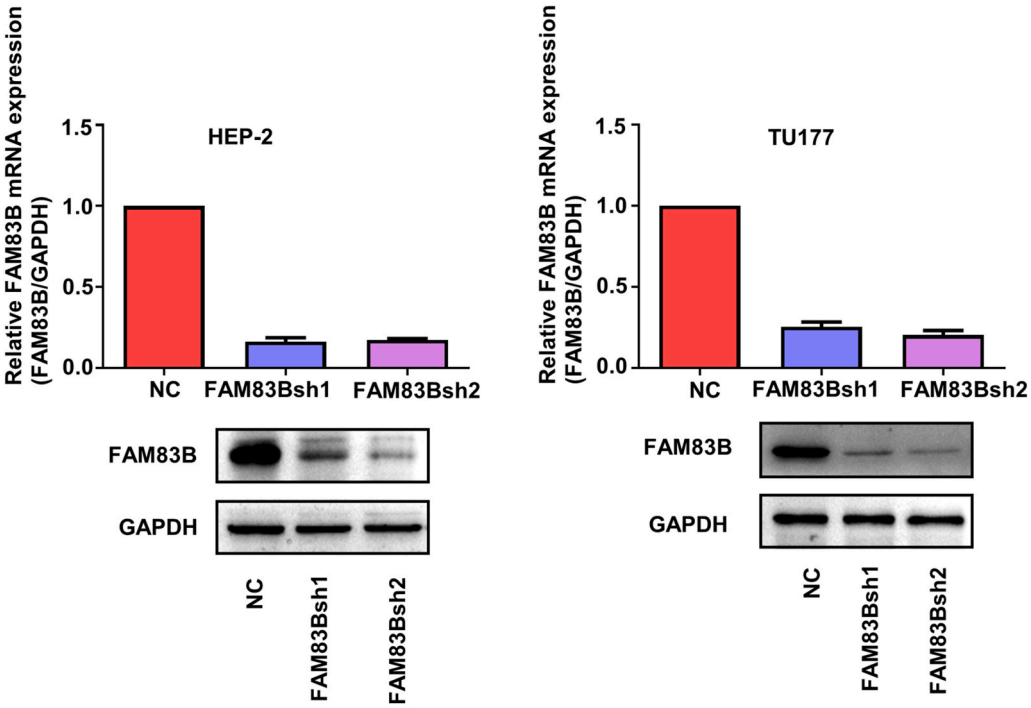

Fig. 2. Knockdown efficiency of FAM83B in HEP-2 and TU177 cell lines was tested by performing qPCR and WB assays. GAPDH was utilized as the control. The experiment data show that the efficiency of knockdown is more than 70 %.

transformation from G1 phase to S phase (Fig. 5A and B and table S3).

3.6. FAM83B knockdown downregulates expression of CDK4/CDK6/CCND1

To identify the potential mechanism the influence of FAM83B on cell growth, we tested the variations in the expression quantity of the cell cycle regulatory proteins cyclin-dependent kinase 4/6 (CDK4/CDK6) and cyclin D1 (CCND1). CCND1 and CDK4/6 are the main mitogens in the G1 phase, playing crucial roles in cell division. Downregulation of CCND1 and CDK4/6 can consequently lead to inhibition of G0/G1 transition [14].The knockdown of FAM83B apparently decreased the level of CDK4/CDK6/CCND1 in HEP-2 and TU177 cell strains. These outcomes suggest that FAM83B may change the growth rate of cells by affecting the expression of CDK4/CDK6/CCND1 (Fig. 6A and B and fig. S1B).

3.7. FAM83B promotes tumour growth in vivo

To further determine the role of tumour promotion of FAM83B in LSCC, 5×10^6 NC or HEP-2cells with stable FAM83B-knockdown were injected into subdermal axilla of female BALB/c nude mice. Two weeks later, the FAM83B knockdown group showed remarkably inhibited tumour growth compared with control subjects (Fig. 7A and B). The average weight of the tumours in the control group was 0.852 g, while that in the knockdown group was 0.378 g (Fig. 7B and table S4). In addition, immunohistochemical analysis of Ki-67 substantiated the noteworthy growth inhibition in FAM83B-knockdown cells, indicating that FAM83B maybe influence LSCC tumour formation in vivo (Fig. 7C and table S4).

4. Discussion

Laryngeal carcinoma is a commonly fatal disease in the field of otolaryngology. However, the understanding of its potential molecular mechanisms is limited. At present, surgery, radiotherapy, and chemotherapy are the main treatment methods [3,4]. In recent years, the application of molecular targeted drugs has gradually increased. Although molecular targeted therapy for laryngeal carcinoma is still in its infancy, it has become a research hotspot. Therefore, exploring the mechanisms of cancer occurrence and development, searching for new effective therapeutic targets, and identifying prognostic markers are crucial.

FAM83B is one of the FAM83 family members, which has been mainly associated with cell transformation [5]. More and more research being done on the role of FAM83B in tumours over the years [15]. Researches have shown aberrant expression of FAM83B in some malignant tumours, such as breast, bladder, lung, thyroid, cervical, ovarian, and endometrial cancers [16–20]. Of these cancer types, the FAM83 family has been studied most in breast cancer [21–24]. FAM83B has been demonstrated to enhance the proliferation

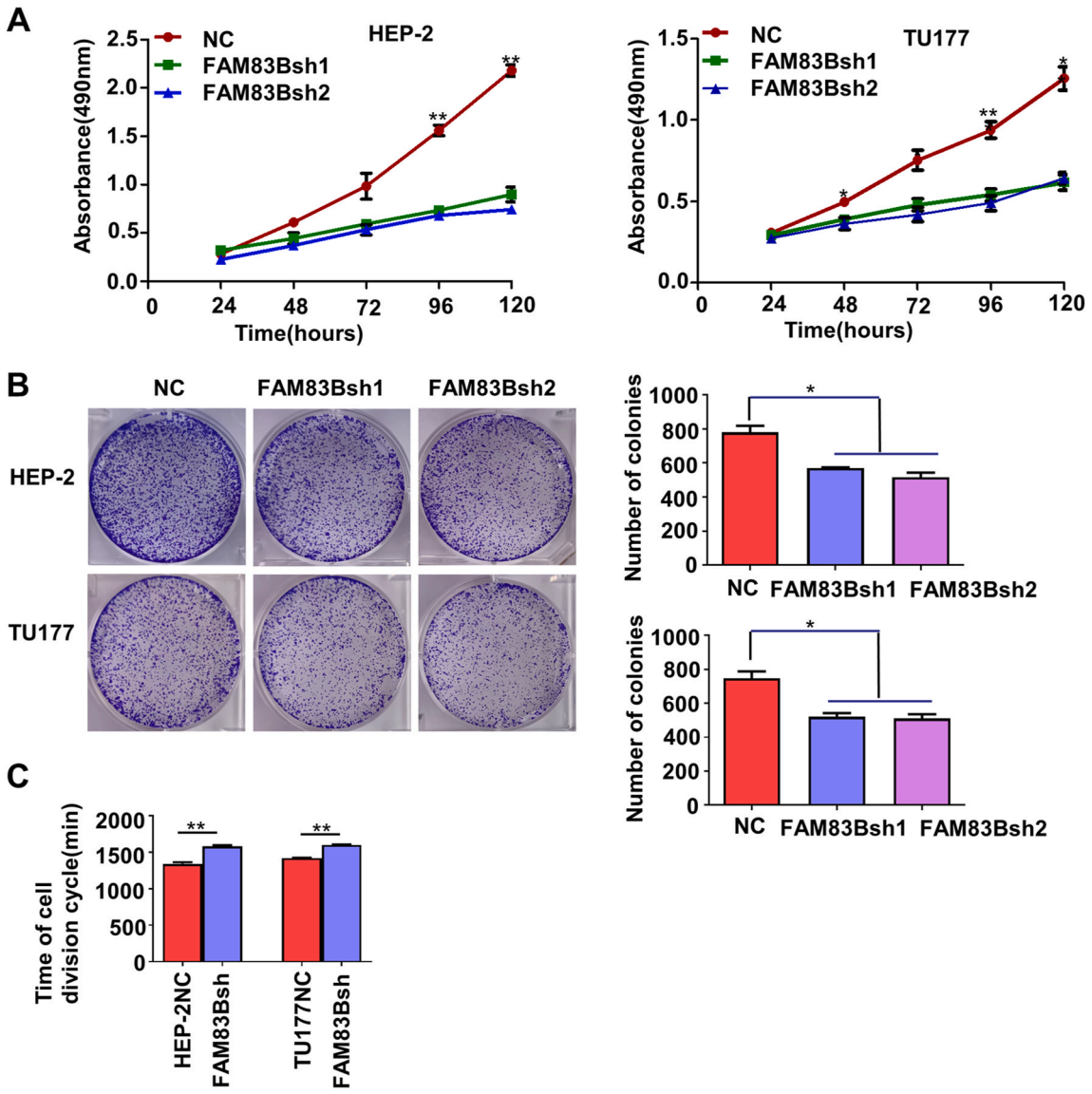

Fig. 3. Effects of FAM83B knockdown on proliferation of laryngeal squamous cell carcinoma (LSCC) cell lines. **(A)** FAM83B knockdown inhibits the growth of HEP-2 and TU177 cell lines. **(B)** The number of colony formation by LSCC cell strains was inhibited in FAM83B knockdown subjects were in comparison with that in the control subjects. **(C)** Analysis of cell cycle by live cell imaging in the control and FAM83B-knockdown of HEP-2 and TU177 cell lines. Trials were replicated three times, and results are shown as the mean ± standard deviations (SD). $^{*}p < 0.05$, $^{**}p < 0.01$.

of breast cancer and tumour growth via activating the MAPK/RAS and mTOR/PI3K signalling pathways. Studies have shown that FAM83B may combine with Axin-1 and APC to promote the Wnt signalling pathway [25–27]. It has been found that the survival of breast cancer patients is shortened and the recurrence rate is increased with an increase in FAM83B expression. The expression quantity of FAM83B in squamous cell lung cancer (SCC) is also remarkably higher than that in normal lung tissue, making it a potential reliable biomarker in diagnosis and prognosis of SCC [28]. Recently, two tests have shown that FAM83B can promote the transformation of endometrial cancer and pancreatic ductal adenocarcinoma (PDAC) cells from G1 phase to S phase [16,17]. The cell cycle is a continuous process, and CCND1 plays an important role as a promoter of the G1-S transition [29]. Moreover, CCND1 binds to CDK4/6 and activates CDK4/6, leading to the induction of E2F target genes, promoting development from the G1 stage to S stage [30]. Dysregulation of the CDK4/CDK6/CCND1 axis is frequently observed in almost all human cancers, contributing to aberrant cell proliferation and consequent tumorigenesis [14]. Notably, CDK4/6 inhibitors have been authorized for the therapy of hormone receptor-positive patients, HER2-negative end-stage of breast carcinoma, and many inhibitors of CDK4/6 are currently being tested in clinical trialsfor the treatment of various cancers [31,32].

Although FAM83B may play critical roles in LSCC, its function and the molecular mechanism by which FAM83B contributes to LSCC tumourigenesis remains indistinct. In this research, we discovered that the expression quantity of FAM83B in LSCC textures was

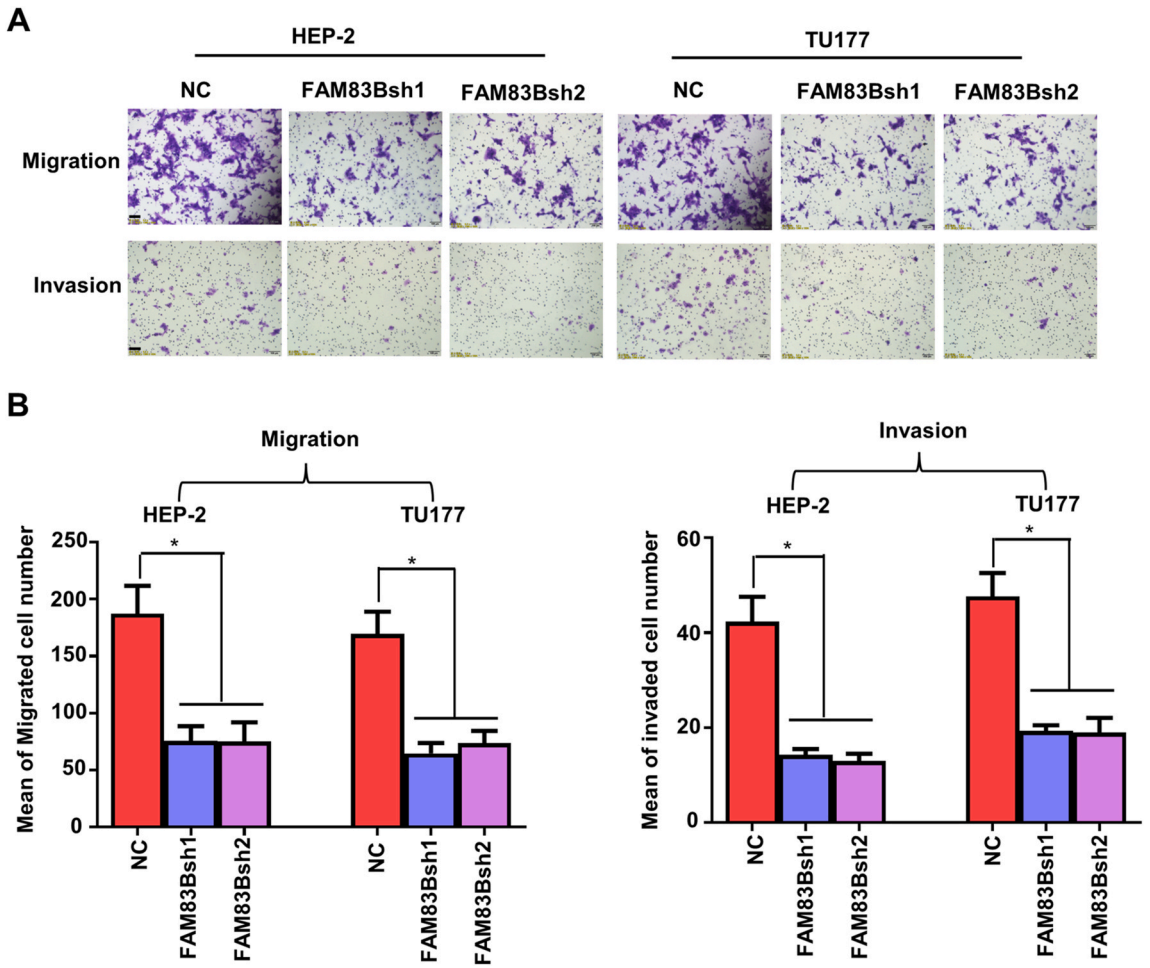

Fig. 4. Transwell assays using HEP-2 and TU177 cell strains handled the lentivirus expressing FAM83B-shRNA. **(A)** FAM83B knockdown notably inhibits HEP-2 and TU177 cell migration ability and invasiveness (scale is 100 μm). **(B)** The statistical quantification analyses. Trials were replicated three times, and results are shown as the mean ± standard deviations (SD). $^{*}p < 0.05$.

higher than the gene in paired adjacent textures. This discovery is accordant with the reported in PDAC, adenocarcinoma of lung, and endometrial cancer [16,17,28]. Moreover, we confirmed the tumour promoting role of FAM83B in LSCC via in vitro and in vivo assays. FAM83B knockdown showed the ability to inhibit tumour growth, migration, and invasion. In particular, we preliminarily probed the influence of FAM83B knockdown on CDK4/CDK6 and CCND1, which are cell cycle regulators, in LSCC cell lines. CDK4, CDK6, and CNCD1 complexes involved in regulating the cell cycle, and can phosphorylate the retinoblastoma protein, thus promoting the cell cycle progression from G1 stage to S stage. Lin et al. [17] and Shen et al. [16] observed that FAM83B knockdown leading to a cell cycle block at the G1 stage in endometrial cancer and PDAC, respectively, which is consistent with our results.

In this study, cell cycle assay by flow cytometry showed that FAM83B knockdown led to an obvious increasement in the G1 phase cells. CDK4/6 can form a complex with CCND1, which is implicated in the adjustment of the G1 phase of the cell cycle [33,34]. WB manifested that FAM83B knockdown contributed to a significant reduction in CDK4/CDK6/CCND1 protein expression, which could have decelerated cell cycle progression.

In our study, a stable knockdown cell line was established via puromycin screening after knockdown of FAM83B in HEP-2 and TU177 cell of LSCC cell strains by lentivirus infection. We used RT-qPCR and WB experiments to verify that the FAM83B knockdown efficiency in LSCC was above 70 %. MTT and hard clone experiments showed that FAM83B knockdown could inhibit LSCC proliferation and clone formation. Transwell experiments showed that FAM83B knockdown could inhibit LSCC cell invasion and migration, a contrast to the findings in PDAC. In PDAC cells, FAM83B exhibited no significant effect on cell invasion, possibly due to the inherent differences between cancer types [16]. Flow cytometry testified that FAM83B knockdown could restrained the shift of LSCC cells from G1 phase to S phase.

The study reported for the first that the expression and role of FAM83B in LSCC. However, further research is required on the interaction between FAM83B and CDK4/CDK6/CCND1 complexes, as well as the specific mechanism by which FAM83B enhanced the invasion and migration of LSCC cell strains. We aim to further explore these research questions in further investigations. In addition,

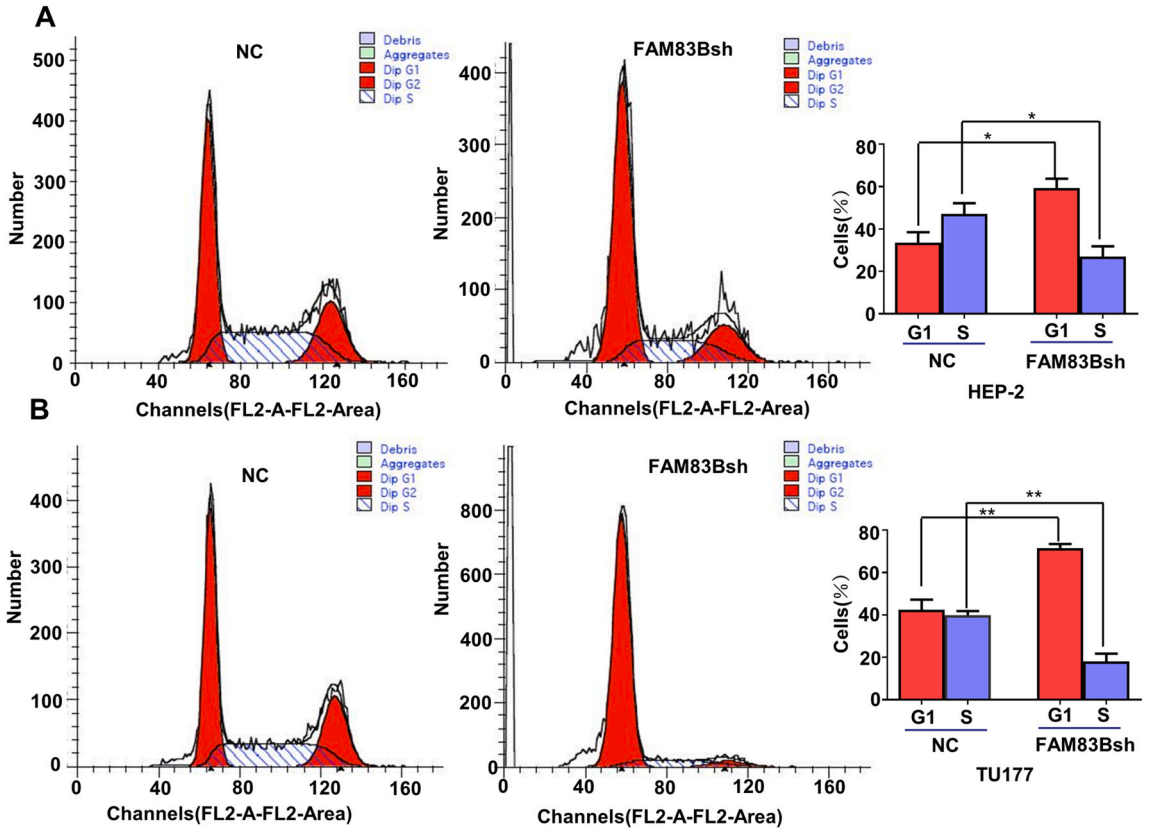

Fig. 5. Cell cycle distribution is analysed by utilizing flow cytometry. **(A)** Photo on the left: Typical cell-cycle pictures of NC and FAM83B-shRNA HEP-2 cell lines. Photo on the right: The quantitative analysis of the statistics in cell-cycle distribution among the HEP-2 cells. **(B)** Photo on the left: Typical cell-cycle pictures of NC and FAM83B-shRNA TU177 cells. Photo on the right: The quantitative analysis of the statistics in cell-cycle distribution among the TU177 cells. Trials were replicated three times, and results are shown as the mean ± standard deviations (SD). $*p < 0.05$, $**p < 0.01$.

we have been actively researching this topic and have recently expanded our LSCC sample collection and conducted an in-depth investigation on the expression of FAM83B in LSCC and it connected with clinical pathology.

Together, our in vivo and in vitro experiment results manifest that FAM83B maybe play an oncogenic role in LSCC by promoting the shift of LSCC cells from G1 phase to S phase. However, the specific mechanism of FAM83B's role in LSCC requires further investigation. These studies will offer insights and an academic foundation for elucidating the mechanism of LSCC occurrence and evolution and for developing LSCC treatment strategies.

In conclusion, our study shows FAM83B as a crucial regulatory factor involved in proliferation of LSCC cells. Our discoveries are of great significance to understand the mechanisms that boost the evolution and advance of LSCC and for developing novel treatment strategies.

The Tree map of the assay is displayed in Supplementary figure 2 (fig. S2).

Ethics statement

All assays were authorized by the ethics committee of Shanxi Provincial People's Hospital Affiliated to Shanxi Medical University (2019-164). All samples were collected before treatment and =were in line with the guidance of the native ethics board, and also gained the informed and consented forms of each participator.

Funding

This study was endorsed by funding from the Natural Science Foundation of Shanxi Province (202103021224368, 202203021211226 and 20210302123353).

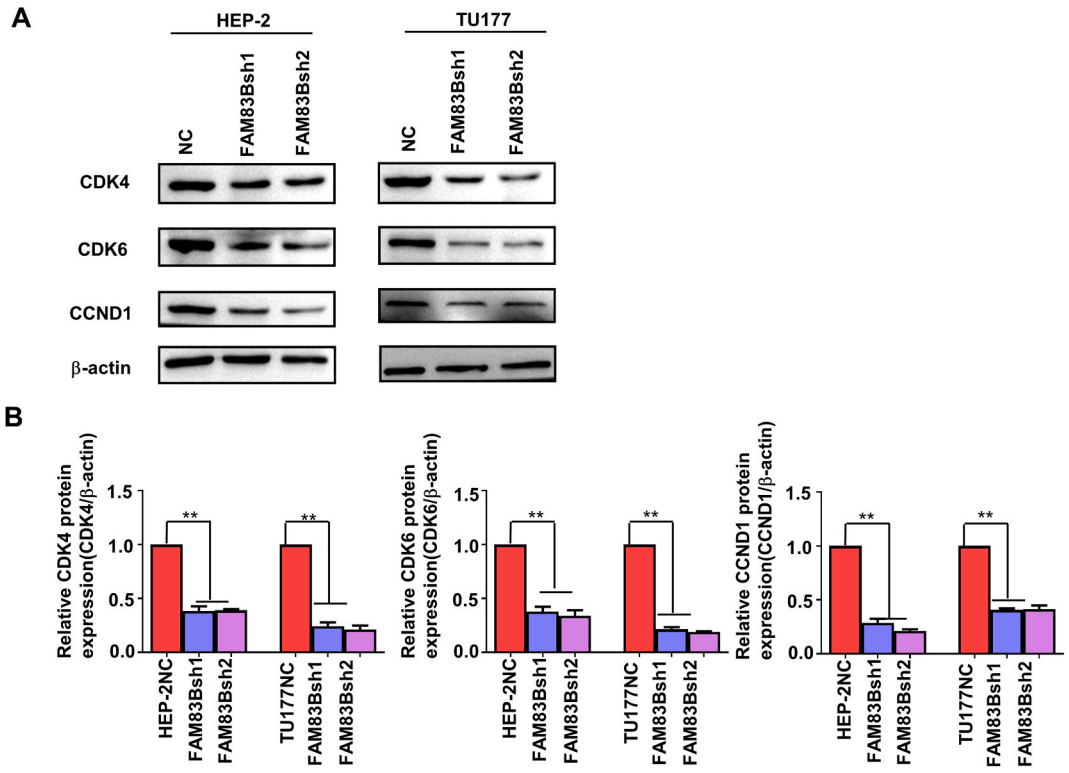

Fig. 6. FAM83B affects cell cycle regulators. **(A)** The protein levels of CDK4, CDK6, and CCND1 were tested by WB. β-actin was employed as a control protein. **(B)** The statistical quantification analyses of CDK4/CDK6/CCND1 expression in FAM83B knockdown cells. Trials were replicated more than three times. $^{**}p < 0.01$.

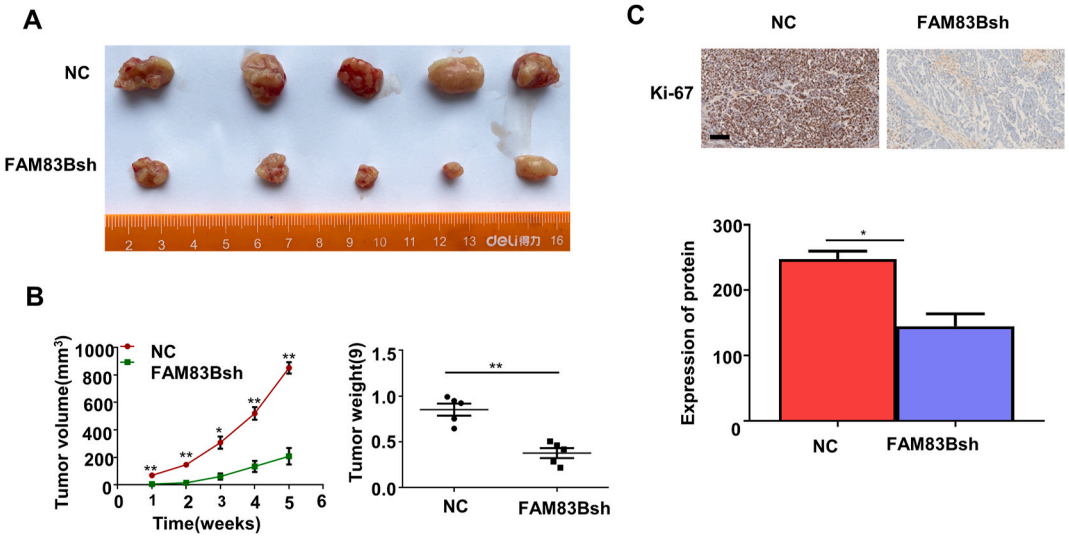

Fig. 7. FAM83B promotes tumour growth and affects cell cycle regulators in nude mice. **(A)** FAM83B knockdown significantly inhibits the growth and weight of xenotransplanted tumours in mice. **(B)** Changes in gross tumour bulk (photo on the left) and weight (photo on the right). **(C)** Typical immunohistochemical pictures showing the expression quantity of Ki-67 in nude mice injected with NC cell lines or FAM83B knockdown cell lines (scale is 100 μm). Underside photo demonstrated the statistical bar graph of Ki-67 protein levels. $^{*}p < 0.05$, $^{**}p < 0.01$.

Data availability

The results required to assess the conclusions in the paper are present in the paper and/or supplements. Attached results was connected with this these perhaps asked by the author.

CRediT authorship contribution statement

Xiaoling Hu: Writing – original draft, Investigation, Funding acquisition, Conceptualization. **Siwei Zou:** Resources, Methodology, Investigation. **Xiaoyu Shi:** Resources, Methodology. **Qiangwei Zhang:** Validation, Methodology, Data curation. **Yanfei Li:** Software, Data curation. **Mengya Wang:** Validation, Methodology. **Tongli Li:** Methodology, Funding acquisition, Data curation. **Xuanping Zhang:** Supervision, Formal analysis. **Guodong Li:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29933.

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