High MEIS3 expression intimates poor prognosis for clinical stage II/III colorectal cancer patients

Ma Jian
The third affiliated hospital of soochow University
Haitao Li
Qindao University Medical College Affiliated Yantai Yuhuangding Hospital https://orcid.org/0000-0002-2193-3905
Meihua Wang
Changzhou Tumor Hospital Affiliated of Soochow University
Ming Zhu
Changzhou Tumor Hospital Affiliated to Soochow University
Ang Li
School of Life Science and Technology, Tongji University
Yang Ling (lingyang2015@sohu.com)
Changzhou Tumor Hospital Affiliated of Soochow University https://orcid.org/0000-0003-2613-0949

Research

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Abstract

Background

The middle stages colorectal cancer (CRC) patients are severely differentiated, so suitable biomarkers are required to distinguish the cohort with high recurrence risk. We hypothesized that among the specimens retained after surgery, those from cohort who relapsed quickly after surgery may be more capable to find the high-risk markers.

Methods

A lable-free analysis was employed to identify candidate proteins markers in CRC tissues relapsed within three years since surgery. Combined with MEIS3 immunohistochemistry characteristic, we analyzed MEIS3 (myeloid ecotropic viral insertion site 3) expression level in clinical stages. Kaplan-Meier Analysis was adopted to analyze the correlation between gene expression and 5-year disease-free survival (DFS) of CRC patients. Finally, cell biology methods were employed to analyze molecular mechanism for tumor cell migration and metastasis.

Results

Through proteomics immunohistochemistry analysis, we identified protein MEIS3 are mainly located in growth front and other highly invasive areas in cancer. And its expression level is closely associated to the clinical stage of CRC. Furthermore, high MEIS3 expression in cancer tissues is closely related to the 5-year DFS expectations of CRC patients. The 5-year DFS of stage II patients with high MEIS3 expression was significantly lower than that of low MEIS3 expression (60.2% % vs 81.1% $p < 0.01$). The 5-year DFS III patients with high MEIS3 expression was also significantly lower than those of low MEIS3 expression (38.1% vs 56.7% $p < 0.01$). The analysis on SW480 cells showed that MEIS3 may promote migration and invasion of CRC cells by regulating the expression of LAMB1.

Conclusion

This study intimates that MEIS3 overexpression is associated to poor prognosis for stage II and III patients, and MEIS3 can promote the CRC cells invasion through regulating LAMB1 expression.

1. Introduction

The CRC prevalence has been increasing in recent decades, and becoming one of the most common malignancies and death factors in the world. In China and the United States, the incidence rate of CRC ranks third in all tumors.[1–3] Up to now, the therapy and prognosis of CRC has become an important project in clinical medicine and biological research. With the benefits from successive insight for CRC, the

survival rate and life quality of stage IV patients have been significantly improved following the targeted treatment of radiotherapy, chemotherapy and related therapy measures.[4]

However, due to the complex pathology, a considerable number of stage II and III CRC patients are prone to relapse. However, it is difficult to identify these patients based on current diagnostic techniques. As a result, they rarely receive the necessary consistent therapy such as and/or chemotherapy radiotherapy after surgery. Ultimately, patients at these stages haven not get enough benefits from new therapy strategy, so these patients present a high risk of cancer recurrence after surgery. Therefore, finding an effective and simple identification method has become a practical urgency for clinical and biological research.

To address this problem, researchers have also done a lot of work to identify specific biomarkers that can effectively indicate the prognosis for CRC patients. [5–8] Through analyzing the function and mechanism of related genes, the expression fingerprints of gene transcriptome and proteome in peripheral blood and/or tumor tissues, the existed large database system, and the perfect bioinformatics technology, researchers and doctors have found some specific mutated or differentially expressed genes to identify the CRC patients with high-risk .[5, 9–11] Mutations in KRAS and TP53 genes, as well as abnormal DNA methylation in promoter regions, have been found to indicate poor prognosis and shortened survival in CRC patients.[12–14] The abnormally expressed genes such as carcino-embryonic antigen, CA 19 − 9 and TP53 also showed poor prognosis for patients with colorectal cancer.[12, 15] In addition, FOXRED1, FOXM1, L1CAM, CKS2 and other genes have been found to be used to evaluate the prognosis of patients with CRC.[16–18] However, the actual effect of these genes in suggestion for clinical adjuvant therapy still needs to be evaluated. Eventually, a large number of candidate biomarkers may be discarded in clinical practice on a large scale. In short, for CRC patients with stage II and III, there is still a need for more clinical indicators and molecular biomarkers that can accurately predict recurrence after surgical operation.

The probable cause of the recurrence of CRC patients is that very early and distal metastasis has occurred before the operation, which is difficult to detect based on current clinical, genetic and protein methods. But the corresponding genes and proteins have been fully expressed in cancer tissues, but these genes or proteins are still in frog waiting to be identified. Therefore, we believe that by proteomic detection of the tissues of relapsed patients, and corresponding analysis, we can find corresponding biomarkers to distinguish patients who are prone to relapse.

For researching the CRC proteomic fingerprints, we selected the tissues in stage II patients who had relapsed after surgery, according to the follow-up investigation in our department over the years. Through the analysis of the relative protein abundance difference, we screened out a batch of proteins which were significantly up-regulated or down-regulated, that is, DEPs in CRC tissues. With the aid of bioinformatics technology and literature review, we have screened a number of DEPs which may participate in the distant metastasis of cancer cells. Then, we verified the relationship of MEIS3 expression level and the clinical stages of CRC patients. We also found that MEIS3 expression level will affect the 5-year DFS of

clinical stage II patients because this gene can promote cancer cell invasion through LAMB1 activation. We expect that parts of stage II patients can receive reasonable radiotherapy and/or chemotherapy in clinic through checking this biomarker.

2. Material And Method

2.1 Clinical Samples

This study was approved by the ethics committee of the Third Affiliated Hospital of Soochow University with No.2017-SY-12, and in accordance with the International Ethical Guidelines for Human Biomedical Research standards, signed informed consent with patients. The clinical staging of patients with colorectal cancer is carried out in accordance with the American Joint Committee on Cancer (ACJJ) standard, that is, the clinical staging is based on the cancer's tissue invasion, lymph node metastasis and distal metastasis[19]. All patients were treated in accordance with the colorectal cancer guidelines of the Chinese Society of Clinical Oncology (CSCO). During follow-up, once the patient was found to relapse, it was recorded as the endpoint of DFS, and if the patient died due to other than colorectal cancer, the data will be deleted. From 2015 to 2018, a total of 403 patients participated in the study, and finally 346 patients were included in the analysis. The characteristics of some cases related to this study are summarized in Table 1. And Kaplan Meier analysis was used to evaluate the 5-year DFS of patients with different factors.

After surgical excision of tumor specimens, one part was stored in paraformaldehyde for histological analysis; one part was stored in TRIZOL to extract total RNA for analyzing gene expression levels, and the remaining part was treated with liquid nitrogen. Store in -80oC refrigerator for Western Blot analysis and other purposes.

Table 1
Clinical and pathological features of CRC patients

Age(years)	Number	Percent
< 50	128	38.32%
50–70	176	52.69%
> 70	30	8.98%
Median age	55 years	
Gender		
male	184	53.18%
Female	162	46.82%
JACC Clinical Stage		
I	45	13.01%
II	109	31.50%
III	137	39.60%
IV	55	15.90%
Tumor size (cm)		
< 2	17	6.91%
2–5	95	38.62%
> 5	134	54.47%

2.2 Lable-free proteomic analysis

In order to study clinical II patients who are prone to relapse after surgery, we selected cancer tissues and para-cancerous tissues of 9 patients from patients who relapsed within three years after surgery, and selected 0.1 g of non-connective solid tissue in each sample. The cancer specimens of every 3 patients are mixed into a new sample, and the same 3 para-tissues are mixed into a new analysis sample. Then put the specimen in 8M urea, 50 mM TEAB lysis buffer for full lysis. The protein lysate was reduced by 10 mM DTT,then 30 mM Iodoacetamide (IAM) to alkylate the sample. Then, the lysis were transfered into the ultrafiltration tube (10K, Millipore), and was centrifuged for 12,000 rpm, 20 minutes to remove the remaining IAM and protein impurities.

The100ug of total protein was digested by trypsin for 8hrs at 37oC. After digestion, the product was lyophilized in vacuum freeze dryer at -80oC, and then the lyophilized powder was dissolved with 30 ul of 10% formic acid. The lyophilized peptide was successively transferred to the Dionex Acclaim PepMap100

C18 nm inverse column (5 m, 100 A, 100um I.D. X 2 cm,Thermo Fisher Scientific, sunnvvale, CA, USA) and RSLC C18 separation column (2um, 100 A, Isolation of peptide on 75 m I.D.X 25 cm (Thermo Fisher Scintific) by nanoflow CHIP-LC (CHIP-nLC, AB Sciex). The eluted peptides are directly transferred to the LTQ orbitrap Elite LC/MS system for analysis. The mass spectrometry data acquisition parameters were as follows: 2.2 kV electrospray, mass spectrum acquisition range (350–2000 m/z), primary detector: Oribit -Trap.

Proteome Discover Version 1.4 (Thermo Fisher Scientific) software was used for mass spectrometry data analysis, Mascot Server (Version 2.3 Matrix Science London, UK) was used for search engine, and Uniprot protein database was used for database retrieval, finally, the protein and relative abundance were defined. [20, 21] The protein abundance difference between cancer tissues and para- tissues was analyzed by t-test. When the ratio was greater than 1.75 times, $P < 0.05$, it was defined as differential expression protein. The differentially expressed proteins were analyzed using online tools such as gene ontology (GO), Visualization and Integrated Discovery (DAVID) and STRING for bioinformatics analysis such as protein clustering and protein interaction network.[22–24]

2.3 Antibodies and Media

In this study, we used the following antibodies:Rabbit anti- MEIS1 (Atlas) ,Rabbit anti-MEIS2 (Proteintech), Rabbit anti-MEIS3 (Atlas), Rabbit anti-LAMB1 (Proteintech), Rabbit anti-POSTN (BosterBio), Rabbit anti-GPNMB (CST), Rabbit anti-ACTB (Proteintech), HRP-conjugated Affinipure Donkey Anti-Mouse IgG(H + L) (Proteintech), HRP-conjugated Donkey Anti-Rabbit IgG(H + L)(Proteintech), Rabbit anti-E-Cadherin (Thermo Fisher Scientific) and Mouse anti-Vimentin (CST). DTT, IAM and TEAB were purchased from SIGMA Co Ltd. Cell culture medium and serum were purchased form Thermo Fisher Scientific.

2.4 WB and Q -PCR

The total RNA in tissues or cells was extracted by Trizol method, and then RNA reverse transcription was performed according to PROMEGA's AMV reverse transcriptase system product manual with RNase-free operation guidelines. The Q-PCR analysis was performed according to the instructions of TOYOBO's SYBR Green Master-mix kit. According to the Ct value generated by the amplification result, the -Δ/ΔCt method was used to quantify the expression abundance of the target sample and the reference sample. The primers involved are shown in table S1.

The Western Blot (WB) was carried out according to the description operation of molecular cloning: A Laboratory Manual.[25] After the development is completed, use Quanitive one-4.40 to analyze the strips according to the relative concentration.

2.5 Immunohistochemistry

The tissues were fixed for 24 hours after 4% PFA, and dehydrated in 70%, 80%, 95%, 95%, 100% and 100% alcohol in turn, and then transparent with xylene, immersed in paraffin wax and embedded. The tissue is cut into slices with a thickness of 8um by the LEICA microtome. The tissue sections were then reverse-treated with 100–50% alcohol from xylene, immersed in sodium citrate antigen repair solution, and

repaired with microwave method. The tissue sections were soaked in 5% H2O2 for 10 minutes, washed in tap water, and added with 10% rabbit serum (PBS dilution), then, 1 hour later, it was replaced by antibody-10% serum and incubated overnight at 4oC. The next day, the primary antibody was discarded, washed with PBS three times, and the secondary antibody diluted in 1 × PBS was added. After incubating for 1 hour at room temperature, the color was developed by SABC method. After dehydration with gradient ethanol and xylene, the slices were sealed with neutral gum, and the results were photographed and recorded by microscope.

2.6 Construction of virus particles

The MEIS3 silencing lentiviral particles and the LAMB1 over-expression adenovirus particles were packaged with the assistance of ZQXZ Bio Tech. (Shanghai) Corp. Ltd., and preserved in liquid nitrogen. The knockdown expression vector is composed of three U6 promoters connected in series, each of which directed a shRNA sequence. The three shRNA sense sequences were as follows: 5'-CUUGGAAGGAGAAUGGCAUUAUCTA-3', 5'-CUGCAAGUCAACAACUGGUUCAUTA-3' and 5'-CUGGUGGAGAAGAUGAGGACUUGGA-3'.

2.7 Cell invasion capabilities analyze

The cells were first cultured in DMEM without FBS for 24 hours. During this period, the Transwell chamber was soaked and moistened with 1 × PBS, then matrigel was added, and the gel was incubated in a CO_2 incubator at 37 °C to solidify the gel. Then add 600 μl of complete medium containing FBS to the lower chamber, add 200 ul of DMEM hanging cells (density 5 × 10E5 cells/ml) to the upper chamber, and continue to culture for 48hrs, stain with 0.5% crystal violet, and count the results.

3. Results

3.1 DEPs in tissues of relapsed CRC patients

For identifying candidate biomarkers that may represent the high recurrence risk of CRC patients in clinical stage II, we selected tissue specimens from surgical resection of stage II patients who relapsed within 3 years after surgery. For proteomic study, Each 3 cancer growth front tissues were mixed in equal quality, and 3 para-cancer tissues were also mixed in equal amounts to form a test sample. Compared with para-cancer tissues, when the abundance of protein in cancer tissue is higher or lower than 1.75 folds, it is selected as DEPs. A total of 136 DEPs were screened, of which 77 proteins were up-regulated and 59 proteins were down-regulated (Table S2).

3.2 Search new candidate biomarkers of prognosis by bioinformatics technology

For the purpose to analyze the distribution characteristics of DEPs in the tissues of patients with recurrence, we used Database for Annotation, DAVID (version 6.8) and the database of GO (http://geneontology.org/) to perform cluster analysis on all the DEPs found.[22, 24, 26] According to the

distribution and clustering of DEPs in tissues and cell component, we found that structure-related proteins such as membranes (GO:0016020,5.90E-10), mitochondrion (GO:0005739, 3.30E-02) extracellular spaces (GO:0005615 9.10E-03), extracellular exosomes (GO:0070062 9.00E-23) and cytoplasm (GO:0005737 3.50E-06) accounted for the highest proportion (Fig. 1A), indicating that these subcellular structures played an important role in cancer metastasis and recurrence. From the biological processes involved in related proteins, it can be seen that the signal pathways such as cell-cell adhesion (GO:0098609,5.60E-03), positive regulator of Cell migration (GO:0030335, 3.30E-03), transcription from RNA polymerase II promoter (GO:0045944,2.20E-02), negative regulation of cell proliferation (GO:0008285, 4.10E-03), Wnt signaling pathway, planar cell polarity pathway (GO:0060071, 8.60E-05) and MAPK cascade (GO:0000165, 1.70E-02) show a strong degree of enrichment (Fig. 1B). Finally, these proteins perform their functions by RNA binding (GO:0003723, 4.80E-02), cadherin binding involved in cell-cell adhesion (GO:0098641, 4.90E-04), actin filament protein (GO:0051015, 3.90E-03), protein binding (GO:0005515, 1.00E-10) and transcription factor binding (GO:0008134, 1.90E-03) (Fig. 1C). Since Wnt signaling pathways participate in the regulation of cell proliferation, migration and invasion, metabolism and other processes in an all-round way, it implies that the DEPs related to the Wnt signaling pathway may play a crucial role in CRC.[27–29]

In order to screen new biomarkers more comprehensively and effectively, we used network analysis tools such as STRING to establish a protein-protein interaction network (PPI) for all DEPs.[23] The results showed that FARSA, MEIS3, RER1, VEZT and PLP2 and other 11 DEPs were not adequately involved in the net (Fig. 1E, Fig. S1). To further confirm this result, we established independent PPIs for individual protein of MEIS3, RER1 and FARSA by employing the STRING again (Fig. 1D, Fig S2, S3). After reviewing literature, we found that there were no report about whether these genes related to the recurrence risk for CRC.

3.3 MEIS3 Protein localization in colorectal cancer

Among these proteins, MEIS3 is a direct target gene of the Wnt/beta-Catenin signaling pathway, which participates cell migration during development.[30, 31] Therefore, we selected MEIS3 as candidate biomarker for prognosis of CRC patients. Firstly, we analyzed the MEIS3 localization in tissues by immunohistochemistry firstly. As shown in Fig. 2, MEIS3 protein was expressed in the nucleus, and not in differentiated tissues (Fig. 2A, B). Histologically, MEI3 were located in growth and invasion front (Fig. 2C, D), which is the main area for tumor growth and invasion into normal tissues. Another major area was the intervening region of the middle-highly differentiated area (Fig. 2E, F), which is full of poorly differentiated cancer cells. Such cells also have strong proliferation and invasion capabilities, and cause cancer progression. In contrast, in tumor cells are densely growing areas, the MEIS3 intensity decreases (Fig. 2G, H). During cancer metastasis, it is not differentiated, but poorly differentiated, especially cancer cells of growth front dominate the process, [32] Thus, the immunohistochemistry results suggested that MEIS3 was related to cancer deterioration.

3.4 MEIS3 expression increases with the CRC stages Progresses

Considering the relationship between ACJJ staging and cancer metastasis, [19] We screened MEIS3 expression in protein level in 346 pairs of tissues (Table 2). As a result, the MEIS3 expression level was significantly increased (2.8 folds) in CRC tissues than para-tissues (Fig. 3A, B). And MEIS3 expression increased with the disease stages progresses (Fig. 3C), which implied that MEIS3 could be a candidate biomarker for surgical prognosis of CRC patients. Although several genes expression were reported to be gender-related, we did not find this trend on MEIS3 (Fig. 3D).

3.5 Association of MEIS3 expression and prognosis in stage II/III patients

The DFS of CRC patients after surgery are related to many factors including the clinical stage, gender, gene mutations and expression levels. At present, the clinical stage is believed to be the main factor. After Kaplan-Meier Analysis, we found that the 5-year DFS of CRC patients in different clinical stages during surgery are significantly different ($p < 0.01$). The 5-year DFS of clinical stage I CRC patients was 87.5% (Fig. 4A). Obviously, the stage IV patients have a poor prognosis, and the 5-year DFS was 23.4% only (Fig. 4A). The 5-year DFS of patients if stage II and stage III are 69.8% and 54.9%, respectively.

The MEIS3 expression was closely correlated with the clinical stages of CRC, so we hypothesized that MEIS3 was also associated with DFS of CRC patients after operation. As shown in the Fig. 4B, in the overall study population, the 5-year DFS of patients with high MEIS3 expression was statistically lower than that of patients with low MEIS3 expression (44.8% vs 59.0% $p < 0.05$).These suggested that although MEIS3 expression level in CRC tissues was significantly correlated with the DFS, but this prognosis results did not show advantage compared with the DFS based on clinical stages (Fig. 4A).

Then, we performed a new 5-years DFS analysis after we simultaneously introduced the clinical stage and MEIS3 expression level into CRC prognostic assessment. Since stage I patients had a good survival, and Stage IV patients have benefited from modern biomedicine, we focused on the stage II and III patients. As shown in the Fig. 4C, the 5-year DFS of stage II patients with low MEIS3 expression was 81.1% (Fig. 4C), which was comparable to that of the overall stage I patients (87.5%) (Fig. 4A). The 5-year DFS of stage II patients with high MIES3 expression was 60.2%, which was significantly lower than those of high MEIS3 expression ($p < 0.01$), and roughly same to that of stage III patients with low meis3 expression (56.7%) (Fig. 4C). However, the expected 5-year disease-free survival of patients with stage III and high expression of MIES3 was significantly lower than those of low MEIS3 (38.1% vs 56.7 $p < 0.01$) (Fig. 4C), indicating a poor prognosis for this part patients.

3.6 Association of MEIS3 and gender on prognosis of stage II and III patients

Since gender is the most stable and convenient factor, and the male and female groups have different responses to CRC, [33] we introduced gender into the survival analysis. As shown in Fig. 4D, the survival rate of female was higher than that of male (45.3% vs 62.7%), but it was not statistically significant ($p > 0.05$). Then, after introducing MEIS3 and gender at the same time, we performed new analysis again. The

stage II female patients with low MEIS3 expression had a significantly high 5-year DFS than those with high MEIS3 expression (90.2% vs 62.5%, $p < 0.05$) (Fig. 5A). While the stage III patients with high MEIS3 expression have a similar survival level to patients with low MEIS3 expression (59.1% vs 40.6%, $p > 0.05$) (Fig. 5B).

In the male cohort, the 5-year DFS of stage II patients with low MEIS3 expression was significantly higher than that of high MEIS3 expression (72.8% vs 50.8%, $p < 0.05$) (Fig. 5C). In the stage III male cohort, the 5-year DFS of patients with MEIS3 low expression was expected to be 52.2%, while that of MEIS3 high expression of was 30.6%, both of which were significantly different (Fig. 5D). In short, regardless of female or male patients, high expression of MEIS3 predicted the deterioration of patients' condition after surgery.

3.7 MEIS3 increases tumor progression tendency through LAMB1

Since MEI3 expression was associated with prognosis of stage II and III CRC patients, located in highly invasive cancer cells, and play a crucial role for inducing cell migration in development, [34, 35] we suspected that MEIS3 may contribute to further cancer progression by enhancing the migration of cancer cells.

Therefore, we constructed lentiviral particles carrying tandem U6 promoter-MEIS3 shRNA and transfected the virus into SW480 cells to silence MEIS3. (Fig. 6A-B) Then, for exploring the downstream regulated genes that promotes cell migration, we returned to the proteomics DEPs again. Among Wnt signaling pathway related proteins, LAMB1, GPNMB and POSTN expression levels were increased (Fig. 1F), which was consistent with the trend of increased cell invasion ability. Accordingly, we also found that LAMB1 expression was most significantly decreased, followed by POSTN and GPNMB (Fig. 6C), indicating that MEIS3 could regulate the expression of POSTN, GPNMB and LAMB1. We also constructed lentiviral particles overexpressing MEIS3 mRNA and transfected the virus into SW480 cells (Fig. 7A). Accordingly, LAMB1 also overexpressed significantly (Fig. 7A), meaning MEIS3 can regulate LAMB1.

The wound healing and transwell assay was used to detect the changes in cell biological function. As MEIS3 knockdown treatment, the invasion and migration ability of cells decreased significantly (Fig. 7B-C). When MEIS3 was overexpressed through lentiviral treated, the migration and invasion ability of SW480 cells has also been significantly enhanced (Fig. 7B-C). These results indicate that MEIS3 can promote the invasion ability of cancer cells through regulating the expression of LAMB1, which may lead to deterioration of patients' condition.

4. Discussion

Prognostic diagnosis is the key to estimate the recurrence risk of CRC patients after therapeutic tumor excision.[5] Today, the prognosis of patients is mainly based on cancer stage, tumor metastasis based on magnetic resonance imaging and/or positron emission tomography, and other markers,such as

histopathological grade, gene stability and expression level.[13, 15, 19, 36] Once the lymphatic metastasis or nerve infiltration signs were detected, the patient have been already in a high-risk state.[19] From the perspective of tissues, cells in stable regions and highly invasive regions in cancer tissues have different activated gene groups.[37] Yet, unless specific sample is selected, genomic, transcriptome, and proteome data are difficult to reflect this characteristic. In theory, the cancer tissues of patients who are prone to relapse may contain more abundant such malignant cells. Therefore, we believed that genes, proteins and other biomarkers components related to CRC duplication can be found more effectively in this type of patient tissue.

In this study, we used proteomics and bioinformatics methods to find DEPs from surgical tissues of relapsed stage II CRC patients. As a result, we found 136 DEPs which may closely relate to the recurrence of CRC patients after surgery excision. Especially, through patient survival retrospective, biological information analysis, and reported studies, it can be shown that certain genes/proteins are closely related to cancer cell energy metabolism, proliferation, metastasis, invasion and immune escape. Therefore, we think there may contain protein biomarkers for poor prognosis of CRC patients in the DEPs.

During cancers development, the highly invasive fronts play important roles for recurrence. These areas contain a large number of pluripotent tumor stem cells, which is responsible for rapid growth, invasion and distant metastasis of the cancer.[38] Several biomarkers have been focused on this area and have shown good clinical effects.

In addition, the regions of differentiated cancer tissues are often separated by connective tissue. Based on connective tissue, accompanied with the entry of blood vessels and nerve tissues, the differentiated areas enter a relatively stable state. Thus, these areas are not easily transformed into highly invasive state, and the patient will not deteriorate due to this type of tumor tissue in a short time. Another case between the differentiated areas is poorly differentiated cells, which are scattered or distributed in a dense state with high invasiveness and proliferation. These cells are also an important factor in the distant metastasis, and this corresponding region often become a key factor for cancer recurrence. Therefore, we think it meaning to pay attention to the biological characteristics of the pathological tissues, especially genes which have positive guiding value for prognosis.

MEIS3, is mainly expressed in invasive fronts and the spaces between differentiation areas of CRC tissues, forming scattered or dense nuclear staining characteristic. The distribution range of these kinds of cells have positively correlated with the MEIS3 expression level. At the molecular level, we confirmed that MEIS3 may promote the migration and invasion of tumor cells by promoting the LAMB1 expression. Therefore, the high expression of MEIS3 is not only significantly related to the clinical stage of CRC patients, but also hinting a high-risk for CRC metastasis and recurrence. The 5-year DFS of clinical stage II patients with high MEIS3 expression was comparable to the overall level of stage III patients. And 5-year DFS expectation of the corresponding stage III patients with high MEIS3 expression was approximately closer to the overall level of the stage IV patients. Especially when combined with gender, the differentiation was further enlarged, and the risk of male was greater than that of female.

In the therapy of patients with advanced CRC, adjuvant chemotherapy and radiotherapy have played an important role in clinical practice, and the expected 5-year DFS rate has been significantly improved.[4, 19] However, due to the severely differentiated outcomes in stage II and III patients, it is controversial whether adjuvant radiotherapy and/or chemotherapy is needed for these patients. Ultimately, the benefits of these patients from modern medicine are far less than advanced stage patients.[5, 39] Here, stage II and III patients with high expression of MEIS3, especially males, had poor prognosis. It is need to treat these patients according the therapeutic measure of high-risk patients to improve their survival rate and life quality.

Of course, we also found that MEIS3 can promote the transformation through activation of LAMB1 which play crucial roles in Epithelial-Mesenchymal Transition,[38, 40] yet, it is still a superficial result. We don't know the specific genes involved in this process, nor the specific regulation process mode between these genes. Therefore, further research is needed to identify the specific molecular mechanism of MEIS3 in determining the fate of tumor/cancer cells.

In addition, we need to study the feasibility of MEIS3 as a biomarker of poor prognosis with larger retrospective CRC patients groups, especially in patients with concurrent therapy and follow-up, before applied in clinical therapy for improving their survival rate and life quality.

5. Conclusion

In summary, MEIS3 is highly expressed in the poorly differentiated areas and growth fronts of tumors/cancers. And the 5-year DFS of stage II and III CRC patients with high MEIS3 expression are significantly lower than those with low MEIS3 expression. In a short word, MEIS3 has great promise as a biomarker for surgical prognosis in patients with colorectal cancer.

Abbreviations

CRC: colorectal cancer, DFS: disease-free survival, DEPs: differentially expressed proteins, MEIS3: myeloid ecotropic viral insertion site 3, ACJJ: American Joint Committee on Cancer, DAVID: Visualization and Integrated Discovery, PPI: protein-protein interaction network, WB: Western Blot, Q-PCR: quantity polymerase chain reaction, ACJJ: American Joint Committee on Cancer, CSCO:Chinese Society of Clinical Oncology

Declarations

Conflict of interest: The authors have no conflict of interest to declare.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LingY and LiHT designed the study; MaJ, WangMH,ChenJ and ZhuM performed the experiments, MaJ, LingY, ChenJ,PKJ, WangMH,LiA and ZhuM analyzed and

interpreted the data; MaJ, LingY, LiTH and LiA wrote the manuscript; all authors

commented and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Consent for publication

Informed consent form for publication was obtained from all authors.

Competing interests

The authors state no competing of interest.

Ethics approval and consent to participate

This study is approved by Medical Ethics Committee of Changzhou Cancer Hospital.

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Supplementary Tables

Table S1 Primers used in this study

Gene	Primer	Primer Sequences	Tm (℃)	Product Length
ACTB	ACTB-F	5'- CGCAAAGACCTGTACGCCAA -3'	58.4	216bp
	ACTB-R	5'- CTCCTGCTTGCTGATCCAC -3'		
MEIS1	MEIS1-F	5'- AAGCAGTTGGCACAAGACAC -3'	55.1	186bp
	MEIS1-R	5'- AATTCCCATATGTTGCTGACCG -3'		
MEIS2	MEIS2-F	5'- CGATAACTTCTGCCACCGAT -3'	57.3	266bp
	MEIS2-R	5'- TGTCTAAACCATCCCCTTGC -3'		
MEIS3	MEIS3-F	5'- GACATCGCTGCCTTTGCCAA -3'	59.8	328bp
	MEIS3-R	5'- TCCCCAAATGTACAGACCCACT -3'		
LAMB1	LAMB1-F	5'- CAGAAGCCGAAAGCCTAGACA -3'	55.8	232bp
	LAMB1-R	5'- AACTGGGATTCTCGCTCCATC -3'		
POSTN	POSTN-F	5'-ATTTGGGGCTTTTCATTAACCAT -3'	56.6	270bp
	POSTN-R	5'- TCTCAAAAGCCTCATTGGTG -3'		
GPNMB	GPNMB-F	5'- ACCAGGAAAAGGATCCGCTA -3'	51.7	259bp
	GPNMB-R	5'- TATCAGTTTCACAACATGGCT -3'		

Tabel S2. Differentially expressed proteins in colorectal cancer tissues

Symbols	Uniprot Accession	Folds	T-Test
CCT5	P48643	1.96	0.001
CDC25B	P30305	1.98	0.001
EIF3B	P55884	2.35	0.001
NME2	P22392	3.09	0.001
RAB2A	P61019	4.31	0.001
EIF3K	Q9UBQ5	0.5	0.001
ETFA	P13804	0.54	0.001
LMNA	Q3BDU5	0.49	0.001
CTNNB1	P35222	2.01	0.000
MEIS3	Q99687	3.07	0.001
PARP1	P09874	1.96	0.001
SNRPF	P62306	6.59	0.001
UBA1	P22314	3.65	0.001
PRELP	P51888	0.32	0.001
LGALS3	P17931	0.26	0.001
PRKACB	P22694	0.34	0.001
MAPK3	P27361	0.27	0.001
GGT5	P36269	0.20	0.001
CDC37	Q16543	1.98	0.002
EMILIN1	A0A0C4DFX3	2.35	0.002
NUCKS1	Q9H1E3	3.25	0.002
PITPNA	I3L471	2.24	0.002
SSBP1	Q04837	3.79	0.002
MT2A	P02795	0.29	0.002
RER1	O15258	2.49	0.002
ETFB	P38117	0.51	0.002
MAPRE1	Q15691	2.85	0.003
POSTN	Q15063	1.96	0.003

PSME2	Q9UL46	3.91	0.003
SUB1	P53999	9.54	0.003
TXNDC17	I3L0K2	1.95	0.003
ILK	Q13418	0.23	0.003
CLDN7	O95471	0.54	0.003
CRYAB	E9PNH7	0.52	0.003
IPO5	O00410	2.73	0.004
PSMC2	P35998	4.07	0.004
THY1	P04216	4.77	0.004
CDKN1A	P38936	0.54	0.004
ECI1	P42126	0.49	0.004
PRDX3	P30048	0.47	0.004
FZD6	O60353	2.43	0.005
HNRNPR	O43390	2.52	0.005
PSMB4	P28070	2.28	0.005
RPL11	P62913	2.23	0.005
SF3B3	Q15393	5.59	0.005
SMPDL3B	F8VWW8	0.37	0.005
MATR3	D6R991	3.05	0.006
SFRP2	Q96HF1	0.36	0.006
PDLIM5	H0Y8Y3	0.47	0.006
SUN2	Q9UH99	0.42	0.006
GPNMB	Q14956	2.21	0.006
MMP1	P03956	2.24	0.007
CALD1	E9PGZ1	1.86	0.008
SULF1	Q8IWU6	9.94	0.008
CAMK2D	Q13557	0.53	0.008
MYLK	Q15746	0.30	0.008
AP2B1	P63010	1.81	0.009

DHX9	Q08211	2.28	0.009
LAMB1	P07942	2.22	0.009
CA2	P00918	0.50	0.009
CAND1	Q86VP6	0.54	0.009
LASP1	C9J9W2	0.18	0.009
BMP4	P12644	1.86	0.010
CDK4	P11802	1.98	0.010
PTBP1	P26599	4.28	0.010
SND1	Q7KZF4	6.23	0.010
LTF	P02788	0.26	0.010
MMP11	P24347	2.99	0.011
PML	H3BVD2	3.35	0.011
B2M	P61769	0.43	0.011
TCF7L2	Q9NQB0	0.43	0.011
FZD5	Q13467	0.18	0.011
CLTA	P09496	1.99	0.012
CSNK2B	P67870	1.99	0.012
HYOU1	E9PJ21	2.62	0.013
RAB7A	P51149	4.42	0.013
RPN2	P04844	5.22	0.013
SELENBP1	Q13228	0.35	0.013
CA1	P00915	0.55	0.013
CCND1	P24385	1.90	0.014
HMGCL	P35914	0.23	0.014
FOXO4	P98177	0.13	0.015
EPCAM	P16422	1.93	0.016
KPNA2	P52292	2.93	0.016
POLR2H	C9JLU1	3.43	0.016
ST13	H7C3I1	7.80	0.016

PSMD11	O00231	0.34	0.016
FBLN5	Q9UBX5	0.11	0.016
RRP12	Q5JTH9	5.11	0.017
FHL1	Q5JXI8	0.53	0.018
CCT2	P78371	1.92	0.019
CLIC1	O00299	1.99	0.019
FARSA	K7EPH2	2.49	0.019
IFI16	Q16666	2.20	0.019
BTD	C9JSN9	0.43	0.019
TPM1	H0YKX5	0.43	0.019
GPX3	A0A087X1J7	0.21	0.019
PLP2	Q04941	3.14	0.020
KHDRBS1	Q07666	0.24	0.020
CCNB1	Q9NPC3	1.88	0.021
C16orf89	Q6UX73	0.51	0.021
APEX1	P27695	2.12	0.022
NME1	P15531	3.02	0.022
VAPA	Q9P0L0	0.49	0.023
ARPC4	P59998	2.12	0.024
CYFIP1	Q7L576	1.90	0.026
H1F0	P07305	2.50	0.026
AQP1	P29972	0.50	0.026
SPINK4	O60575	0.39	0.027
GSN	P06396	0.32	0.027
RPL8	P62917	4.73	0.029
SOD3	P08294	0.38	0.029
ASPN	Q9BXN1	1.85	0.030
SFXN1	Q9H9B4	0.48	0.032
SNX2	O60749	0.48	0.032

CCNB1IP1	P27695	2.13	0.033
SLC1A5	Q15758	5.79	0.033
STT3A	P46977	8.62	0.033
ANKRD22	Q5VYY1	1.81	0.034
CKB	P12277	0.55	0.034
CRABP2	Q5SYZ4	1.99	0.035
AKR7A3	O95154	0.55	0.035
GANAB	Q14697	2.67	0.036
ARHGDIB	F5H3P3	1.82	0.037
WDR1	O75083	0.48	0.037
LRPPRC	P42704	2.91	0.039
ADH1B	D6RHZ6	0.53	0.040
ALDH1A1	P00352	0.53	0.040
MGST3	O14880	0.38	0.040
MVP	Q14764	0.44	0.043
SAE1	Q9UBE0	2.19	0.045
CDC20	Q12834	1.96	0.046
IPO9	Q96P70	2.21	0.048
CALM1	P62158	0.50	0.048
VDAC1	P21796	1.89	0.049
VEZT	F8W8C2	3.31	0.049

Figures

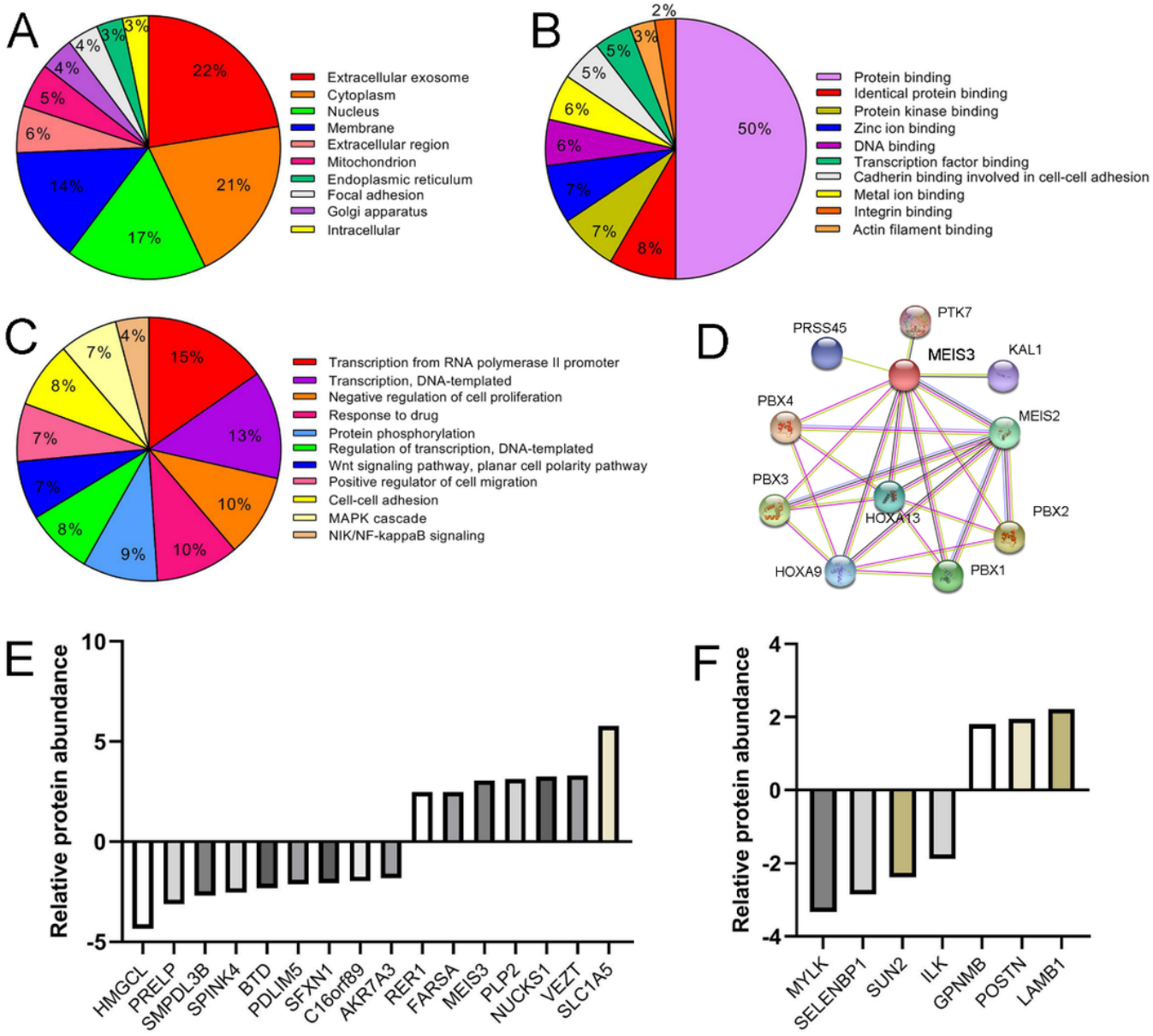

Figure 1

Bioinformatics analysis of DEPs in the tissue proteome of CRC (A) Enrichment of DEPs localization in cells and tissues. (B) Enrichment of DEPs participated biological processes. (C) Enrichment of DEPs participated molecular function. (D) PPI between proteins that can interact with MEIS3. (E) The abundance of proteins not involved in PPI net in DEPs. (F) Protein abundance related to the Wnt pathway.

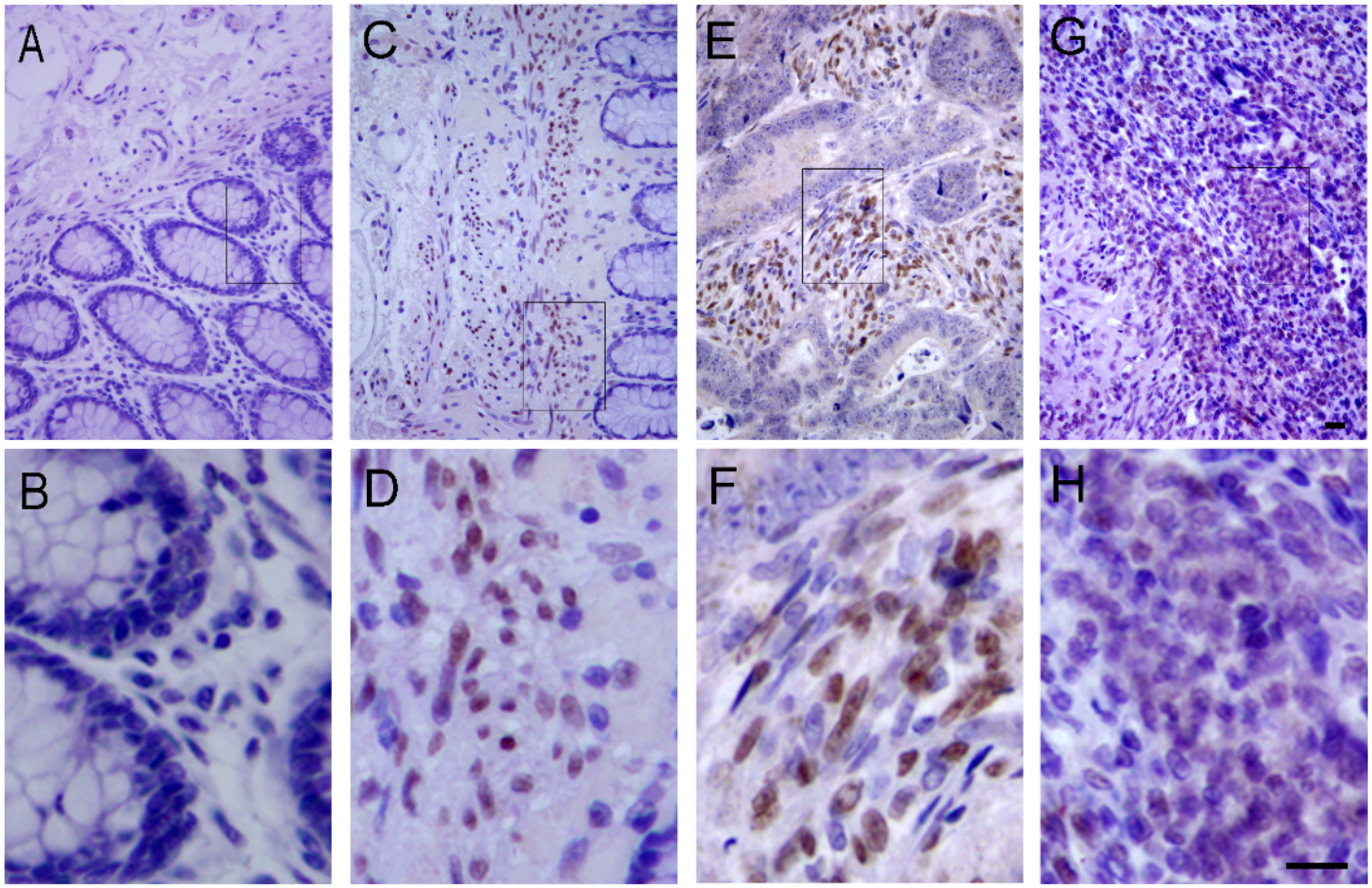

Figure 2

Localization of MEIS3 expression in CRC tissues MIES3 is not significantly detected expression in differentiated CRC tissues (A and B), has the highest expression level in the cancer growth front (C, D) and regions between differentiated tissues (E, F), and weakly expressed in the area of vigorous growth. These suggests that the gene is associated with invasion and metastasis of cancer cells. Among them, B, D, F and H are the screenshots within the boxes of A, C, E and G diagrams respectively. The bar means 50 μm.

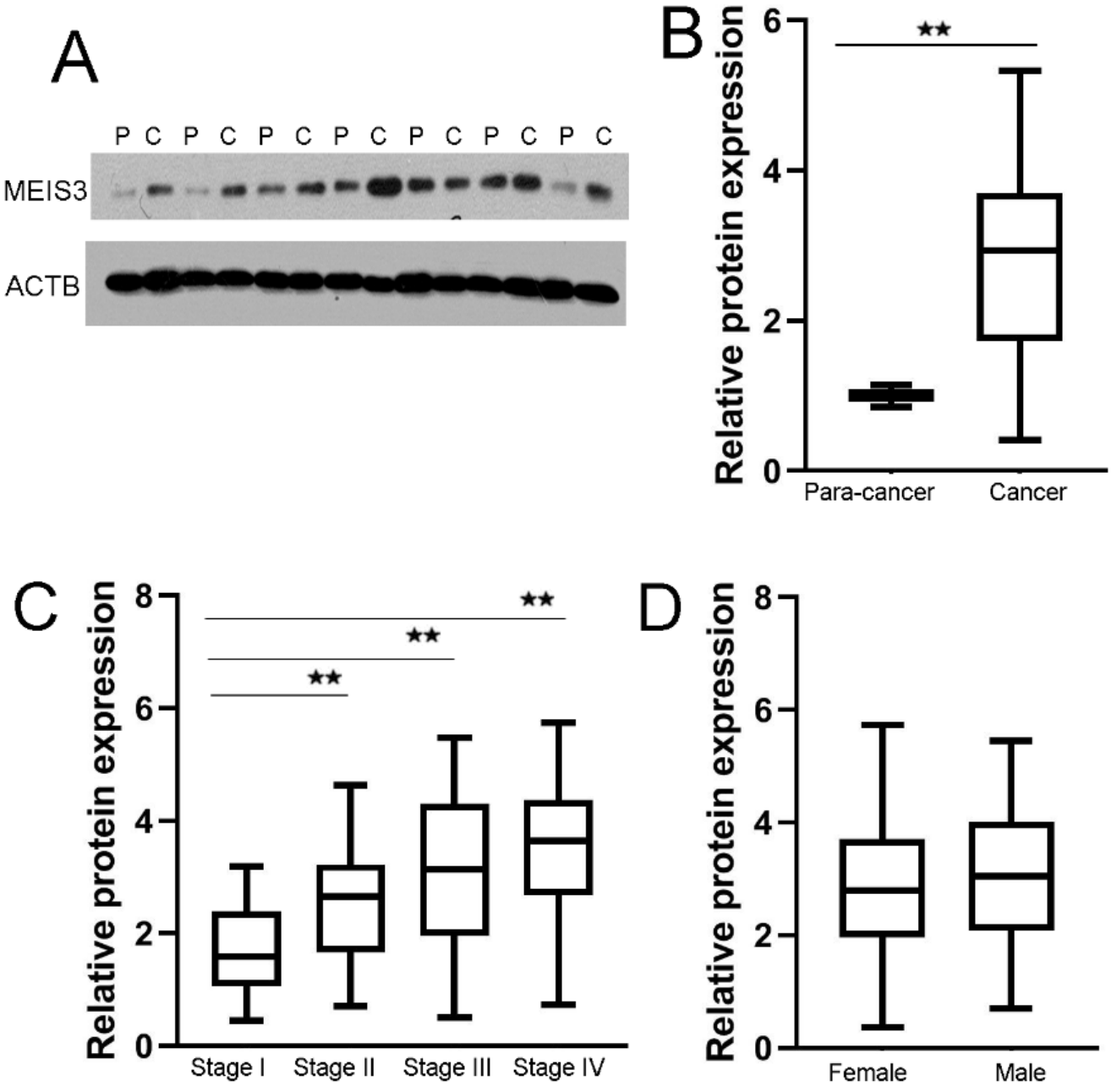

Figure 3

Expression of MEIS3 in colorectal cancer (A)The expression of MEIS3 in cancer and para-cancer tissues. (B) The relative expression of MEIS3 at the protein level in CRC tissues and para-cancer tissues. (C) MEIS3 Expression in cancer tissues of different clinical stages patients. (D) Expression level of MEIS3 in cancer tissues of patients of different genders One asterisk means $p<0.05$, and two asterisks mean $p<0.01$.

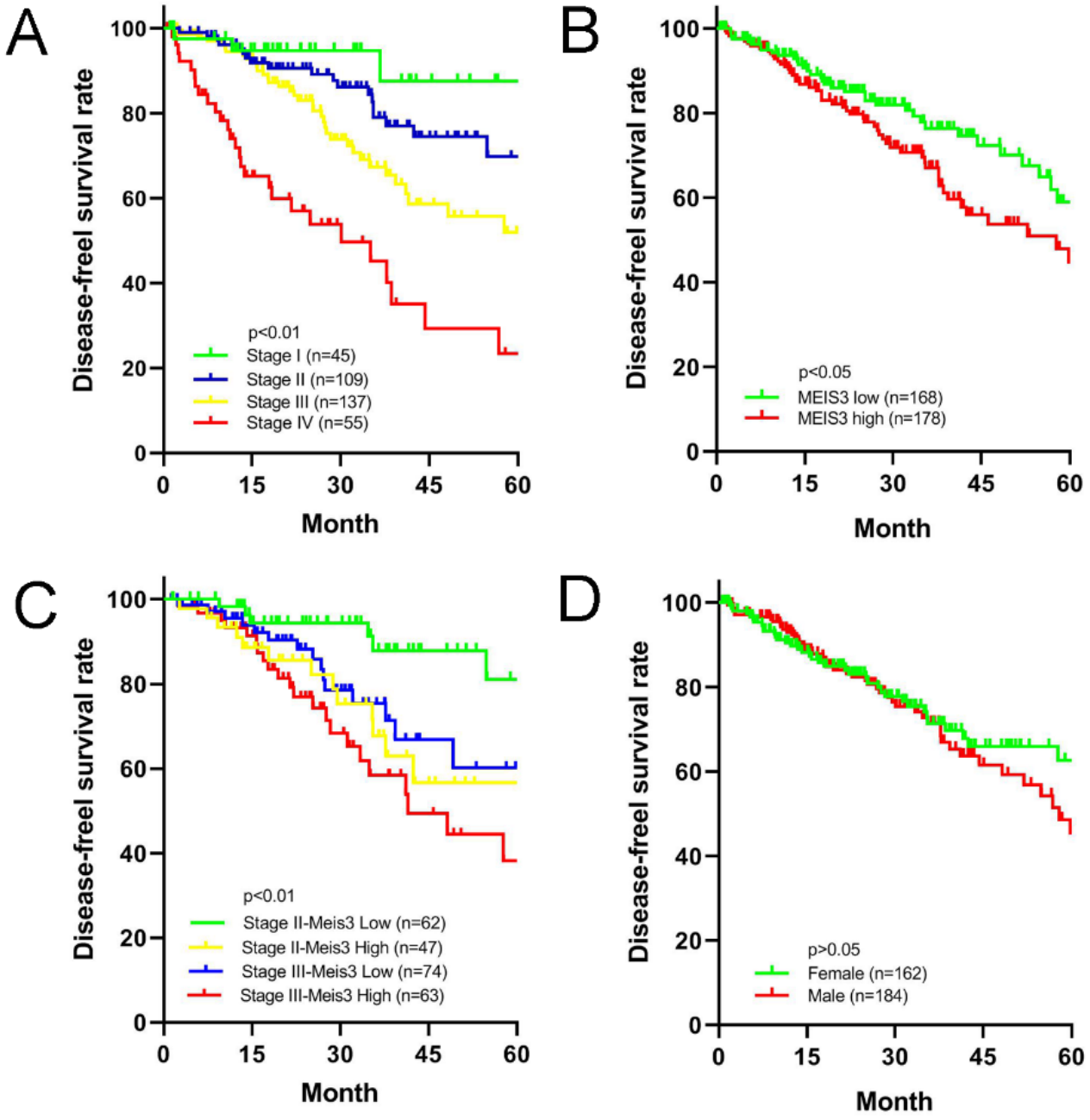

Figure 4

Disease-free survival of patients with different factors (A) The 5-year DFS of patients at different clinical stages (p<0.01). (B) The 5-year DFS of patients with high and low MEIS3 expression (p<0.05). (C) The 5-year DFS of patients with high and low MEIS3 expression in Clinical Stage II and III (P <0.01). (D) The 5-year DFS of patients with colorectal cancer of different genders (p>0.05).

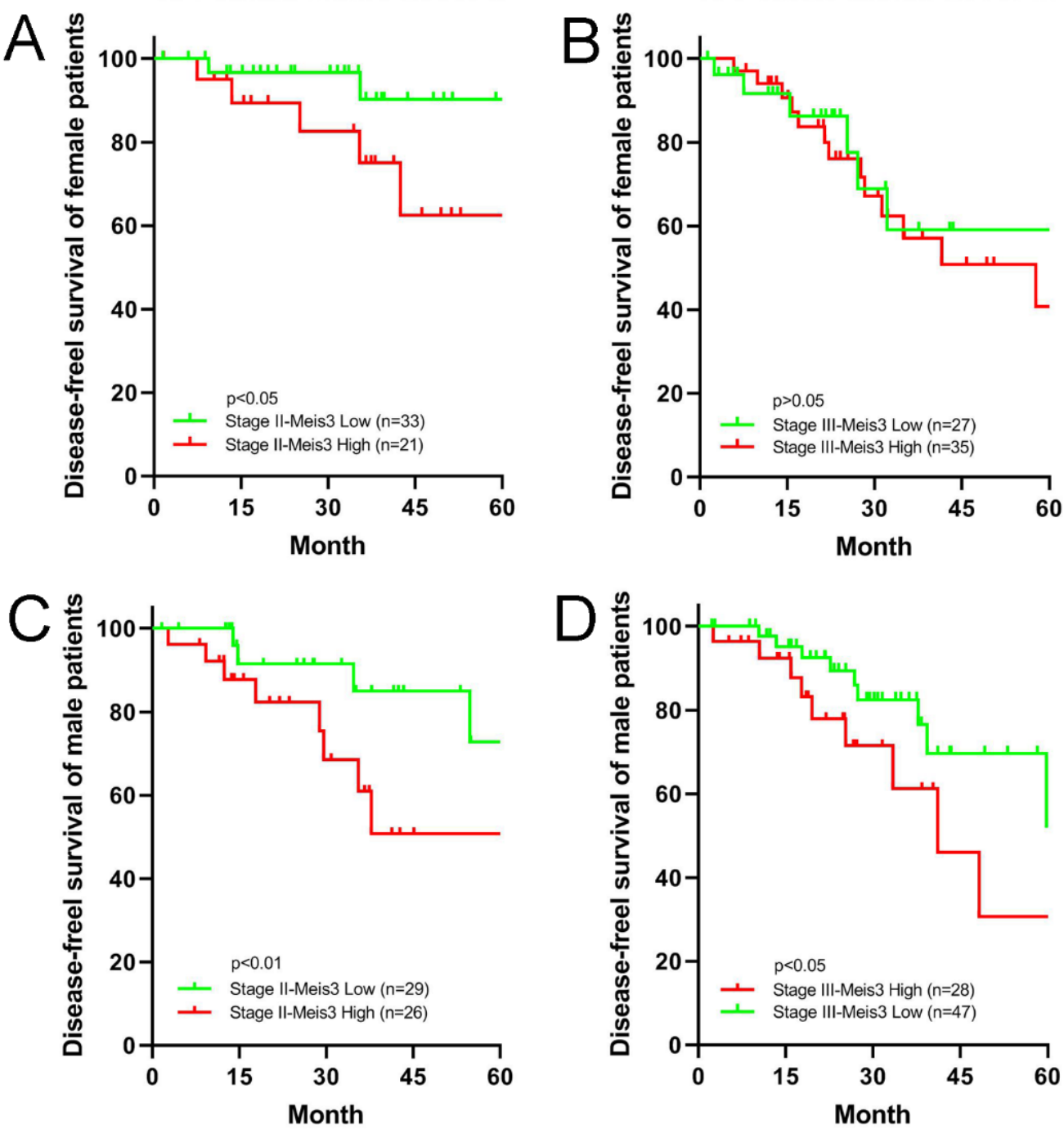

Figure 5

Association of MEIS3 expression level and gender on the 5-year DSF of clinical stage II and III patients (A) For female patients in stage II with high MEIS3 expression, the 5-year DFS was significantly lower than those with low expression ($p<0.05$).(B) There was no significant difference in the 5-year DFS between stage III female patients with high and low MEIS3 expression ($p>0.05$). (C) The 5-year DFS in stage II male patients was significantly lower in the MEIS3 high expression group than those with the low expression ($P <0.05$). (D) For male patients in stage III with high MEIS3 expression, the 5-year DFS was significantly lower than that of low expression ($p<0.05$).

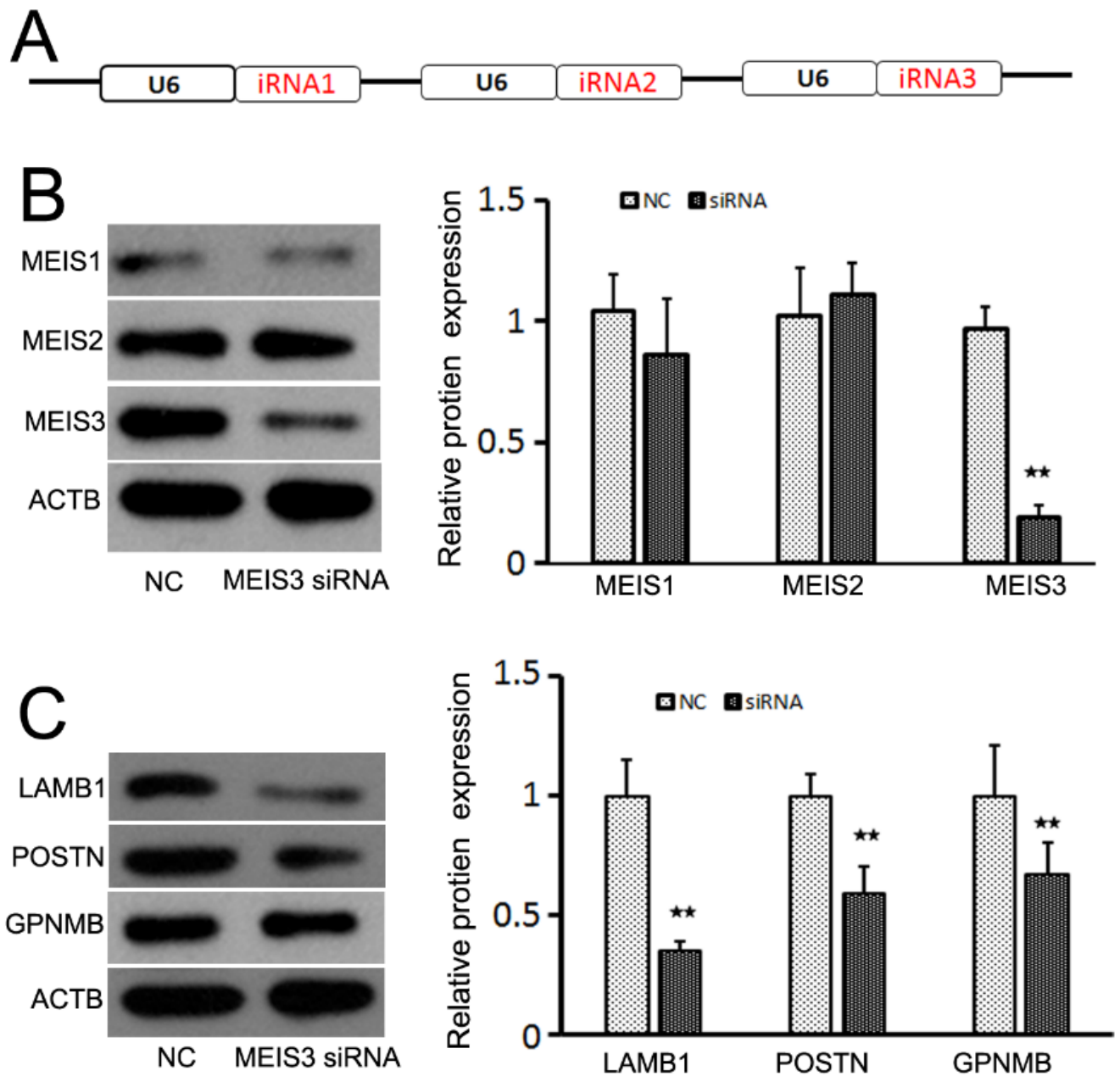

Figure 6

MEIS3 regulates the genes expression (A) Construction of tandem U6-shRNA virus expression vector (B) Effect of transfection of MEIS3 silenced lentivirus on the expression of MEIS family member genes (C) MEIS3 silencing resulted in the decrease of LAMB1, POSTN and GANMB gene expression levels

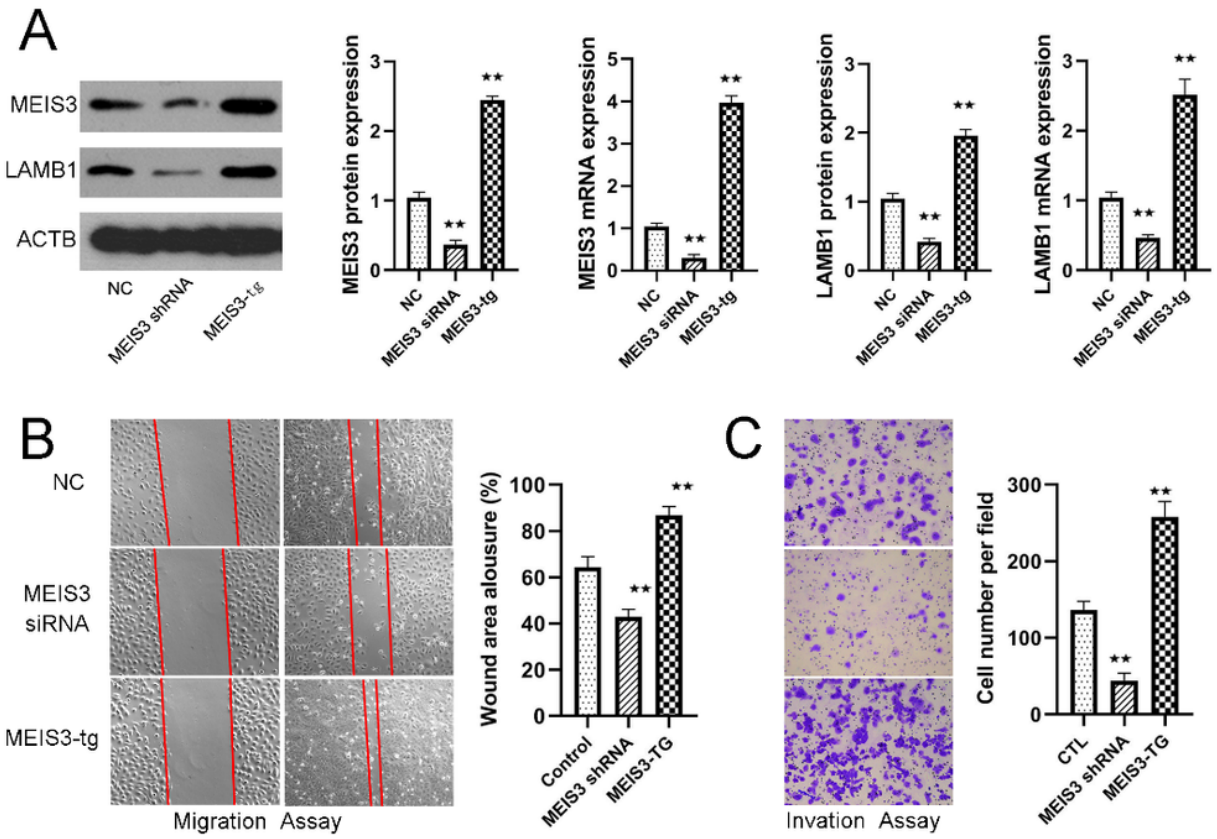

Figure 7

MEIS3 regulates the migration and invasion of cancer cells through LAMB1 (A) MEIS and LAMB1 expression when MEIS3 was silenced and overexpressed. (B) The cells migration level was significantly reduced when MEIS3 was silenced, and it was enhanced after MEIS3 overexpression. (C) The cells invasion ability was significantly reduced for MEIS3 silencing, and it was enhanced when MEIS3 overexpressed.

Supplementary Files

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- S1.jpeg
- S2.png
- S3.png