High MEIS3 level in colorectal cancer of stage II/III indicates poor prognosis

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Research

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Abstract

Background:

The wnt/beta-catenin signaling pathway plays indispensable roles in tumor budding and EMT process. Myeloid ecotropic viral insertion site 3 (MEIS3), a direct target of wnt/ β -catenin, promotes vagal neural crest cells migrating into the gut tissue during development, while its role in cancer process remains unclear. So, we wonder the roles MEIS3 plays in CRC progression.

Methods: We analyzed the association between MEIS3 protein expression and clinical stages of CRC patients. The cell biology methods were employed to analyze tumor cell migration and invasion activity. Finally, Kaplan-Meier analysis was used to exhibit the relationship between the proteins level and disease-free survival (DFS) and overall survival (OS) of CRC patients.

Results: We found that MEIS3 was mainly located in highly invasive areas including growth front of CRC tissues. And its expression level increased with the CRC progression according clinical stages. In vitro analysis showed that MEIS3 may promote CRC cells metastasis by enhancing migration and invasion process. Stratified by expression level, the 5-year DFS of patients with high expression was lower than MEIS3 low expression patients (40.6% vs 61.7%, $p < 0.0001$). We found that MEIS3 levels could be used to stratify patients at specific clinical stages. The 5-year DFS of stage II patients with MEIS3-high expression was significantly deteriorative than that of MEIS3-low expression (53.4% vs. 67.3%, $p = 0.0174$). The 5-year DFS III patients with high MEIS3 expression was also significantly deteriorative than those of low MEIS3 expression (30.9% vs 49.5%, $p = 0.0038$).

Conclusion: This study intimates that MEIS3 may be a biomarker for recurrence of stage II/III patients, and promote the cancer cells metastasis process.

1. Introduction

In recent decades, radical surgical resection and subsequent adjuvant radiotherapy and chemotherapy have been the most important treatment for CRC patients.[1, 2] Yet, cancer metastasis and recurrence have always been the dominant mortality reason in CRC patients. At least 50% of the patients have in situ and/or distant metastasis in liver, lung, bone and other tissues after 2-5 years since surgery.[1, 3, 4] Even 25% of CRC patients have already undergone clinical metastases at the time of diagnosis or surgical resection.[3, 4] Currently, the recurrence risk prognosis is mainly based on metastasis into lymph node, vascular and nerve infiltration found in operation, results of computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), pathological report form histopathological grading, gene stability during surgery.[1, 2, 5] Based on these results, clinicians decide whether to give adjuvant radiotherapy and chemotherapy, targeted therapy, palliative treatment or other measures to patients. However, it's difficult to identify early micro-metastases through these clinical indicators, resulting in failing to receive appropriate intervention for part patients with cancer metastases. Therefore, we still need to find markers for clinical detection.

During cancer progression, dysproliferation makes tumours growing continuously, while cell migration and invasion leads to cancer recurrence in situ or distant tissues. It has been confirmed that epithelial-mesenchymal transition (EMT) and tumour budding determine the transformation of differentiated cancer cells from stable characteristics to active invasion activity, which are the leading events of cancer metastasis, and then dominate the process of cancer recurrence.[6, 7] The structural genes which participate in EMT and tumour budding display regulatory roles through the alteration of protein level and activity. In body fluids and/or cancer tissue of patients, this process is accompanied by changes in abundance level or tissue localization pattern of the protein. So, these proteins have potential characteristics as early biomarkers of tumor metastasis.[8-10]

During EMT and tumour budding processes, wnt/ β -catenin, TGF- β , MAPK, Notch, Sonic Hedgehog (SHH), related regulation factors and downstream genes play essential and crucial roles.[7, 11] After transfers from cell membrane to the nucleus, β -catenin form complex with wnt protein, and bind to the promoter region of target genes to regulate gene expression which induce cell invasion activity.[11] Multiple transcription factors, including three amino acid loop extension homeobox (TALE) proteins, are required to form transcription complex with wnt and/or β -catenin when promoting cell migration and invasion.

MEIS3, a homeobox protein of TALE family which are expressed with a spatial and temporal pattern, and indispensable for early embryological development.[12, 13] TALE family consists of MEIS and pre-B cell leukemia (PBX) family genes.[14] The MEIS family includes MEIS1, MEIS2, MEIS3, Prep1 and Prep2 genes, which are essential for embryonic development and cancer progression.[13, 14] MEIS1 plays crucial roles for erythropoiesis, megakaryopoiesis and hematopoietic stem cell activity, and its overexpression lead to myeloid leukemia.[15-17] The MEIS2 is indispensable for development of heart and cranial nerves, closely associated to leukemia with overexpression.[18, 19] The MEIS3 protein promotes the posterior neural cell migrating into hindbrain and gut tissues. And MEIS3 deficient causes Hirschsprung's disease in infants and children, a genetic disease with congenital megacolon as the main symptoms.[20] As a target gene of wnt/ β -catenin, MEIS3 plays such an important role in regulating cell migration, yet we still lack its information about gut tumor progression.[21, 22]

Based on the function MEIS3 played in gut development, we hypothesized that MEIS3 may play important roles in CRC progress, metastasis, recurrence and survival of patients. Therefore, we analyzed the location pattern of MEIS3 in CRC tissues, the association between overall protein expression level and cancer progression, CRC metastasis, and possible roles in CRC cells migration, invasion through in vitro cell experiments. Further, we stratified the patients by protein level and clinical stage to verify the value of MEIS3 as a prognosis biomarker for CRC recurrence after radical resection.

2. Material And Method

2.1 Clinical Samples

This study was approved by the ethics committee of the Third Affiliated Hospital of Soochow University, and in accordance with the International Ethical Guidelines for Human Biomedical Research standards,

signed informed consent with patients. The clinical staging of CRC patients was carried out in accordance with the American Joint Committee on Cancer (ACJJ) standard, meaning, the clinical staging was based on the cancer's tissue invasion, lymph node metastasis and distal metastasis[1]. All patients were treated in accordance with the CRC guidelines of the Chinese Society of Clinical Oncology (CSCO). During follow-up, once the patient was found to recurrence, it was recorded as the endpoint of DFS, and if the patient died due to other than CRC, the data was deleted. From 2015 to 2020, a total of 323 patients participated in the study, and finally 292 patients were included in the analysis.

2.2 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-MMP2 (Proteintech), Rabbit anti-E-Cadherin(CST), Mouse anti-Beta Catenin(Proteintech), Mouse anti-Vimentin (Proteintech), 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). Cell culture medium and serum were purchased form Thermo Fisher Scientific.

2.3 Western Blot

The Western Blot (WB) was carried out according to the description operation of molecular cloning Laboratory Manual.[23] After the development is completed, use Quantitative one 4.40 to analyze the strips according to the relative concentration.

2.4 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% to 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 running water for 1 minute, and incubated in 10% rabbit serum for 1 hour. Then, the serum was replaced by antibody and incubated overnight at 4°C. In next day, the slices were washed with PBS three times, and incubated in HRP-conjugated IgG 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.

2.5 Construction of virus particles

The lentiviral particles for MEIS3 silencing were packaged with the assistance of ZQXZ Bio Tech. (Shanghai) Corp. Ltd.. 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'.

2.6 Cell invasion capabilities analyze

The cells were first cultured in DMEM without FBS for 24 hours. During this period, the Transwell chambers were soaked and moistened with 1×PBS, then matrigel was added, and the gel was incubated in a CO₂ incubator at 37°C to solidify the gel. Then add 600µl of complete medium containing FBS to the lower chamber, add 200µl of DMEM hanging cells (density 5×10⁵ cells/ml) to the upper chamber, and continue to culture for 24hrs, stain with 0.5% crystal violet, and count the results.

2.7 Public database data

The patients MEIS3 mRNA level, DFS and OS analysis of TCGA database was performed on the GEPIA portal (<http://gepia.cancer-pku.cn/>).[24] The data of GSE17537 in the GEO database was downloaded from GenomicScape (<http://www.genomicscape.com/>) and analyzed again with Graphpad.[25] And independent OS analysis was also implemented on this platform.

2.8 Statics analysis

We chose GraphPad Prism 8.0 (GraphPad Software, CA, USA) to perform the statistical analysis. The T-test and/or One-way ANOVA were used to analyze the difference of comparisons in protein level, cell migration and invasion. The 5-year DFS and OS of patient survival analysis with different factors were estimated with the Kaplan–Meier curves, log-rank tests.

3. Results

3.1 MEIS3 location in CRC tissues

Firstly, we analyzed localization pattern of MEIS3-positive cell in CRC tissues by immunohistochemistry. Although regulated by the wnt/ β -catenin protein, distribution pattern of MEIS3-positive cells was not consistent with the characteristics of β -catenin.[26, 27] As shown in Fig.1, MEIS3 protein was not found in tumor bulk cells which were well differentiated (Fig. 1A, B), but in cell nucleus of cancer invasion front (Fig.1C, D). The invasion fronts dominate the process of cancer invasion into para-tissues and metastasis into distant organ.[28-30]

The second region containing abundant strong MEIS3-positive cell was tumour–stroma interface (Fig. 1E, F), which distribution was similar to that of vimentin in CRC tissues.[30, 31] And MEIS3-positive cells were widespread in undifferentiated regions where cancer cells proliferate vigorously (Fig.1G, H), yet the intensity in nucleus was much weaker than the invasion fronts and tumour–stroma interface. We also found tumour budding cells in the MEIS3 positive area, which have active migration ability (Fig. 1D, F). Thus, the immunohistochemistry results suggested that MEIS3 was related to cancer metastasis and recurrence.

3.2 Association between MEIS3 expression and colon cancer metastasis

Considering the association between clinical stages and cancer metastasis, we used clinical stages as CRC metastasis indicator. We employed the ratio of protein abundance between cancer and para-tissues to evaluate MEIS3 protein level. We found that the MEIS3 level was significantly increased in CRC tissues than para-tissues (2.81 ± 1.45 folds, $p < 0.01$, Fig.2A, 2B), and incrementally increased with CRC progresses (stages I/II/III/IV: $1.71 \pm 0.94/2.52 \pm 0.97/3.16 \pm 1.43/3.42 \pm 1.31$ folds, $p < 0.01$, Fig. 2C).

To verify the results, we employed the GSE4107 cohort data to analyze the MEIS3 expression in CRC tissues.[32] As shown in Fig.2D, MEIS3 mRNA level in cancer tissues were significantly increased to about 3 folds than that in normal control ($p = 0.015$). Then, we employed the GEPIA platform to analyze the association between MEIS3 level and clinical stage in TCGA cohort.[24] We found that MEIS3 mRNA level in CRC tissues incrementally increased with clinical staging progression (Fig 2E). Although a quantity of genes were found to be gender-related expression, we did not find this trend on MEIS3 in our cohort (female vs male: 2.86 ± 1.29 vs. $3.05 \pm 1.1.19$, $p = 0.43$, Fig.2F). These results shew that MEIS3 expression was positively correlated with CRC metastasis progression, and could be a candidate biomarker for surgical prognosis of CRC patients.

3.3 MEIS3 enhances CRC cell metastasis

Based on the distribution characteristics of MEIS3-positive cells (Fig.1), the correlation between its level and CRC progress (Fig. 2) in tissues, and promoting neural cell migration into gut tissue during development, we suspected that MEIS3 may contribute to CRC metastasis by regulating cancer cell migration and invasion.[21, 22] Therefore, we constructed lentiviral particles carrying three tandem U6-promoter-MEIS3-shRNA which employed to silence MEIS3 expression in SW480 and SW1116 cells (Fig.S1A). Through analyzing the mRNA and protein levels of MEIS1, MEIS2 and MEIS3, we determined that the virus particle could specifically and effectively silence the MEIS3 expression (Fig.S1B-C Table S1).

The scratch wound-healing assay was used to analyze the ability of cell migration. When the wound area of the control cells was healed by $67.1 \pm 7.82\%$, only $34.3 \pm 4.73\%$ region was covered by SW480 cells with silenced MEIS3 expression ($p < 0.001$ Fig.3A). The migrated area of SW116 cell was also significantly decreased for MEIS3 silence (Wound area clousure, shCTL vs. shMEIS3: $63.7 \pm 5.12\%$ vs. $41.5 \pm 3.10\%$, $p < 0.001$ Fig.3B).

The invasion process of cancer cells relies on degrading the surrounding matrix and penetrating into adjacent tissues, so we employed Matrigel covered transwells to analyze its invasion ability. When MEIS3 was silenced, the penetration level of SW480 cells was reduced by 61.8% (Transwell cell number, shCTL vs. shMEIS3: 101.3 ± 6.03 vs. 38.7 ± 3.05 , $p < 0.001$) ability (Fig.3C). The same treatment reduced the invasion level of SW116 cells by 44.2% (shCTL vs. shMEIS3: 97.3 ± 5.03 vs. 54.3 ± 6.11 , $p < 0.001$ Fig. 3D). It shew that the migration and invasion ability of CRC cells was significantly down-regulated for MEIS3 silencing.

Furthermore, we analyzed the proteins levels of LAMB1, E-Cadherin, VIM, MMP2 and β -catenin which were responsible for migration and invasion of tumor cell in EMT and tumour budding process.[6, 7, 11] We found that the protein expression of LAMB1, VIM and MMP2 were significantly decreased, the expression level of E-Cadherin also increased, and β -catenin level did not show significant change corresponding to MEIS3 silencing (Fig. 3E). Therefore, high MEIS3 expression may increase CRC metastasis from enhance EMT and/or tumour budding process.

Then we made an expression association analysis between MEIS3 and genes that regulate tumor cell invasion and proliferation in TCGA cohort on GEPIA platform.[24] MEIS3 expression was positively correlated with genes performing cell migration and invasion with medium to strong level, such as VIM($R=0.78$, $p<0.001$), MMP2 ($R=0.71$, $p<0.001$), LN1($R=0.63$, $p<0.001$), LAMB1($R=0.15$, $p=0.013$); genes regulating EMT and tumour budding process including twist1 ($R=0.66$, $p<0.001$), twist2 ($R=0.72$, $p<0.001$), snail1 ($R=0.53$, $p<0.001$), snail1 ($R=0.71$, $p<0.001$), and TGFB($R=0.77$, $p<0.001$); yet weakly negative or even no correlation with the genes regulating cell proliferation: cyclin D1($R=0.15$, $p=0.011$), PCNA ($R=-0.12$, $p=0.023$) and MYC ($R=-0.1$, $p=0.083$) (Fig. S2). These results also suggested that MEIS3 might lead to cancer metastasis by regulating the migration and invasion capabilities of CRC cells.

3.4 High MEIS3 expression and poor prognosis of CRC patients

Then we used the Kaplan–Meier curve to analyze 5-years DFS of CRC patients stratified by MEIS3 level. As shown in the figure 4A, in the overall study population, the 5-year DFS of patients with high and low MEIS3 expression were 61.7% and 40.6% respectively($p<0.0001$, $HR=2.441$, 95% CI:1.493 - 3.989 Fig.4A, Table S2). However, when grouped by gender, age, tumors volume and location factors, the 5-year DFS of patients showed no significant difference (Table S2).

To verify this result, we analyzed the recurrence risk of CRC patients from TCGA cohort on GEPIA platform.[24] The 5-year-DFS of high MEIS3 expression patients was significantly depressed than that of low expression cohort with a cutoff 50% ($p=0.0026$, Fig 4B). And the GSE17537 cohort also showed the same trend ($p=0.0073$, Fig 4C). Therefore, MEIS3 can be employed to independently assess the recurrence for CRC patients after surgery.

We also analyzed the 5-year OS rate grouped by MEIS3 level. The 5-year OS of high MEIS3 expression cohort was 42.6%, which was significantly pessimistic than that of low level group (62.3%, $p<0.001$, Fig 4D). As to TCGA cohort, 5-year OS of patients with high MEIS3 level was also significantly pessimistic than that of the low level with a cutoff 45% ($p=0.0084$, Fig 4E). Another independent survival analysis for GSE17537 cohort also showed similar results ($p=0.00012$, Fig 4F). In conclusion, the high expression of MEIS3 was strongly correlated with poor prognosis in CRC patients.

3.5 High MEIS3 expression indicate recurrence-risk in stage II/III patients

Currently, recurrence prognosis for postoperative CRC patients was dominantly based on very clinical stages but not gene expression level.[1] We wondered whether can make a more precise prognosis when

MEIS3 level was combined with clinical stage. So, we performed a multivariate analysis according to MEIS3 level with ACJJ stage and other independent factors (Table 1). As shown in Fig.5, the 5-year DFS of stage II patients with high MEIS3 expression was significantly deteriorative than cohort with low MEIS3 level (53.4% vs. 67.3%, HR=2.38, 95% CI: 0.9843 -5.786, p=0.0174, Table 1, Fig. 5B). The 5-year DFS of stage III patients with high MEIS3 level was also significantly deteriorative than cohort with low MEIS3 level (30.9% vs. 49.5%, HR=2.817, 95% CI: 1.370-5.792, p=0.0038 Table 1, Fig. 5C). Moreover, the 5-DFS was comparable between cohort of stage II with high MEIS3 expression and cohort of stage III with low MEIS3 expression (53.4% vs.49.5%), and between cohorts with high MEIS3 expression in stage III and overall stage IV patients (30.9% and 29.6% Fig.5C, Table S2). However, when grouped by MEIS3 level, there was no prognostic difference in stage I or IV patients (Fig.5A, D, table 1).

4. Discussion

It's well known that during tumor/cancer progression, hyperactive cell proliferation, migration and EMT are the leading factors which also occurs during embryonic development under orderly regulation. [31] Genes that play important roles in embryonic development also tend to regulate cancer progression. [9, 31] Clinical cases have shown that deletion of 19q13.32 region where the MEIS3 gene is located leads to symptoms such as developmental delay, short stature, and Hirschsprung's disease.[20] MEIS3 promotes neural cell to migrate into gut tissue during development and is overexpressed in invasion front of CRC tissues, especially in tumour budding cell which present high metastasis activity.[22] It is also highly over-expressed in tumour stroma, which is consistent with the expression characteristics of vimentin.[30, 31] Highly expressed vimentin promote cell invasion which is one of the hallmark events of EMT and positively correlated with cancer deterioration.[30]

We found that MEIS3 expression increased with the metastatic CRC progression in multiple patient cohorts. In our CRC cohort, the MEIS3 protein level was significantly higher in cancer than adjacent tissues, and incrementally increased with the deterioration of cancer metastasis. In TCGA cohort, the MEIS3 mRNA level was also increased with the progression of cancer stages. Although the evaluation indicators and results in our and TCGA cohorts were different, the trends were similar. Therefore, MEIS3 level in cancer tissue reflects the cancer metastasis risk in patients.

Reported results have shown that MEIS3 was essential in migration of nerve cells in gut.[21, 22] We found that the migration and invasion capabilities of cancer cells were significantly reduced for MEIS3 silence, confirming the value of this gene in cancer metastasis. These results were also consistent with MEIS3 expression pattern in CRC tissues, meaning IHC signal intensity of MEIS3 in cells with high metastatic activity was stronger than that in active proliferation cells, and negative in differentiated tumor stroma cells.

The wnt/ β -catenin signaling pathway is an indispensable factor in cell metastasis. Studies have shown that β -catenin protein directly binds to MEIS3 promoter region, while Wnt3a is a necessary condition for MEIS3 transcription.[12] Then, translated MEIS3 protein participates DNA-binding complex formation with

Pbx and/or Hox to control the transcription factors accommodation on promoters of target genes. [21] Finally, MEIS3 regulates nerve cells migration during gut development by activating the expression of SHH, primary nerve cell differentiation through FGF3 and FGF8 activity.[12, 22, 33] In this study, we found that MEIS3 can promote the gene expression, such as VIM, MMP2, LamB1, which work as behavioral factors in cell migration and invasion.[6, 7, 11, 31] Moreover, MEIS3 are significantly and positively correlated with MMP2, Vimentin, LamB1, FN1 at high or moderate levels in CRC tissues, suggesting that it may promote cancer metastasis with directly or indirectly pattern. These genes are also functional members in EMT and tumour budding process, but we need more detailed and molecular-based studies to determine how MEIS3 plays crucial role in these processes.

The Prognostic diagnosis of recurrence risk was mainly based on the clinical stages, pathological assay including Chest X Ray and PET report of the patient, which were the basement whether to give adjuvant radiotherapy and chemotherapy, targeted drugs, or palliative care.[1, 2, 5, 34] Molecular and cellular biological abnormalities are the main causes of tumor metastasis, protein expression pattern highly correlated with cancer metastasis are expected to be prognosis biomarkers for recurrence.[5, 8]

When stratified the postoperative patients by MEIS3 level, we found that the DFS and OS of high expression cohort were significantly worse than patients with low expression. These results were also supported by the cohorts from ATGC and GSE17537. When we stratified patients in a specific clinical stage by MEIS3 level, we found that patients could be regrouped. Among stage II patients, sub-cohort with MEIS3-high expressing had a similar recurrence risk to stage III patients with low MEIS3 expression, while those with low MEIS3 expression had a recurrence risk closer to stage I patients. Similarly, among stage III patients, recurrence risk of those with MEIS3-high expression was almost same as overall stage IV patients. Thus, when stratified by MEIS3 protein level and clinical stages, we could detect patients with higher recurrence risk in stage II and III cohorts, but not discovered with current clinical methods. In contrast, ECA, CA 19-9 and other biomarkers are not more discriminative than clinical staging in predicting recurrence after operation.[8, 35, 36] Therefore, the combination of gene expression and clinical stage to predict postoperative recurrence may be a more effective method, which can effectively screen out patients with high recurrence risk after radical resection. Thus, clinicians could give appropriate adjuvant radiotherapy and chemotherapy in time to improve their survival and life quality.

Ordinarily, we utilize IHC, WB, and Q-PCR to analyze expression characteristics for specific genes. Yet, IHC can be easily affected by the personal judgment of the observer, Q-PCR reflect the transcriptional activity, while WB analyze protein level which is biologically functional product of the target gene. Moreover, MEIS3 transcription is repressively regulated by its protein, therefore, it might be a more objective analyze the MEIS3 expression employing WB technique.[37]

Open databases and analysis platforms such as TCGA and GEO provided abundant clinical and corresponding gene expression data most of which were mRNA levels based on gene chips and high-throughput sequencing. [24, 25] These make our research based on a more solid and reliable foundation, although the expression of mRNA and protein is not a simple linear relationship. Our research has also

benefited from public platforms such as TCGA and GEO, but we need larger retrospective cohort to study the feasibility of MEIS3 as a biomarker for high recurrence risk, before applied in clinical for improving their survival rate and life quality.

We also found that MEIS3 can promote the metastasis through activating functional genes, such as LAMB1, vimentin and LN1 which also play crucial roles in EMT and tumour budding process.[29, 38] Yet, we don't know the specific genes and regulation process. We need further research to identify the molecular mechanism of MEIS3 promoting cancer cells metastasis.

5. Conclusion

In summary, we found that high MEIS3 expression indicated a high metastasis risk for CRC and was a great promise biomarker of CRC recurrence for postoperative patients.

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Abbreviations

CRC: colorectal cancer; MEIS3: myeloid ecotropic viral insertion site 3; ACJJ: American Joint Committee on Cancer; WB: Western Blot; DFS: disease-free survival; HR: Hazard ratio; CI: confidence interval,

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.

Tables

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%

Figures

Figure 1

Localization of MEIS3 protein in CRC tissues

MEIS3 was not detected in well differentiated CRC tissues (A and B), deeply stained in the cancer nucleus of growth front (C, D) and tumour–stroma interface regions (E, F), widely distributed in poorly differentiated areas, but the intensity was weak (G, H). These suggested that MEIS3 is associated with 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. Arrows point to tumor budding cells. The bar means 50 μ m.

Figure 2

Expression level of MEIS3 in colorectal cancer

The MEIS3 protein in CRC cancer and para-cancer tissues were detected by WB (A) based on which we analyzed the relative level of MEIS3 protein in CRC tissues as to para-cancer tissues in our (B) and GSE4107 cohort (D) and tendency of MEIS3 Expression in cancer tissues according to clinical stages in our (C) and TCGA cohort (E). The relative protein expression of MEIS3 in CRC tissues was also analyzed according genders (F).

p<0.01.

Figure 3

Silencing MEIS3 expression resulted in significant decrease of cell metastasis

After treatment with lentivirus particles expressing negative control shRNA and shMEIS3, we analyzed the wound area clousure of SW480 (A) and SW116 cells (B) by scratch assay, and crystal violet stained SW480 (C) and SW116 (D) cells in transwell analysis and counted. MEIS3 silencing also resulted in the decrease of vimentin, LAMB1, and MMP2, increasing of E-cadherin protein level (F). shCTL, negative control; shMEIS3, MEIS3 silencing lentivirus;

p<0.01

Figure 4

Disease free survival and MEIS3 level of CRC patients

The 5-year DFS of our cohort were stratified by MEIS3 level (A), and verified through independent analysis in GEPIA portal (B) and GSE17537 dataset(C). The relationship of 5-year OS of patients and MEIS3 level are shew from our cohort (D), GEPIA platform of TCGA cohort (E) and GSE17537 by GenomicScape portal (F).

Figure 5

Disease free survival in different clinical stages of CRC patients

The 5-year DFS of CRC patients post operation were stratified by MEIS3 level according clinical Stage I (A), stage II (B), stage III (C) and stage IV (D).

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