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Hsa-miR-1248 suppressed the proliferation, invasion and migration of colorectal cancer cells via inhibiting PSMD10

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Abstract

Background: Lymph node metastasis (LNM) is a critical event during the colorectal cancer (CRC) development and is indicative of poor prognosis. Identification of molecular markers of LNM may facilitate better therapeutic decision-making.

Methods: Six pairs of CRC tissues and corresponding adjacent tissues [3 pairs diagnosed as pT1N0M0 (M_Low group) and 3 pairs diagnosed as pT4N2M0 (M_High group)] collected from CRC patients who underwent surgical resection were used. MicroRNA sequencing was performed to screen differential microRNAs involved in CRC LNM. The selected microRNAs were validated in CRC tissues and cell lines using qRT-PCR. The functions of candidate hsa-miR-1248 were evaluated by CCK-8, colony formation, and Transwell assay. The binding of hsa-miR-1248 with its target PSMD10 was confirmed by luciferase activity assay, and the expression of PSMD10 in tissues was detected by droplet digital polymerase chain reaction.

Results: Ninety-five miRNAs were downregulated in carcinoma tissues (M_Low and M_high groups) compared with the normal group. Their expression in M_High group was significantly lower compared with M_Low group. The top 3 were hsa-miR-635, hsa-miR-1248, and hsa-miR-668-3p. After validation in tissues/cell lines, only hsa-miR-1248 was decreased in high metastatic tissues or SW620 cells compared to low metastatic tissues or SW480 cells. Hsa-miR-1248 was found to inhibit CRC cell viability, proliferation, invasion, and migration. The tumor suppressor effect of hsa-miR-1248 in CRC cells was attenuated or enhanced by up-regulating or down-regulating PSMD10, respectively.

Conclusion: Hsa-miR-1248 may act as a tumor suppressor gene in CRC by targeting and inhibiting PSMD10, which provides a clue for CRC treatment.

Keywords: Hsa-miR-1248, Colorectal cancer, PSMD10, Invasion, Migration, Lymph node metastasis

Introduction

Globally, colorectal cancer (CRC) is the 3rd most common cancer and the second leading cause of cancer-related deaths [1]. Lymph node metastasis (LNM) is the most critical event during the progression of CRC and is indicative of poor prognosis. LNM also plays an essential role in the therapeutic decision-making for CRC. Unfortunately, approximately 20% of all CRC patients with no histopathological evidence of LNM

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(stage I or II disease) develop recurrence within 5 years [2]. Moreover, there is emerging evidence indicating that lymph node micro-metastasis is associated with poor prognosis [3]. Therefore, identification of molecular markers of LNM will help improve therapeutic decision-making in patients with CRC.

MicroRNAs (miRNAs) are short endogenous non-coding RNAs (length 19–21 nucleotides) which regulate their target gene expression post-transcriptionally [4]. MiRNAs are remarkably stable and have been shown to play an essential role in different types of cancer development via competing endogenous RNA (ceRNA) mechanisms. The binding of 5' nucleotides 2–7 of miRNAs with the 3'UTR of target mRNAs results in the suppression of the expression of target mRNAs [5, 6]. In other words, if miRNAs have been verified to have an abiding site with the predicted mRNAs, the expression of the mRNAs shows a negative correlation with miRNAs. For example, hsa-miR-1248 was reported to bind with cyclin D2 and tumor-promoting gene tripartite motif-containing protein 24 to regulate the progression of non-small cell lung cancer (NSCLC) [7, 8]. Hsa-miR-1248 was also found to regulate the expression of C3A in lung squamous carcinoma cells [9]. However, the role of hsa-miR-1248 in the context of CRC is not well characterized.

Proteasome 26S subunit non-ATPase 10 (PSMD10), also named Gankyrin, has been reported to be over-expressed in CRC and correlated with TNM stage and metastasis [10]. In our recent study, CRC patients with higher PSMD10 expression exhibited a higher risk of occult liver metastases and lower progression-free survival (PFS) rate [11]. However, the relationship between PSMD10 and CRC lymph node metastasis is not clear. Moreover, several miRNAs have been reported to regulate the expression of PSMD10. For example, miR-214 was shown to decrease the proliferation and metastasis of thyroid carcinoma cells through binding with PSMD10 [12]. MiR-1254 was shown to inhibit CRC progression by binding with PSMD10 [13]. However, the relation between hsa-miR-1248 and PSMD10 is unclear.

In this study, we explored the differentially expressed miRNAs among paratumoral normal colon tissues, low lymph node metastatic carcinoma (T1N0M0), and high lymph node metastatic carcinoma (T4N2M0) via miRNA sequencing. Our results indicated that hsa-miR-1248 as a critical miRNA may participate in the CRC LNM. Then, we explored the function of hsa-miR-1248 and its binding site of PSMD10. Hsa-miR-1248 was found to decrease CRC cell proliferation and metastasis by inhibiting PSMD10. This study may provide new insights for CRC therapeutic strategies.

Materials & methods

Tissue samples

In this study, we collected 6 pairs of CRC tissues and corresponding paratumoral normal tissues from CRC patients who received surgical resection at the Department of Gastrointestinal Surgery at the Jiangmen Central Hospital, Guangdong Province, China between 2019 and 2020. None of the patients received radiotherapy, chemotherapy or immunotherapy before operation. Patients were diagnosed following the introduction of AJCC 8th Edition: Colorectal Cancer. The samples included three pairs of low metastasis CRC tissues (without lymph node metastasis) diagnosed as pT1N0M0 and their corresponding adjacent tissues, three pairs of high metastasis CRC tissues (with lymph node metastasis) diagnosed as pT4N2M0 and their corresponding adjacent tissues. Informed consent was obtained from all patients. All procedures were approved by the Institutional Research Ethics Committee of the Jiangmen Central Hospital (decision no. JXY202229).

miRNA sequencing

Low metastasis CRC tissues (M_Low group), high metastasis CRC tissues (M_High group), and their corresponding adjacent tissues were collected for miRNA sequencing, and each group contained three samples. After RNA isolation, RNA from three samples in the same group were mixed, and the RNA from the adjacent normal tissues of the two groups was also mixed as the Normal Control group (Normal group). Then libraries were prepared following the protocol of the TrueSeq Small RNA Sample Prep Kit (Illumina). Subsequently, the libraries were used for sequencing on the Illumina platform. The sequencing data were analyzed using the R package.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Tissues and cells were collected for RNA isolation using TRIzol (Thermo). Then RNA was reversed into cDNA following the protocol of HiScript[®] Reverse Transcriptase (Vazyme, Nanjing, China). PCR was performed on the ABI 7900 platform using AceQ[®] qPCR SYBR Green (Vazyme) as per the manufacturer's instructions. For calculating the relative miRNA expression, U6 was used as the internal standard; for calculating the relative PSMD10 expression, GAPDH was used as the internal standard. The cycling conditions were as follows: initial denaturation at 95°C for 10 minutes, then 40 denaturations at 95°C for 1 minute, and annealing/extension at 56°C for 1 minute. All samples were made in triplicate and standardized according to the internal control. The fold-changes or relative gene expression levels were

calculated based on the $2^{-\Delta\Delta Ct}$ method. The primer sequences used in this study are shown in Table 1.

Droplet digital polymerase chain reaction (ddPCR) assay

The PSMD10 expression was also detected by ddPCR. DdPCR was performed as described in a previous study with a few modifications [14]. In brief, RNA was extracted from tissues and reverse-transcribed into cDNA following the protocol of HiScript[®] Reverse Transcriptase (Vazyme, Nanjing, China), which was used as template. DdPCR was performed on the MicroDrop-100 droplet digital PCR system (Forevergen, China). The primers are shown in Table 1.

Cell culture

Human low metastasis colon cancer cell line SW480 and Human high metastasis colon cancer cell line SW620 were purchased from the Chinese Academy of Science Cell Bank (Shanghai, China). All cells were cultured in DMEM medium (Gibco, Grand Island, NY, USA),

containing 10% FBS at 37°C in a 5% CO₂ atmosphere. The cells were passaged at a ratio of 1:4. SW480 and SW620 cell line were used in all cell experiments in this study.

Plasmid and transfection

The has-miR-1248, miR-1248-sponges, PSMD10, and sh-PSMD10 were PCR-amplified from cDNA and cloned into lentiviral vectors (Yeasen Biotechnology, Shanghai, China). All primers used in the reactions for clone PCR are shown in Table 2. Cell transfection was performed using the Lipofectamine 3000 reagent (Thermo Fisher Scientific, Waltham, MA, USA) as per the manufacturer's instructions. Briefly, cells were digested, seeded, and cultivated for 24 h before transfection until the attainment of 60–70% confluence. Then, lipofectamine 3000 and relative plasmid were diluted in Opti-MEM individually followed by mixing at 37°C for 5 min. The medium was replaced with a new medium containing the DNA-lipid

Table 1 Primers used in the reactions for real-time RT-PCR

Gene	Sequence (5'–3')
GAPDH-up	GAGTCAACGGATTGGTCGT
GAPDH-dn	GACAAGCTTCCC GTTCTCAG
PSMD10-up	CATGTTACTGGAAGGCGGGG
PSMD10-dn	AGGAGTGTTACCCCTCAGTGTCT
hsa-miR-635-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCGACTGGATACGACGGACAT ACTTGGGCACTGAAACAAT
hsa-miR-1248-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCGACTGGATACGACTTTAGC ACCTTCTTGATAAGCACTGTGC
hsa-miR-668-3p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCGACTGGATACGACGTAGTG TGCACTCGGCTCGGCCCA
U6-up	CGCTTCGGCAGCACATATAC
U6-dn	CGAATTTGCGTGTCACTCTTG

Table 2 Primers used in the reactions for clone PCR

Gene	Sequence (5'–3')
MiR-1248-up	GAGGATCCCCGGGTACCGGTTTCGAATTCTGGTGAAAAAGG
MiR-1248-dn	CACACATTCCACAGGCTAGCTAATTCATCTGTTAGGTCTAATG
MiR-1248-sponges-up	GAGGATCCCCGGGTACCGGTTTTAG
MiR-1248-sponges-dn	CACACATTCCACAGGCTAGCACCTTCT
PSMD10-shRNA-up	CCGGCAGGTTGGTCTCCTCTTCATACTCGAGTATGAAGAGGAGACCAACCTGTTTTTG
PSMD10-shRNA-dn	AATTCAAAAACAGGTTGGTCTCCTCTTCATACTCGAGTATGAAGAGGAGACCAACCTG
PSMD10-CDS-up	ATGGAGGGGTGTGTGTCTAAC
PSMD10-CDS-dn	TTAACCTTCACCACTTCTTTGAG
PSMD10-WT-up	ACACTTAGCCTGTGATGAGGAGAG
PSMD10-WT-dn	GGGGACAACAACAACATACAAAG

mixture. After forty-eight hours, cells were collected for further analysis.

Colony formation assay

For colony formation assay, vector-transfected CRC cells were plated in 6-well plates at a density of 500 cells per well and cultured in a humidified atmosphere containing 37°C for 2 weeks. Cell colonies were washed with phosphate-buffered saline (PBS), fixed with methanol for 30 min, and stained with 0.1% crystal violet (1 mg/mL) for another 30 min. The colony morphologies were captured and manually counted under a light microscope.

CCK-8 analysis

Cell viability was assessed using Cell Counting Kit-8 (CCK-8) assay. In brief, the indicated CRC cells were seeded on 96-well plates containing complete medium at a density of 1000 cells per well. After cell attachment, the medium was changed, and cells were transfected with relevant vectors, and cultured for 5 days. Cell proliferation was detected every 24 h. Then 10 μ L CCK-8 solution was added to each well and the cells were incubated at 37°C for 4 h. CCK-8 absorbance was measured at 450 nm.

Invasion and migration assays

Transwell assays were performed to assess the invasion and migration of SW480 and SW620 cells. SW480 and SW620 cells transfected with relevant vectors were first starved for 24 hours. On the next day, the cells were suspended in serum-free L-15 medium, and 4000 cells were seeded in the upper chamber. The lower chamber was filled with 600 μ L of L-15 medium containing 20% FBS to induce cell invasion and migration. Following incubation for 48 hours, the membranes with the cells invaded or migrated were fixed with methanol and stained with crystal violet. Then the cells in the membranes were counted under a microscope.

Luciferase activity assay

miRDB was used to predict the potential binding site in target mRNA PSMD10 of has-miR-1248. The wild-type sequence of PSMD10 (PSMD10-WT) and mutant type sequence of PSMD10 (PSMD10-MUT) were cloned into the pmirGLO vector (Promega) respectively, which was performed by General Biol (Anhui, China). Subsequently, PSMD10-WT or PSMD10-MUT was co-transfected with anti-miR-1248 or anti-miR-NC into SW480 cells. They were also co-transfected with miR-1248 or miR-NC into SW620 cells as well. After transfection for 48 hours, the luciferase activity was measured following the procedure of TransDetect® Double-Luciferase Reporter Assay Kit (Transgen, Beijing, China). PSMD10-Mut can be used as a comparison of the PSMD10-WT, which more

intuitively reflects that PSMD10-WT can improve the luciferase activity of SW480 cells and reduce the luciferase activity of SW620 cells.

Western blot analysis

Cells were lysed by RIPA buffer (Beyond) containing protease inhibitor. Then, protein concentration was evaluated using the BCA method. Protein (25 μ g) was separated by 10% SDS-PAGE gel and was transferred to PVDF membrane. Afterward, the membrane was blocked with 5% skim milk and incubated overnight with PSMD10 (ab182576, Abcam), α -tubulin (CST, 3873 s, 1:2000) at 37°C. Secondary antibody (SA0004-1, Porteintech) was added to the membrane and incubated for 2 h at 25°C. ECL plus (PE0010, Solarbio) and Tanon-5200 CE (Biotanon, Shanghai, China) were used to observe the protein bands. α -tubulin served as the loading control.

Statistical analysis

All data were analyzed using Graphpad 8.0 (GraphPad Software Inc., San Diego, CA, USA) and SPSS 10.0 (IBM, Armonk, NY, USA). Continuous variables are presented as mean \pm standard deviation and between-group differences were assessed using the Wilcoxon test. *P* values < 0.05 were considered indicative of statistical significance.

Results

Hsa-miR-1248 showed a relationship with CRC lymph node metastasis

To explore the miRNA changes between high metastasis (with lymph node metastasis) and low metastasis (without lymph node metastasis) CRC tissues, we performed miRNA sequencing in the three groups, i.e., high metastatic carcinoma tissue (M_High group), low metastatic carcinoma tissue (M_Low group), and adjacent normal colorectal tissue (Normal group). As shown in Fig. 1A, compared to the Normal group, 382 miRNAs were down-regulated in the M_Low group, while 1462 miRNAs were down-regulated in the M_High group. Compared to the M_Low group, 942 miRNAs were down-regulated in the M_High group. As shown in the Venn graph (Fig. 1B), 95 miRNAs were down-regulated in carcinoma tissue (M_Low and M_high groups) when compared with the Normal group, and these were also down-regulated in M_High group when compared with the M_Low group. According to the fold-change (log₂) in the M_High group compared with the M_Low group, we ranked these 95 miRNAs and harvested the first three, i.e., hsa-miR-635, hsa-miR-1248, and hsa-miR-668-3p (Fig. 1C). According to the miRNA profile, we screened out hsa-miR-635, hsa-miR-1248, and hsa-miR-668-3p as the potential miRNAs affecting CRC LNM.

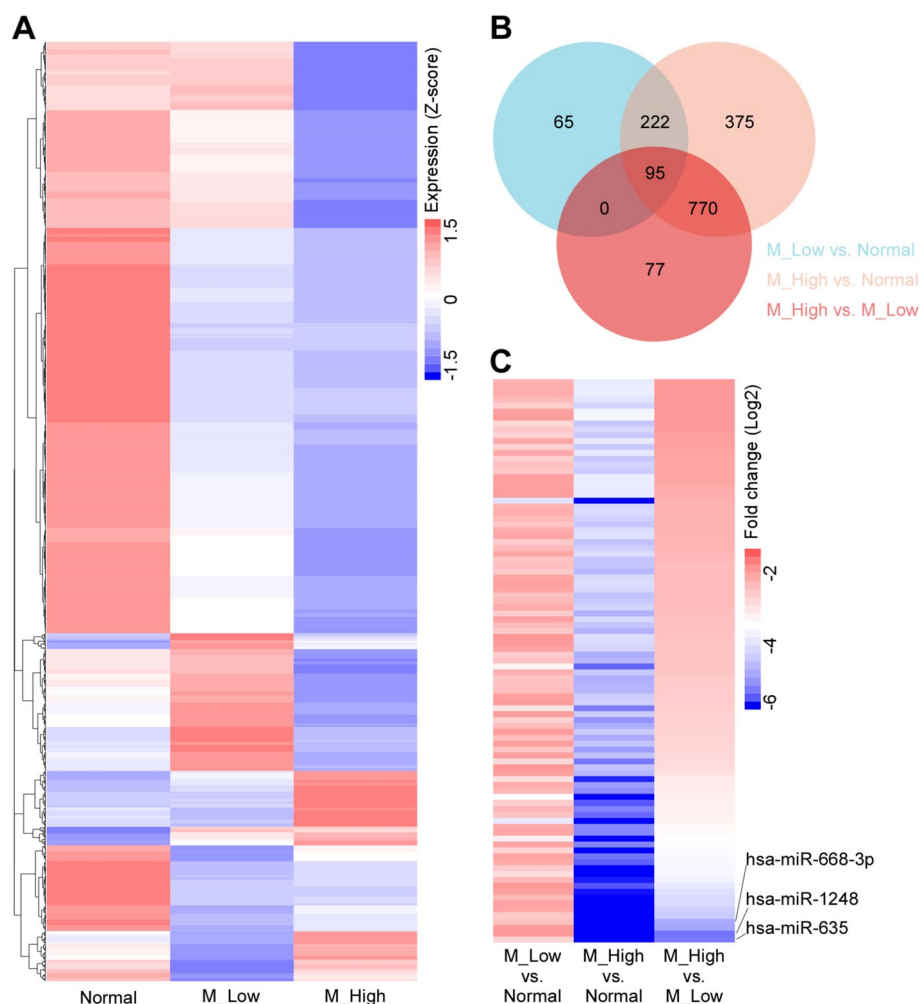


Fig. 1 Sequencing results showed a relation of has-miR-1248 with CRC lymph node metastasis. MiRNA sequencing was performed in three groups, i.e., high lymph node metastatic carcinoma tissue group (M_High), low lymph node metastatic carcinoma tissue group (M_Low), and adjacent normal colorectal tissue group (Normal). The expression (z score) is shown in the heatmap (Fig. 1A). There are 95 miRNAs down-regulated in carcinoma tissue (M_Low and M_high groups) when compared with the adjacent normal tissue (NC group), and they were further down-regulated in M_High groups when compared with M_Low groups (Fig. 1B). According to the Log₂ Fold change in the M_High vs. M_Low, the top 3 differentially expressed miRNAs were hsa-miR-635, hsa-miR-1248, and hsa-miR-668-3p (Fig. 1C). Consequently, we screened out hsa-miR-635, hsa-miR-1248, and hsa-miR-668-3p as the potential miRNAs affecting CRC lymph node metastasis

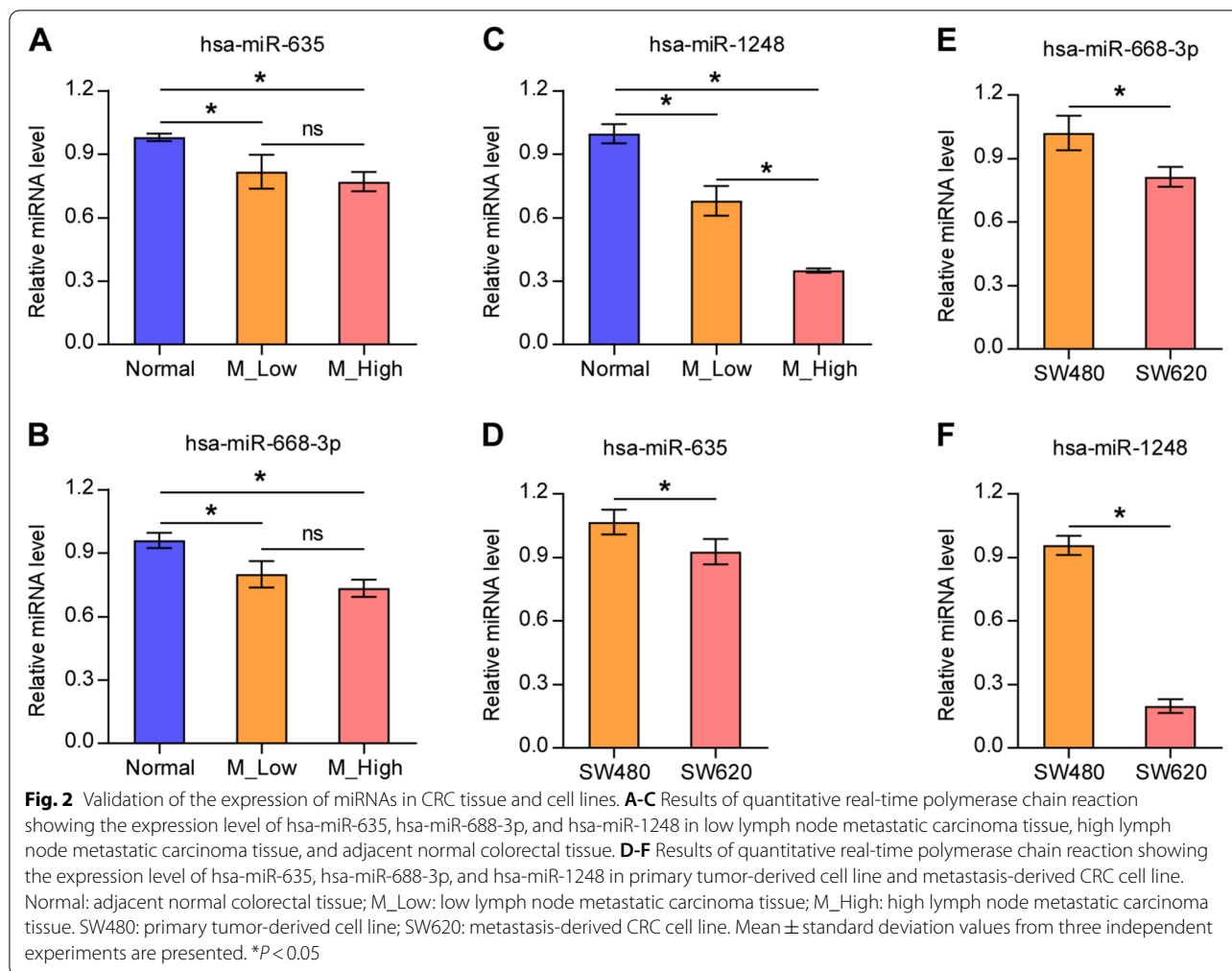
Validation of the miRNA expression in CRC tissues and cell lines

Then, we performed qRT-PCR assay to verify the expression of these three miRNAs in the sequencing samples. As shown in Fig. 2, hsa-miR-635, hsa-miR-668-3p, and hsa-miR-1248 expression levels in the M_Low and M_High groups were significantly lower than those in the Normal group. These results were consistent with the sequencing data. However, only the hsa-miR-1248 expression level was significantly lower in the M_High group compared with the M_Low group. We also tested the expression of these three miRNAs in human colon cell lines: primary tumor-derived SW480 and

metastasis-derived SW620. We found that the expression of hsa-miR-635, hsa-miR-668-3p, and hsa-miR-1248 in SW620 were significantly lower than those in the SW480. Among these, the decrease in hsa-miR-1248 expression was most remarkable. Altogether, only hsa-miR-1248 presented a consistent relationship with CRC LNM, both in CRC tissues and cell lines. Hence, we selected hsa-miR-1248 for further analysis.

Hsa-miR-1248 inhibits the proliferation, migration, and invasion of CRC cells

As shown in Fig. 3, anti-miR-NC or anti-miR-1248 was transfected into low metastasis colon cell line-SW480,



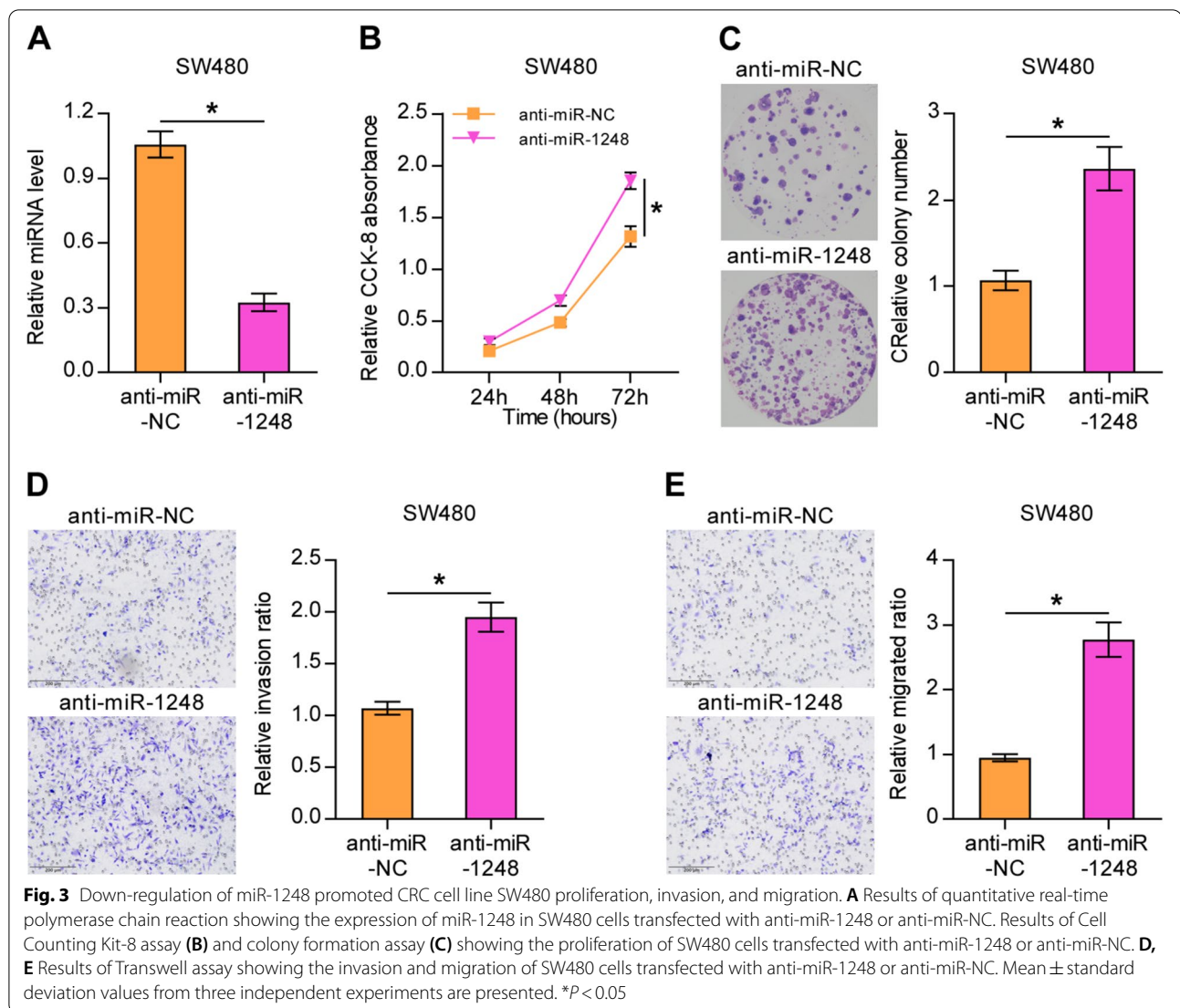
which had a high expression level of miR-1248. The expression of miR-1248 was assessed using qRT-PCR. MiR-1248 expression showed a significant decrease in the anti-miR-1248 group. The CCK-8 assay suggested that SW480 cells transfected with anti-miR-1248 showed much stronger viability at 72 hours. The BrdU cell proliferation assay showed that down-regulation of miR-1248 expression increased the colony formation ability of SW480 cells. In addition, Transwell assays showed that low-expression of miR-1248 had a positive effect on cell invasion and migration.

Moreover, as shown in Fig. 4, miR-NC mimic and miR-1248 mimic were also transfected into high metastasis colon cell line-SW620, which had a low expression level of miR-1248. The qRT-PCR results showed a significantly higher miR-1248 expression in the miR-1248 mimic group SW620 cell line. The CCK-8 assay, BrdU cell proliferation assay, and Transwell assay were also performed to evaluate the cell viability, proliferation, invasion, and migration. The results indicated that upregulation of

hsa-miR-1248 decreased the viability and proliferation of SW620 cells, and inhibited their migration and invasion. These data suggest that hsa-miR-1248 inhibited the proliferation, migration, and invasion of CRC cells, in turn affecting CRC progression and metastasis.

Hsa-miR-1248 direct binding with PSMD10 and regulate its expression

We next investigated the relationship between *PSMD10* expression levels and miR1248 (Fig. 5). We also performed ddPCR to measure the expression of *PSMD10* in the CRC tissues and their corresponding adjacent tissues collected for sequencing. We found that *PSMD10* expressed a high copy number in CRC tumor tissues compared with the adjacent normal tissue, and the copy number was further elevated in M_High tissue compared with M_Low tissue, which showed an inverse correlation with miR-1248. We further verified the regulatory relationship between miR-1248 and *PSMD10*. The binding site was predicted by miRDB, then confirmed by

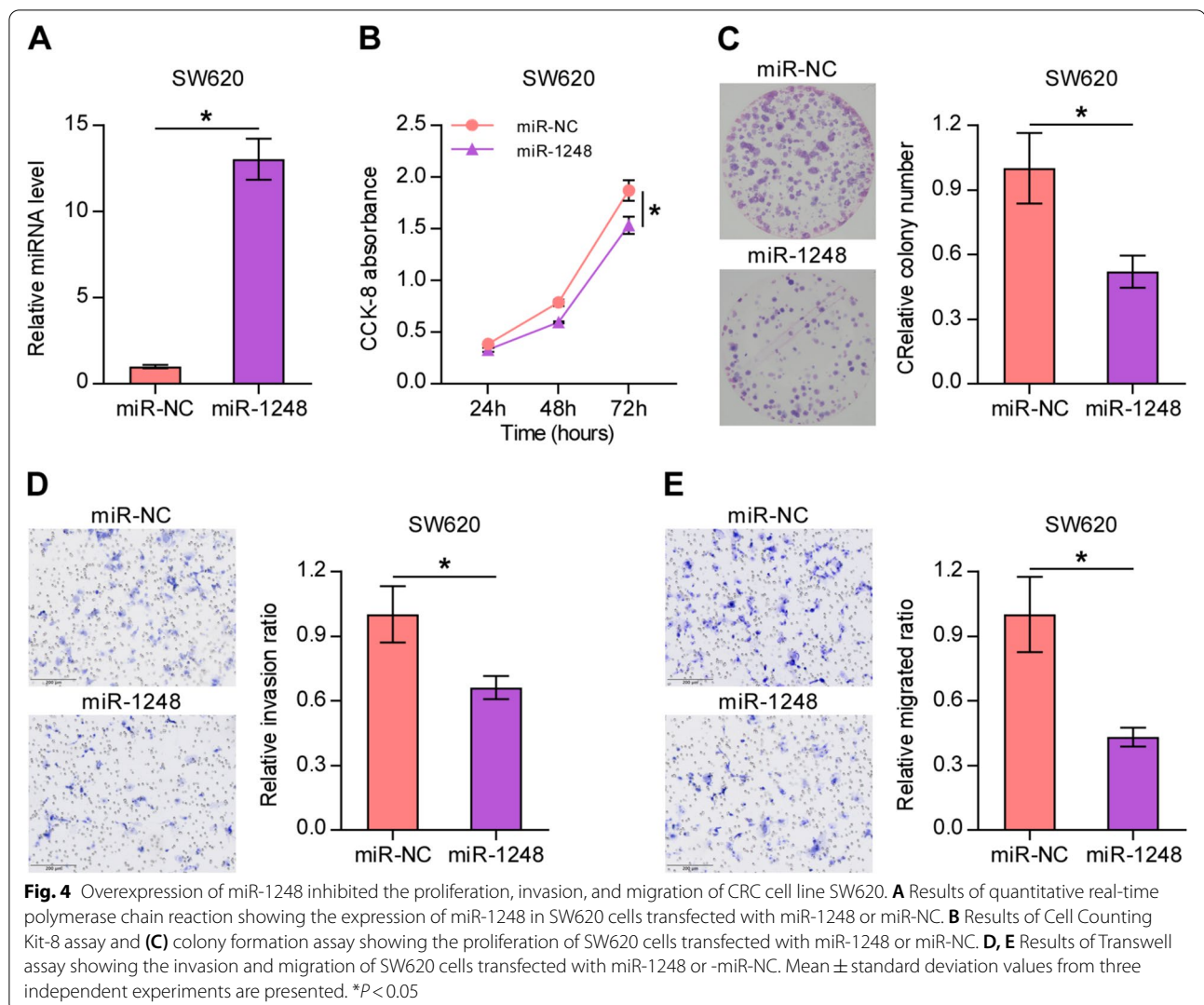


luciferase activity assay. Anti-miR-1248 co-transfected with PSMD10-WT increased the luciferase activity of SW480 cells compared with that co-transfected with PSMD10-Mut. Moreover, miR-1248 co-transfected with PSMD10-WT decreased the luciferase activity of SW620 cells compared with that co-transfected with PSMD10-Mut. These results revealed that hsa-miR-1248 showed direct binding with *PSMD10* and regulated its expression.

Has-miR-1248 influences the viability, migration, and invasion of CRC cells by regulating PSMD10

In previous experiments, hsa-miR-1248 had been confirmed to inhibit the viability, proliferation, migration, and invasion of CRC cells. As PSMD10 was verified as the target of hsa-miR-1248, the expression of PSMD10 was suppressed in the hsa-miR-1248 mimics group and was enhanced in the anti-hsa-miR-1248 group. Then

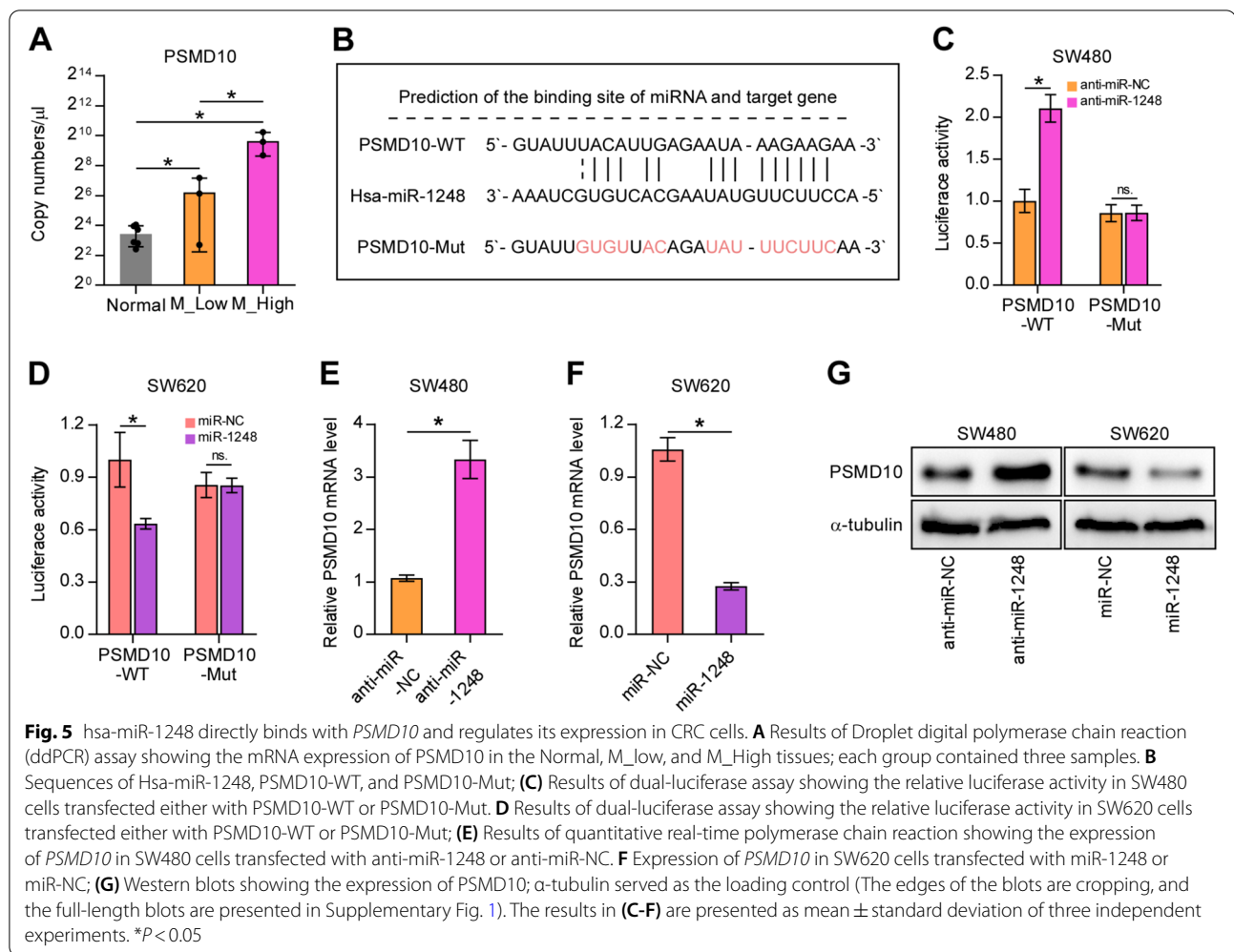
we performed CCK-8 assay, BrdU cell proliferation assay, and Transwell assay in CRC cell lines transfected with various vectors. As shown in Fig. 6, suppression of miR-1248 significantly enhanced the viability, proliferation, invasion, and migration of SW480 cells, but these effects were attenuated when the SW480 cells were co-transfected with shPSMD10. In addition, we also performed the same experiments in SW620 cells. Upregulation of miR-1248 significantly weakened the viability, proliferation, invasion, and migration of SW620 cells. However, these effects were attenuated when SW620 cells were co-transfected with PSMD10-mimic, which was consistent with the results obtained above. To summarize, hsa-miR-1248 was considered to regulate the proliferation, migration, and invasion of CRC cells partly through PSMD10.



Discussion

Lymph node metastasis (LNM) is the most common path of metastasis of CRC. LNM is associated with a poor prognosis and is a key determinant of the therapeutic strategy. However, the traditional pathological diagnosis of LNM may not satisfy the requirement of individualized treatment. Therefore, identification of potential molecular biomarkers that can accurately predict LNM and provide a reference for advanced intervention is a key research imperative. MiRNAs play an important role in the development of cancer; moreover, some studies have indicated the predictive value of miRNAs for LNM in CRC [15, 16]. In the present study, we adopted a sample mixture strategy prior to sequencing, which attenuated the variability between individual samples. The relationship between the candidate micro-mRNA and CRC LNM was further validated in tumor tissues.

After sequencing, we found that 95 miRNAs were down-regulated not only in the carcinoma tissue compared with the adjacent normal tissue, but also further decreased in the high lymph node metastasis CRC tissue compared with low lymph node metastasis one. The top 3 miRNAs were hsa-miR-668-3p, hsa-miR-635, and hsa-miR-1248. Their relationship with tumorigenesis has previously been reported. For example, miR-635 was shown to be a potential inhibiting factor in NSCLC and gastric cancer [17, 18]. MiR-668-3p expression was shown to be significantly increased in hepatocellular carcinoma [19]. Hsa-miR-1248 was found to be down-regulated in NSCLC and lung squamous carcinoma cells [7–9]. However, there is a paucity of studies about the role of miR-635, miR-668-3p, and hsa-miR-1248 in CRC. Our tissue and cell line validation experiments found that these three miRNAs were downregulated in

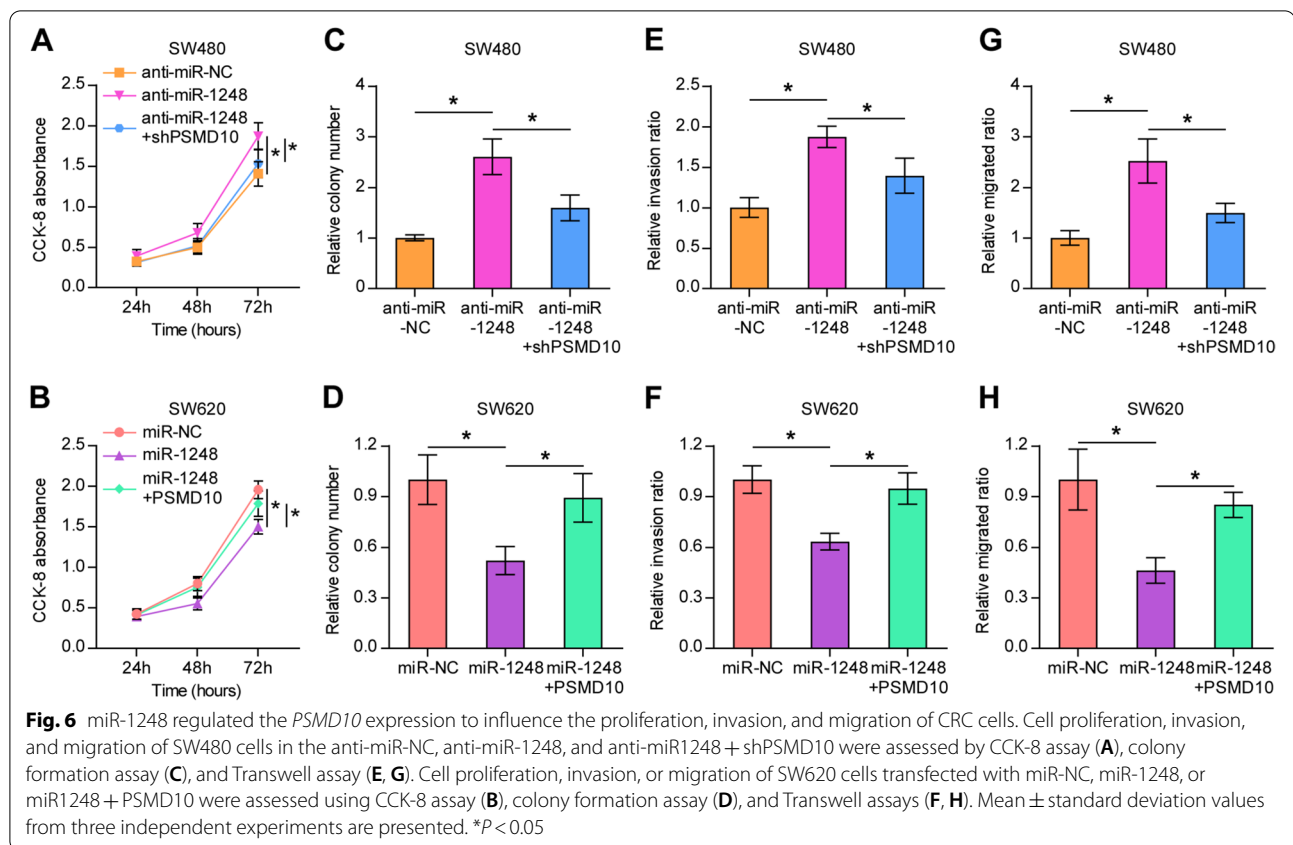


CRC tumor tissues and exhibited a lower expression in the high metastasis CRC cell line SW620.

Interestingly, in addition to the significant downregulation in SW620 cells, only the expression of hsa-miR-1248 was further decreased in the high lymph node metastasis CRC tissue. Furthermore, we also confirmed that hsa-miR-1248 was associated with cell viability, proliferation, migration, and invasion. Therefore, the molecular mechanism of hsa-miR-1248 was worth further elucidation.

As miRNAs often regulate mRNA expression to promote or inhibit cancer, their roles in carcinogenesis and tumor progression vary in different types of tumors. For instance, hsa-miR-1248 was shown to be upregulated in gastric cancer [20] and esophageal squamous cancer tissue [21] and to act as an oncogene. However, its expression level was decreased in NSCLC tissue and it acted as a tumor suppressor gene [7, 8]. The differential expression of critical genes in these tumors contributes to this divergence. Hence, the key target mRNAs of hsa-miR-1248 in CRC were further predicted. *PSMD10* was

earlier shown to be a potential target for cancer therapy [22]. It was also found to act as an oncogene in many types of cancer, such as hepatocellular carcinoma [23], clear cell renal cell carcinoma [24], papillary thyroid carcinoma [25], testicular cancer [26], and osteosarcoma [27]. *PSMD10* was earlier reported to be overexpressed in CRC and its expression level correlated with TNM stage and metastasis [10]. In our recent research, *PSMD10* was shown to be a promising biomarker of occult liver metastasis in CRC patients [11]. Furthermore, in the present research, we also found a potential association of *PSMD10* with LNM. This was also consistent with the relationship between hsa-miR-1248 and CRC LNM. Then, the luciferase activity assay confirmed the binding of hsa-miR-1248 to *PSMD10*, and the CRC cell line transfected with hsa-miR-1248 relative vector resulted in the regulation of *PSMD10*. Collectively, these findings indicated that *PSMD10* was a target of hsa-miR-1248. Moreover, the antitumor effect of hsa-miR-1248 was suppressed or enhanced by up-regulating or down-regulating



the expression of *PSMD10*. These results implied that miR-1248 plays a tumor suppressor role in CRC partially via *PSMD10*.

Some limitations of our study need to be acknowledged. Only a small number of clinical samples were used in this study. Future studies should confirm the expression of hsa-miR-1248 in CRC using a larger number of clinical samples. Secondly, we did not assess the correlation between hsa-miR-1248 and clinical features of CRC. Moreover, in vivo studies are required to assess the function of the hsa-miR-1248/*PSMD10* axis in animal models.

Conclusion

We observed decreased expression of hsa-miR-1248 in CRC tissues with lymph metastasis. The hsa-miR-1248 was found to bind with *PSMD10* and inhibit its expression. *PSMD10* was overexpressed in CRC and showed a positive correlation with LNM. Therefore, hsa-miR-1248 overexpression can inhibit the proliferation and motility of CRC cells by inhibiting the expression of *PSMD10*. Collectively, this study identified the potential involvement of a new axis hsa-miR-1248/*PSMD10* in the development of CRC. Our findings may provide new insights for CRC therapy.

Abbreviations

LNM: Lymph node metastasis; CRC: Colorectal cancer; miRNAs: MicroRNAs; ceRNA: Competing endogenous RNA; NSCLC: Non-small cell lung cancer; *PSMD10*: Proteasome 26S subunit non-ATPase 10; TNM: Tumor node metastasis; miRDB: miRNA target prediction and functional annotations online database.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12885-022-10028-1>.

Additional file 1.

Additional file 2.

Additional file 3.

Additional file 4.

Acknowledgments

All authors would like to express their sincere thanks to the participants who provided colon cancer cell and colorectal tissue samples for this study and ensuring the successful completion of the study.

Authors' contributions

YH, JZ, CW and BW conceived and designed the experiments. CW, BW, WL (Weixing Lin), ZM, WW, MW, YL and YH performed the experiments. WL (Weijun Liang), CZ, WL (Weixing Lin) and XL analyzed the data. All authors prepared the figures and tables, and also drafted the work or revised it critically for important content.

Funding

This study was supported by grants from the Guangdong Administration of Traditional Chinese Medicine Research Foundation (no. 20221433), Guangdong Basic and Applied Basic Research Foundation (no. 2021A1515220175), Guangdong Medical Research Foundation (no. B2021057) and Jiangmen Planned Project of Science and Technology (no. 2021030103670007327). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Availability of data and materials

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

Declarations

Ethics approval and consent to participate

Informed consent was obtained from all patients. All procedures were approved by the Institutional Research Ethics Committee of the Jiangmen Central Hospital (decision no. JXY202229). All methods of this study were carried out in accordance with relevant guidelines and regulations (declaration of Helsinki).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 11 May 2022 Accepted: 22 August 2022

Published online: 26 August 2022

References

- Siegel RL, et al. Cancer statistics, 2021. *CA Cancer J Clin.* 2021;71(1):7–33.
- Baxter NN, et al. Lymph node evaluation in colorectal cancer patients: a population-based study. *J Natl Cancer Inst.* 2005;97(3):219–25.
- Faerden AE, et al. Lymph node micrometastases and isolated tumor cells influence survival in stage I and II colon cancer. *Dis Colon Rectum.* 2011;54(2):200–6.
- Ventura A, Jacks T. MicroRNAs and cancer: short RNAs go a long way. *Cell.* 2009;136(4):586–91.
- Pasquinelli AE. MicroRNAs and their targets: recognition, regulation and an emerging reciprocal relationship. *Nat Rev Genet.* 2012;13(4):271–82.
- Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell.* 2009;136(2):215–33.
- Yue Q, et al. Circ-PITX1 promotes the progression of non-small cell lung Cancer through regulating the miR-1248/CCND2 Axis. *Onco Targets Ther.* 2021;14:1807–19.
- Yang T, et al. Downregulation of circEPST11 represses the proliferation and invasion of non-small cell lung cancer by inhibiting TRIM24 via miR-1248 upregulation. *Biochem Biophys Res Commun.* 2020;530(1):348–54.
- Li G, Guo X. LncRNA STARD13-AS blocks lung squamous carcinoma cells growth and movement by targeting miR-1248/C3A. *Pulm Pharmacol Ther.* 2020;64:101949.
- Tang S, et al. Overexpression of a novel gene gankyrin correlates with the malignant phenotype of colorectal cancer. *Cancer Biol Ther.* 2010;9(2):88–95.
- Wang C, et al. Gankyrin as potential biomarker for colorectal Cancer with occult liver metastases. *Front Oncol.* 2021;11:656852.
- Liu F, et al. miR-214 regulates papillary thyroid carcinoma cell proliferation and metastasis by targeting PSMD10. *Int J Mol Med.* 2018;42(6):3027–36.
- Chu YM, et al. MicroRNA-1254 inhibits the migration of colon adenocarcinoma cells by targeting PSMD10. *J Dig Dis.* 2017;18(3):169–78.
- Zhou H, et al. TMEM2 binds to CSNK2A3 to inhibit HBV infection via activation of the JAK/STAT pathway. *Exp Cell Res.* 2021;400(1):112517.
- Ozawa T, et al. A MicroRNA signature associated with metastasis of T1 colorectal cancers to lymph nodes. *Gastroenterology.* 2018;154(4):844–848 e7.
- Wada Y, et al. A liquid biopsy assay for noninvasive identification of lymph node metastases in T1 colorectal Cancer. *Gastroenterology.* 2021;161(1):151–162 e1.
- Tai G, Zhang M, Liu F. Circ_0000735 enhances the proliferation, metastasis and glycolysis of non-small cell lung cancer by regulating the miR-635/FAM83F axis. *Exp Lung Res.* 2021;47(3):136–48.
- Cao FY, et al. miR-635 targets KIFC1 to inhibit the progression of gastric cancer. *J Investig Med.* 2020;68(8):1357–63.
- Ma H, et al. Circular RNA circ_0014717 suppresses hepatocellular carcinoma tumorigenesis through regulating miR-668-3p/BTG2 Axis. *Front Oncol.* 2020;10:592884.
- Zhang Y, et al. CircHNF1 affects cell proliferation and migration in gastric cancer. *J Clin Lab Anal.* 2020;34(10):e23433.
- Du F, Guo T, Cao C. Restoration of UPK1A-AS1 expression suppresses cell proliferation, migration, and invasion in esophageal squamous cell carcinoma cells partially by sponging microRNA-1248. *Cancer Manag Res.* 2020;12:2653–62.
- Fujita J, Sakurai T. The Oncoprotein Gankyrin/PSMD10 as a target of Cancer therapy. *Adv Exp Med Biol.* 2019;1164:63–71.
- Hao P, et al. Inhibiting effect of MicroRNA-3619-5p/PSMD10 axis on liver cancer cell growth in vivo and in vitro. *Life Sci.* 2020;254:117632.
- Wang C, et al. Blocking the autocrine regulatory loop of Gankyrin/STAT3/CCL24/CCR3 impairs the progression and pazopanib resistance of clear cell renal cell carcinoma. *Cell Death Dis.* 2020;11(2):117.
- Sui G, et al. The lncRNA SNHG3 accelerates papillary thyroid carcinoma progression via the miR-214-3p/PSMD10 axis. *J Cell Physiol.* 2020;235(10):6615–24.
- Camacho-Moll ME, et al. The oncogene Gankyrin is expressed in testicular cancer and contributes to cisplatin sensitivity in embryonal carcinoma cells. *BMC Cancer.* 2019;19(1):1124.
- Cheng L, et al. Gankyrin promotes osteosarcoma tumorigenesis by forming a positive feedback loop with YAP. *Cell Signal.* 2020;65:109460.

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