MicroRNA-875-5p inhibits tumor growth and metastasis of hepatocellular carcinoma by targeting eukaryotic translation initiation factor 3 subunit a

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Received March 24, 2020; Accepted July 8, 2020

DOI: 10.3892/or.2020.7743

Abstract. Accumulating evidence has demonstrated that aberrant microRNA (miRNA) expression is involved in hepatocellular carcinoma (HCC) progression. Previous findings suggested that miRNA (miR)-875-5p participates in the development of various types of cancer. However, the expression and function of miR-875-5p in HCC remains largely unclear. The analysis of clinical samples in the present study demonstrated that miR-875-5p expression was downregulated in HCC tissues compared to adjacent non-tumor tissues, which was associated with a large tumor size, venous infiltration, advanced tumor-node-metastasis stage and unfavorable overall survival. In vitro experiments revealed that ectopic expression of miR-875-5p suppressed, whereas inhibition of miR-875-5p promoted HCC cell proliferation, migration, invasion and epithelial-to-mesenchymal transition (EMT) progression. Overexpression of miR-875-5p restrained HCC tumor growth and metastasis in vivo. Mechanistically, eukaryotic translation initiation factor 3 subunit a (eIF3a) was identified as the downstream target of miR-875-5p in HCC. Further experiments demonstrated that the expression of eIF3a was upregulated and negatively correlated with that of miR-875-5p in HCC tissues. In addition, miR-875-5p negatively regulated the luciferase activity of wild-type, but not mutant 3'-untranslated region (3'UTR) of eIF3a mRNA. miR-875-5p suppressed eIF3a expression at the mRNA and protein level in HCC cells. Additionally, eIF3a exerted an oncogenic role, and knockdown of eIF3a inhibited the proliferation, motility and EMT of HCC cells. In addition, eIF3a overexpression abolished the inhibitory effects of miR-875-5p on the proliferation, motility and EMT in HCC cells. In conclusion, miR-875-5p, which was downregulated in HCC, may inhibit tumor growth and metastasis by eIF3a downregulation via targeting its 3'UTR and may be a promising prognostic and therapeutic strategy in HCC.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors and the fourth leading cause of cancer-related death worldwide (1). Although progress has been achieved in the diagnosis and therapy over the last decades, the long-term survival rate is unsatisfactory due to the high recurrence and metastasis rates (2). Resection is the most widely used curative treatment for patients with early HCC; however, a number of patients diagnosed at an advanced stage are ineligible for surgery (3). Therefore, it is imperative to unravel the molecular mechanisms underlying the occurrence and development of HCC to identify novel therapeutic strategies for this malignancy.

As conserved single-stranded non-coding RNAs of ~22 nucleotides in length, microRNAs (miRNAs) serve pivotal regulatory roles by protein-coding gene cleavage or translation repression via interacting with the 3'-untranslated region (3'UTR) of target mRNAs with imperfect complementarity (4,5). Accumulating evidence has demonstrated that miRNAs capable of controlling cell proliferation, metabolism, invasion and angiogenesis serve crucial roles in the initiation, progression and metastasis of various types of cancer, including HCC (6,7). Recent findings have demonstrated that miRNA (miR)-1468, miR-876-5p, miR-532-3p, miR-3194-3p and miR-519c-3p regulate the growth and metastasis of HCC via different underlying molecular mechanisms (8-12). Previous results have suggested that miR-875-5p is dysregulated in various types of cancer, such as colorectal carcinoma, lung and prostate cancer (13-16). In those studies, miR-875-5p was demonstrated to function as a tumor suppressor or as an oncogenic factor involved in the proliferation, migration, invasion, apoptosis, chemotherapeutic sensitivity and radiation response of cancer cells. However, limited information

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Key words: hepatocellular carcinoma, tumor growth, tumor metastasis, microRNA-875-5p, eukaryotic translation initiation factor 3 subunit a
is currently available about the role of miR-875-5p and its underlying molecular mechanisms in HCC.

Eukaryotic translation initiation factor 3, which serves a central role in translation initiation, comprises 13 subunits, among which eukaryotic translation initiation factor 3 subunit a (eIF3a) is the largest one (17). In humans, eIF3a appears to be ubiquitously expressed and involved in cellular processes such as translation initiation, cell cycle, and DNA synthesis and repair (18). Previous findings have revealed that eIF3a is aberrantly expressed and involved in the tumorigenesis of lung cancer, ameloblastoma, pancreatic cancer and HCC (19-22). In HCC, eIF3a regulates the translation of hypoxia-inducible factor 1α (HIF-1α), mediating HIF-1α-dependent glycolytic metabolism in HCC (22). However, the exact functions of eIF3a on tumor growth and metastasis and the mechanisms underlying the aberrant expression of eIF3a in HCC remain unclear.

Current findings demonstrated that miR-875-5p was downregulated in HCC cells and tissues, which significantly correlated with short survival and progressed clinical features. The results of the loss- and gain-of-function experiments confirmed that miR-875-5p inhibited tumor growth and metastasis in vitro and in vivo. Mechanistically, miR-875-5p interacted with the 3'UTR of eIF3a mRNA, downregulating the expression of eIF3a, which exhibited oncogenic activities in HCC. Thus, the present study validated that miR-875-5p inhibited tumor growth and metastasis by targeting eIF3a in HCC and may represent a potential target for HCC treatment.

RNA extraction and reverse transcription-quantitative (RT-q) PCR. RNA was extracted from tissues and cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and Qiagen AllPrep DNA/RNA FFPE kit (cat. no. 80234) according to the manufacturer's instructions. For the detection of miRNA and miRNA expression, CDNA was synthesized using a cDNA Synthesis kit (Thermo Fisher Scientific, Inc.). qPCR was performed with SYBR®-Green Premix PCR Master Mix (Roche Diagnostics GmbH). miR-875-5p primers (MQPS0002239-1-200) and eIF3a primers were purchased from Guangzhou RiboBio Co., Ltd. U6 snRNA and GAPDH were used as normalization controls. The primer sequences for eIF3a were: 5'‑ACA GGC AGT GTT TGG ACC TTC‑3' (forward) and 5'‑CTT  ACG CGT GTA TTG GAG GCA‑3' (reverse). The primer sequences for GAPDH were: 5'-GGTATGCAACG AATTGGGC-3' (forward) and 5'-GAGCAGCAGGTACTT TATTG-3' (reverse).

Cell proliferation assays. Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc.) assay was used to assess cell viability. Hep3B and HCCLM3 cells were seeded in a 96-well plate (3x10^4 cells/well); 10 µl CCK-8 solution was added to each well at 0, 24, 48 and 72 h and incubated at 37°C for 1 h. A microplate reader (Bio-Rad Laboratories, Inc.) was used to read the absorbance at 450 nm.

An EdU kit (cat. no. C10310-1; Guangzhou RioBio Co., Ltd.) was used to detect cell proliferation. HCC cells were seeded in a 24-well plate (5x10^4 cells/well). Following 4-h incubation with EdU solution, the nuclei were stained with DAPI. Images were captured under a Zeiss fluorescence photomicroscope (Carl Zeiss AG) in at least five random fields for quantification.

Transwell invasion and migration assays. Serum-free DMEM containing cells pre-starved for 12 h were added into the upper chambers of the Transwell inserts (5x10^4 cells/well) with or without pre-coating with Matrigel, and DMEM with 10% FBS was added into the lower chambers. Following 24-h incubation, the remaining cells in the upper chamber were removed, and the invaded or migrated cells were fixed with formalin and stained using crystal violet for 20 min. Cells from at least five random fields were counted under a light microscope with 100-fold magnification.

In vivo experiments. Male BALB/c nude mice were housed under pathogen-free conditions in the Centre of Laboratory Animals at The Medical College of Xian Jiaotong University. Animal experiments were performed according to the protocol approved by the Ethics Review Committee of Xian Jiaotong University. Human endpoints included i) the body condition score was 1/5; ii) the body condition score was 2/5 and the mouse was profoundly lethargic; iii) the tumor affected...
the mouse's gait or normal posture, ability to eat, urinate, or defecate. No mice met these criteria and were euthanized before the end of the experiment.

Mice (4-6 weeks old) were randomly grouped for animal experiments (n=4 mice per group) for animal experiments. The mice were housed with filtered air, 12-h light/dark cycle, constant temperature (25˚C) and had free access to food and water. A subcutaneous xenograft model was established for evaluating the tumor growth of HCC cells. HCCLM3 cells (3x10^6) transfected with miR-875-5p clones in lentiviral vectors or control vectors were suspended in 100 µl PBS and inoculated subcutaneously into the flank of the mice. Tumor volumes were determined every 3 days as length x width x width/2. The mice were sacrificed by cervical dislocation under 10% ether inhalant anesthesia at 3 weeks after implantation, and the xenograft tumor tissues were dissected for further examination. The pulmonary metastatic model was established to investigate the metastatic ability of HCC cells; transfected HCCLM3 cells (1x10^6) were injected into the tail vein. Mice were sacrificed at 10 weeks after injection, and the lung tissues were examined microscopically following hematoxylin and eosin (H&E) staining.

Bioinformatics analysis. The microRNA.org website (2010 version; http://www.microrna.org/microrna/home.do) and TargetScan Human 5.1 (http://www.targetscan.org/vert_72/) were used by entering ‘miR-875-5p’ into the search box to predict potential miRNA target genes and the binding sites.

Immunohistochemical (IHC) staining. IHC was performed as previously described (23). Briefly, formalin-fixed paraffin-embedded sections were dewaxed, dehydrated and rehydrated. Antibodies against Ki-67 (cat. no. ab92742; Abcam), which is the proliferation marker, were added to the sections and incubated at 4˚C overnight, followed by the addition of the streptavidin peroxidase-conjugated secondary antibody (cat. no. SP-9001; OriGene Technologies, Inc.). The slides were counterstained with hematoxylin and inspected under a microscope.

Table I. Clinical correlation of miR-875-5p expression in HCC (n=90).

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Cases (n)</th>
<th>miR-875-5p^high (n=45)</th>
<th>miR-875-5p^low (n=45)</th>
<th>P-value</th>
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<tr>
<td>Age (years)</td>
<td></td>
<td></td>
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<tr>
<td>&lt;50</td>
<td>29</td>
<td>16</td>
<td>13</td>
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<td>≥50</td>
<td>61</td>
<td>29</td>
<td>32</td>
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<tr>
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<td></td>
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</tr>
<tr>
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<td>39</td>
<td>37</td>
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</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>6</td>
<td>8</td>
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<tr>
<td>Tumor size (cm)</td>
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<td>III+IV</td>
<td>18</td>
<td>4</td>
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<td>0.033*</td>
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<tr>
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<td>21</td>
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*P<0.05.
miR-875-5p is downregulated in HCC and associated with the progression and survival of HCC. To determine whether miR-875-5p was dysregulated in HCC, RT-qPCR was performed in 90 pairs of tumor and adjacent non-tumor tissues. The results revealed that miR-875-5p expression was significantly downregulated in HCC tissues compared with that in the adjacent non-tumor tissues (P<0.001; Fig. 1A). Subsequently, the patients were divided into two groups according to the median value of miR-875-5p expression in HCC tissues, the high miR-875-5p group (n=45) and the low miR-875-5p group (n=45). The Chi-square test revealed that low expression of miR-875-5p was associated with larger tumor size (P=0.006), venous infiltration (P=0.033) and advanced TNM stage (P=0.016) (Table I). Kaplan-Meier and log-rank analysis further revealed that patients with HCC with low miR-875-5p expression presented with unfavorable OS (P<0.05, Fig. 1B). Consistent with the results from the HCC tissue samples, RT-qPCR results demonstrated that miR-875-5p expression levels were downregulated in HCC cell lines compared with those in the immortalized hepatic cell line THLE-3 (P<0.05 or P<0.01, respectively; Fig. 1C). Thus, the above results suggested that miR-875-5p was downregulated in HCC tissues and cell lines, and that low miR-875-5p expression was associated with tumor progression and poor OS of patients with HCC.

miR-875-5p suppresses HCC cell proliferation. To determine the effects of miR-875-5p on HCC cell proliferation, we transfected miR-875-5p inhibitors in Hep3B cells which exhibited relatively high endogenous miR-875-5p level and overexpressed miR-875-5p in HCCLM3 cells which exhibited relatively low endogenous miR-875-5p to obtain satisfactory transfection efficiency and obvious biological effects. As determined by RT-qPCR, the expression of miR-875-5p was decreased in Hep3B cells (P<0.01; Fig. 2A) transfected with the miR-875-5p inhibitors compared with that in the negative control group and upregulated in HCCLM3 cells.
miR-875-5p restrains proliferation in HCC cells. (A) miR-875-5p knockdown notably decreased the expression of miR-875-5p in Hep3B cells. (B) miR-875-5p overexpression significantly increased the expression of miR-875-5p in HCCLM3 cells. (C) CCK8 assay revealed that miR-875-5p knockdown increased the viability of Hep3B. (D) miR-875-5p overexpression restrained the viability of HCCLM3 as detected by CCK8 assay. (E) miR-875-5p knockdown enhanced the proliferation of Hep3B as detected by EdU assay. (F) EdU assay showed that miR-875-5p overexpression suppressed the proliferation of HCCLM3. Scale bars, 20 µm. *P<0.05; **P<0.01.

(P<0.01; Fig. 2B) transfected with the miR-875-5p mimics compared with that in the control group. CCK-8 assay results revealed that the miR-875-5p inhibitors enhanced the viability of Hep3B cells (P<0.05; Fig. 2C), whereas the viability of HCCLM3 cells transfected with the miR-875-5p mimics was significantly reduced (P<0.05; Fig. 2D). In addition, an EdU
miR-875-5p inhibits HCC cell migration and invasion. To determine whether miR-875-5p served a role in the motility of HCC cells, Transwell assays were performed. The results demonstrated that Hep3B cells transfected with the miR-875-5p inhibitors exhibited increased migratory and invasive abilities (P<0.05; Fig. 3A), whereas the miR-875-5p mimics reduced the number of migrated and invasive HCCLM3 cells (P<0.05; Fig. 3B). As epithelial-mesenchymal transition (EMT) is a classical phenomenon of morphology change in HCC cells and serves a pivotal role on HCC cell migration and invasion, the present study further investigated whether miR-875-5p inhibited HCC cell motility via suppressing the EMT progression. Western blot analysis revealed that the miR-875-5p inhibitors decreased the expression levels of E-cadherin and increased those of vimentin and N-cadherin in Hep3B cells (P<0.05; Fig. 3C). By contrast, the miR-875-5p mimics increased the expression levels of E-cadherin and decreased those of vimentin and N-cadherin in HCCLM3 cells (P<0.05; Fig. 3D). Therefore, these results indicated that miR-875-5p inhibited HCC cell migration and invasion by suppressing the EMT.

miR-875-5p inhibits HCC tumor growth and metastasis in vivo. To further confirm the inhibitory effects of miR-875-5p on HCC in vivo, HCCLM3 cells stably overexpressing miR-875-5p were established and injected subcutaneously into nude mice. We found that the miR-875-5p had no detectable effect on the body weight in mice for xenograft model (Fig. S1A). The tumor growth curves revealed that miR-875-5p overexpression induced HCC growth restriction in mice (P<0.05; Fig. 4A). The weight of the tumors formed by miR-875-5p-overexpressing HCCLM3 cells was decreased (P<0.05; Fig. 4B). RT-qPCR results confirmed higher miR-875-5p expression in tumor tissues harvested from the miR-875-5p-overexpressing group compared with those from the control group (P<0.05; Fig. 4C). Immunohistochemistry results demonstrated that overexpression of miR-875-5p decreased the positive rate of the proliferation marker Ki-67 staining (P<0.05; Fig. 4D). Additionally, to determine the metastatic potential in vivo, a lung metastasis model was established by tail vein injection with HCCLM3 cells overexpressing miR-875-5p. The weight loss was lower in the mice injected with miR-875-5p-overexpressing cells compared with that with control, while the difference was not significant (Fig. S1B). RT-qPCR results validated that miR-875-5p expression level in HCCLM3 cells to be injected into the tail vein and in the metastatic nodules was higher in miR-875-5p-overexpressing group compared with that in control (P<0.05; Fig. S1C and D). The results revealed that the miR-875-5p overexpression group exhibited fewer and smaller foci in the lungs of nude mice compared with those in the control group (P<0.05; Fig. 4E). Together, these results suggested that miR-875-5p inhibited HCC growth and metastasis in mice.

eIF3a is the downstream target of miR-875-5p in HCC. To investigate the exact mechanism underlying the inhibitory function of miR-875-5p in HCC, the candidate downstream targets of miR-875-5p were identified using bioinformatics tools (microRNA.org, TargetScan). Comprehensive analysis of a previous study identified eIF3a as an oncogenic molecule in HCC (22), and bioinformatics analysis predicted miR-875-5p binding sites in the eIF3a mRNA 3’UTR (Fig. 5A); thus, eIF3a was selected as the potential target of miR-875-5p. A luciferase reporter assay was performed, and the results demonstrated that the miR-875-5p inhibitors enhanced, whereas the mimics reduced the
luciferase activities of plasmids carrying the wt, but not the mt eIF3a 3’UTR (Fig. 5B). In addition, RT-qPCR and western blotting results demonstrated that the miR-875-5p inhibitors increased the expression of eIF3a at the mRNA and protein level in Hep3B cells (P<0.05; Fig. 5C and D).

By contrast, the miR-875-5p mimics decreased eIF3a mRNA and protein expression in HCCLM3 cells (P<0.05; Fig. 5E and F). Consistently, RT-qPCR results revealed that eIF3a mRNA expression was significantly upregulated in HCC tissues compared with that in adjacent non-tumor

Figure 4. miR-875-5p inhibits HCC growth and metastasis in vivo. (A) HCCLM3 cells stably overexpressing miR-875-5p and control were subcutaneously injected into nude mice. Tumor volume was measured every 3 days from the 6th day after implantation. (B) The nude mice were sacrificed and tumors were harvested and weighed at the 21st day after subcutaneous injection. (C) Xenograft tissues were subjected to RT-qPCR for miR-875-5p expression. (D) Immunostaining of Ki-67 in xenograft tissues arising from miR-875-5p overexpression group and control group. (E) HCCLM3 cells stably overexpressing miR-875-5p and control were administered into mice via tail vein injections. Hematoxylin and eosin (H&E) staining was performed to identify the metastatic nodules from the mouse lung tissues. Black arrows indicate metastatic nodules. Scale bars, 100 µm. *P<0.05.
tissues (P<0.001; Fig. 5G), and eIF3a mRNA expression was significantly negatively correlated with miR-875-5p expression in HCC tissues (r=-0.4105, P<0.0001; Fig. 5H). Western blotting results demonstrated that eIF3a expression was lower in HCC tissues with high miR-875-5p expression compared with those with low miR-875-5p expression (P<0.05; Fig. 5I).

Figure 5. eIF3a is a downstream target of miR-875-5p in HCC cells. (A) Results from TargetScan (http://www.targetscan.org) and miRanda (microRNA.org) showed the predicted miR-875-5p binding sites in 3'UTR of eIF3a mRNA. (B) Luciferase reporter assay indicated that alteration of miR-875-5p expression inversely regulated luciferase activities of wild-type (wt) but not mutant (mt) eIF3a 3'UTR plasmids. (C and D) RT-qPCR and western blotting were performed to identify the expression of eIF3a mRNA and protein in Hep3B cells transfected with miR-875-5p inhibitors. (E and F) HCCLM3 cells with miR-875-5p knockdown. (G) RT-qPCR was performed to detect eIF3a mRNA expression in 90 pairs of HCC tumor tissues and corresponding adjacent non-tumor tissues. (H) Pearson's correlation analysis disclosed the negatively correlation between eIF3a mRNA and miR-875-5p in HCC tissues. (I) Western blotting was performed to detect eIF3a expression of HCC tissues with low miR-875-5p expression and high miR-875-5p expression. (J) Western blotting was performed to determine eIF3a expression in mice xenograft tumor tissues. *P<0.05.
Additionally, the protein expression of eIF3a was downregulated in miR-875-5p-overexpressing xenograft tumor tissues compared with that in the control group (P<0.05; Fig. 5J). Collectively, these results confirmed that miR-875-5p downregulated eIF3a expression by directly targeting the 3'UTR of eIF3a mRNA in HCC.
Figure 7. eIF3a restoration reverses the inhibitory effects of miR-875-5p on proliferation and motility of HCC cells. (A) miR-875-5p overexpressing HCCLM3 cells transfected with empty vector (EV) or eIF3a plasmid were subjected to western blotting for eIF3a. (B) CCK8 assay manifested that eIF3a overexpression reversed suppression functions of miR-875-5p on viability of HCCLM3 cells. (C and D) Edu assay showed that eIF3a overexpression rescued the proliferation of HCCLM3 cells inhibited by miR-875-5p. (E and F) Transwell assay revealed that miR-875-5p inhibits the migration and invasion of HCCLM3 cells while eIF3a overexpression abolished the effects. (G) Western blotting revealed that miR-875-5p increased E-cadherin and reduced N-cadherin and vimentin expression in HCCLM3 cells, while the overexpression of eIF3a blocked these effects. Scale bars, 20 µm. *P<0.05.
**elF3a-knockdown inhibits HCC cell proliferation, migration and invasion.** To determine the role of elF3a in the proliferation and motility of HCC cells, HCCLM3 cells were used to establish an elF3a-knockdown cell line. Western blotting was performed to validate the transfection efficiency (P<0.05; Fig. 6A). CCK-8 assay results revealed that elF3a-knockdown significantly decreased the viability of HCCLM3 cells (P<0.05; Fig. 6B). In addition, elF3a-knockdown reduced the number of active proliferating HCC cells as determined by the EdU assay (P<0.05; Fig. 6C). To explore the effects of elF3a on the migratory and invasive abilities of HCC cells, Transwell assays were performed, and the results demonstrated that knockdown of elF3a decreased the migration and invasion of HCCLM3 cells (P<0.05; Fig. 6D). In addition, elF3a-knockdown inhibited the EMT progression in HCCLM3 cells (P<0.05; Fig. 6E). Taken together, these results suggested that elF3a promoted the proliferation and motility of HCC cells.

**elF3a mediates the inhibitory effects of miR-875-5p on HCC cell proliferation and mobility.** Based on the functions of miR-875-5p and elF3a, and the correlation between them, the present study further examined whether elF3a may mediate the inhibitory functions of miR-875-5p in HCC. RT-qPCR results revealed that miR-875-5p expression was significantly upregulated by miR-875-5p mimics but not affected by elF3a overexpression plasmid in HCCLM3 cells (P<0.05; Fig. S1E). Western blot analysis validated that elF3a expression was significantly restored by an elF3a overexpression plasmid in miR-875-5p-overexpressing HCCLM3 cells (P<0.05; Fig. 7A). Consistent with the promoting effects of elF3a on HCC cell proliferation, elF3a accelerated the proliferation of HCCLM3 cells inhibited by miR-875-5p, determined by the CCK-8 and EdU assays (P<0.05; Fig. 7B-D). The Transwell assay results demonstrated that elF3a overexpression reversed the inhibitory effects of miR-875-5p on HCCLM3 cell migration and invasion (P<0.05; Fig. 7E and F). In addition, elF3a overexpression rescued the miR-875-5p mimic-inhibited EMT progression of HCCLM3 cells (P<0.05; Fig. 7G). In summary, these results supported the role of elF3a in mediating the tumor suppressor function of miR-875-5p in HCC cells.

**Discussion**

miRNAs are small non-coding RNAs that serve pivotal roles in the majority of types of cancer, affecting numerous cancer-associated processes, such as cell proliferation, cell cycle, apoptosis, differentiation, migration and metabolism (24-25). Previous studies have observed the dysregulation of miR-875-5p in various types of cancer (13-15). For instance, miR-875-5p acts as a tumor suppressor by curbing the epidermal growth factor receptor (EGFR)-ZEB1 axis, repressing the EMT and increasing radiation response in prostate cancer (14). In addition, miR-875-5p promotes the proliferation and motility of non-small cell lung cancer cell lines by targeting SATB1 homeobox 2 (15). By contrast, miR-875-5p exerts a tumor suppressor role by inhibiting cell proliferation and metastasis and accelerating apoptosis via targeting EGFR in colorectal carcinoma (13). To determine the role of miR-875-5p in HCC, which remains largely elusive, the present study demonstrated that miR-875-5p was downregulated in HCC, and low expression of miR-875-5p was significantly associated with an unfavorable prognosis and clinical features including large tumor size, venous infiltration and an advanced TNM stage. Consistent with the clinical analysis, the results of the loss- and gain-of-function experiments further revealed that miR-875-5p suppressed the proliferation, migration and invasion of HCC cells. Additionally, *in vivo* experiments demonstrated that miR-875-5p overexpression inhibited tumor growth and metastasis. In a previous study, a hypoxic microenvironment was demonstrated to modulate the expression levels of miR-187-3p, miR-204, miR-1296, miR-671-5p (26-29) and long non-coding RNA AGAP2 antisense RNA 1 (30), which sponges miR-16-5p and promotes cell proliferation and metastasis in HCC. Therefore, whether hypoxia is responsive for the downregulation of miR-875-5p in HCC requires further investigation.

As the crucial component for translation initiation, elF3a serves a vital role in various physiological and pathological processes, such as the cell cycle and DNA synthesis (31-33). Regarding its role in cancer, elF3a has been reported to be involved in decreasing the expression of DNA repair proteins, resulting in enhanced chemotherapeutic sensitivity in lung cancer (16). Consistently, elF3a negatively regulates the resistance to cisplatin via suppressing the cellular synthesis and activity of nucleotide excision repair proteins in nasopharyngeal carcinoma (34). In addition, elF3a exerts an oncogenic role by accelerating cell proliferation and inhibiting apoptosis in ameloblastoma (21). Accumulating evidence has demonstrated that elF3a participates in the development of ovarian, urinary bladder and pancreatic cancer, as well as HCC (20,22,35,36). elF3a is upregulated in HCC and facilitates the translation of HIF-1α, which, in turn, regulates glycolytic metabolism (22). Additionally, a serum anti-elF3a autoantibody has been identified as a potential diagnostic biomarker for HCC, further supporting the role of elF3a in HCC (37). However, the mechanism underlying the upregulation of elF3a and the functions of elF3a on the proliferation and metastasis in HCC remains unclear. In the present study, elF3a expression levels were upregulated and negatively correlated with those of miR-875-5p in HCC tissues. Knockdown of elF3a inhibited the proliferation, migration and invasion of HCC cells. Additionally, the results of the present study demonstrated that elF3a was a downstream target of miR-875-5p in HCC. Firstly, miR-875-5p negatively modulated the luciferase activity of reporter vectors carrying wild-type, but not mutant 3UTR of elF3a. Secondly, altering miR-875-5p expression negatively regulated the mRNA and protein expression of elF3a in HCCLM3 cells. Lastly, overexpression of elF3a reversed the suppressive effects of miR-875-5p on HCC cell proliferation and mobility. Therefore, the present study confirmed that miR-875-5p served a tumor suppressor role by downregulating elF3a in HCC. The majority of HCC cases arise from liver fibrosis or cirrhosis (38). Of note, elF3a has been reported to be involved in the fibrosis of various organs, including the lung, kidney, heart, skin and liver (39-43). The aforementioned studies demonstrated that elF3a, the expression of which is upregulated by the transforming growth factor β1 (TGF-β1)/Smad3 signaling pathway, mediated the TGF-β1-induced fibrosis. Thus, further studies are required to determine whether liver fibrosis participates in the elF3a-induced tumorigenesis in HCC.
In summary, the downregulation of miR-875-5p serves a crucial role in tumor growth and metastasis in HCC and may be a valuable prognostic marker and potential therapeutic target for HCC.

Acknowledgements

We would like to thank Dr Qing-An Jia (Liver Cancer Institute, Zhongshan Hospital, Fudan University, Shanghai, P.R. China) for kindly providing Human HCC cell lines (MHCC-97L, MHCC-97H, HCCLM3).

Funding

This study was supported by grants from the National Natural Science Foundation of China (grant no. 81874069).

Availability of data and materials

The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

Authors' contributions

TC contributed to writing the manuscript, study conception and design, and collection and analysis of data. LS, BY, and LW collected and interpreted data. YW, YN, RL, HM contributed to data analysis and interpretation and drafting the manuscript. ZL and KT contributed to study conception and revised the manuscript. QL contributed to study conception and design as well as revising and approving the final version of the manuscript. All authors have read and approved the final version of this manuscript.

Ethics approval and consent to participate

All procedures involving human participants were in accordance with the ethical standards of the Research Ethics Committee of The First Affiliated Hospital of Xian Jiaotong University and with the Declaration of Helsinki as revised in 2013. Written informed consent to participate in the study was obtained from patients with HCC prior to sample collection. Animal experiments were performed according to the protocol approved by the Ethics Review Committee of The First Affiliated Hospital of Xian Jiaotong University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References


