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MicroRNA-129-5p inhibits hepatocellular carcinoma cell metastasis and invasion via targeting ETS1

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ABSTRACT

MiR-129-5p is deregulated in various human cancers and has been associated with hepatocellular carcinoma (HCC) progression. However, the underlying mechanisms of miR-129-5p involvement in the development and progression of HCC and the effects of miR-129-5p deregulation on the clinical characteristics observed in HCC patients remain poorly understood. We therefore investigated the correlation between low miR-129-5p expression and vascular invasion, intrahepatic metastasis, and poor patient survival. Ectopic restoration of miR-129-5p expression in HCC cells suppressed cellular migration and invasion and the expression of v-ets erythroblastosis virus E26 oncogene homolog 1 (ETS1), while inhibition of endogenous miR-129-5p caused an increase in these parameters. We identified the ETS1 gene as a novel direct target of miR-129-5p. SiRNA-mediated ETS1 knockdown rescued the effects of anti-miR-129-5p inhibitor in HCC cell lines, while the effects of miR-129-5p overexpression were partially phenocopied in the knockdown model. In addition, miR-129-5p levels inversely correlated with those of ETS1 in HCC cells and tissues. Taken together, our findings indicate an important role for miR-129-5p in the molecular etiology of invasive HCC and suggest that miR-129-5p could have potential therapeutic applications in HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer death worldwide [1]. Although surgical and treatment strategies have been improved over time, the prognosis of advanced HCC remains poor due to recurrence, early invasion into blood vessels, and intra-hepatic metastases followed by subsequent extra-hepatic metastases [2,3]. Although many genes have been reported to be related to these processes, the molecular mechanisms responsible for the progression of HCC remain largely unknown.

MicroRNAs (miRNAs), a class of small non-coding RNAs, can bind to complementary sequences in the 3′ untranslated region (-UTR) of target mRNAs and negatively regulate gene expression by translational inhibition and destabilization of mRNAs [4]. MiRNA expression profiles have been used to classify cancers. Several studies have shown that miRNA expression, including miR-150-5p, miR-135b, and miR-520g, is dysregulated in HCC and that both loss and gain of miRNA function contribute to HCC metastasis and invasion [5–8]. In previous studies, we have found that miR-338-3p and miR-145, downregulated in HCC, inhibited the invasion and migration of cultured HCC cells by regulating the expression of SMO (frizzled class receptor) and ADAM17 (a disintegrin and metalloprotease domain 17), respectively [9,10].

We have also previously shown that miR-129-5p is downregulated in HCC tissues samples by microRNA multianalyte suspension array (mirMASA) technology [11]. Further, it has been reported that miR-129-5p is downregulated in several tumor types including gastric cancer [12], bladder cancer [13], and colorectal cancer [14]. Hitherto, only one study reported that VCP/p97

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(valosin-containing protein gene) was downregulated by miRNA-129-5p to regulate HCC progression [15]. However, to the best of our knowledge, a study into the relationship between miR-129-5p and clinical features in HCC patients has never been reported.

In the present study, we aimed to investigate the underlying mechanisms of miR-129-5p involvement in the development and progression of HCC by observing the expression of various genes, including that of v-ets erythroblastosis virus E26 oncogene homolog 1 (ETS1), in HCC patient tissues and in cell lines under varying miR129-5p status. We also studied the effects of miR-129-5p upon the clinical characteristics observed in HCC patients in terms of vascular invasion, intrahepatic metastasis, and overall patient survival.

2. Materials and methods

2.1. Human tissue specimens

Paraffin-embedded specimens originally collected between March 2007 and December 2013 from 173 HCC patients were obtained from the First Affiliated Hospital of Sun Yat-sen University (Guangzhou, China). All patient diagnoses of primary HCC were confirmed by at least 2 pathologists. None of the patients received any pre-operative cancer treatments. The patients' clinicopathological characteristics are shown in Supplementary Table 1. The patients were divided into 2 groups based on median miR-129-5p expression: low expression (n = 86) and high expression (n = 87). The median follow-up interval was 30.8 months. This study was reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University. Clinical samples were collected from patients after obtaining informed consent.

2.2. Cell lines and transfection

Five human HCC cell lines (MHCC-97H, SMMC-7721, Huh-7, HepG2 and BEL-7402) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 100 U/ml of penicillin–streptomycin in a humidified atmosphere of 5% CO_2 at 37 °C. Pre-miR miRNA-129-5p precursor (pre-miR-129-5p), anti-miR miRNA-129-5p inhibitor (anti-miR-129-5p), pre-miR miRNA precursor molecules - negative control (AM17110), and anti-miR miRNA inhibitors - negative control (AM17010) were purchased from Ambion (Austin, TX, USA). Furthermore, 3 siRNA duplex oligonucleotides against human snail1 mRNA were synthesized by RiboBio (Guangzhou RiboBio Co., Ltd., Guangzhou, China). Snail1 siRNA1 was used due to its ability to effectively inhibit endogenous snail1 expression. SiRNA against ETS1 and a matched control siRNA were also synthesized by RiboBio (Table S2). MHCC-97H and SMMC-7721 Cells were transfected with miRNA (100 nM) or siRNA (50 nM) using Lipofectamine 2000 Transfection Reagent (Invitrogen, CA, USA) according to the manufacturer's protocol.

2.3. Scratch migration assay and transwell invasion assays

Migration and invasion assays were performed using hepatic immortalized cell line (LO2) cells. Scratch migration assays were performed as described previously [9]. Briefly, HCC cells (5×10^5) were seeded into 24-well plates and cultured in growth medium overnight and then transiently transfected. After 48 h, at around 90% confluence an artificial homogenous wound was created on the cell monolayer with a 200-μL plastic micropipette tip; cell debris was removed by washing twice with PBS. Images of cell migration were taken under an inverted Olympus BX50 microscope (BX50, Olympus, Tokyo, Japan), with a 100 × objective lens.

Transwell invasion assays were performed according to a previously described protocol [9,10]. An 8-μm pore size filter was inserted into each Transwell chamber (Corning, NY, USA) and coated with 50 μL Matrigel (Sigma, MO, USA). The transiently transfected HCC cells (5×10^3) were then seeded into the upper chamber of a 24-well plate in serum-free medium. After 24 h incubation at 37 °C in 5% CO_2, invasive cells, defined as those on the lower surface of the membrane, were stained with a 0.1% solution of crystal purple dye. Cells from 3 randomly selected fields per filter were counted under a microscope at 100 × magnification. All experiments were conducted in triplicate and performed twice.

2.4. Reverse transcription polymerase chain reaction (RT-PCR) and real-time quantitative RT-PCR (qPCR)

Total RNA was extracted from both tissue and cells as described previously [11]. The expression of mature miR-129-5p was assayed by real-time RT–PCR with TaqMan miRNA assay kit (Applied Biosystems, CA, USA); RNU6b was used as an internal control for miRNA. ETS1 mRNA was detected using TaqMan qRT–PCR and normalized to β-actin (Applied Biosystems). Real-time PCR was performed as described previously [16]. The PCR primers for ETS1 and β-actin were designed with Premier Primer 5.0 software (Table S2). Data were acquired using a HT-7900 TaqMan instrument (Applied Biosystems). All experiments were performed in triplicate and conducted twice.

2.5. Protein extraction and western blotting

Protein extraction rom both tissue and cells and western blotting were performed as described previously [10]. Primary antibodies were obtained from Santa Cruz Biotechnology (CA, USA) and used for Ets1 (1:500) and GAPDH (1:1000) detection) a secondary antibody of goat anti-rabbit horseradish peroxidase–labeled antibody (1:3000, Bio-Rad Laboratories, Inc., CA, USA) was then used.

2.6. Luciferase reporter assay

The ETS1 3′-UTR luciferase reporter vector construct (ETS1 3′-UTR WT) was generated by cloning the ETS1 mRNA 3′-UTR sequence downstream of the luciferase gene in PEZX-MT01-report construct (GeneCopoeia™, MD, USA). The wild type and mutant ETS1 3′-UTR sequences were generated by PCR using the following primers: 5′-GCTAGCATGGCACTGAAGGGGCTGGGG-3′ (sense) and 5′-GCGGCCGCCATTATGAATGAAATTCTTTG-3′ (antisense). PCR products were cloned into the PEZX-MT01 vector at the same sites, amplified, and confirmed by sequencing. There were two predicted miR-129-5p target sites in the 3′-UTR of ETS1 mRNA, nucleotides 430–437 and 3443–3450. To confirm binding between miR-129-5p and the 3′-UTR of ETS1, 3 mutants of 3′-UTR of ETS1 mRNA were constructed by deleting the 2 targets sites, either alternately (PEZX-MT01-ETS1-3′-UTR m1, PEZX-MT01-ETS1-3′-UTR m2) or together (PEZX-MT01-ETS1-3′-UTR m3), to generate 3 reporter vectors. These mutant reporters were transfected into MHCC-97H cells together with pre-miR-129-5p. Forty-eight hours after transfection, the cells were harvested and measured with a Dual-Luciferase Reporter Assay System Kit (Promega, Madison, WI, USA). Firefly luciferase activity was then normalized to Renilla luciferase activity. The experiments were performed in triplicate and repeated twice.

2.7. Immunohistochemistry

Immunohistochemical staining was performed as previously described [16], with antibodies specific for Ets1 (1:200, Abcam Inc,

Cambridge, MA, USA). Negative controls included parallel sections treated with primary antibody omitted.

2.8. Statistical analysis

Statistical analyses were performed using SPSS, version 13.0 (SPSS, Chicago, IL, USA). Statistical differences in univariate survival analyses were compared using the Kaplan–Meier method and the log-rank test. We used the Cox proportional-hazards regression model for multivariate analysis. Differences between the groups were calculated with Student's *t*-test. Pearson's correlation test was used to assess the correlation between ETS1 mRNA expression and miR-129-5p expression. $P < 0.05$ was considered statistically significant.

3. Results

3.1. The clinicopathological significance of downregulated miR-129-5p expression in HCC

Although, in a previous study, we observed lower miR-129-5p expression in HCC tissues than in corresponding adjacent non-tumorous tissue (NT) by a bead-based miRNA expression profiling method [11], the role of downregulated miR-129-5p in the HCC patients remains unknown. To further determine the significance of miR-129-5p expression in HCC patients, miR-129-5p levels were examined in the tissues of 173 HCC patients by qRT-PCR. Patients with low miR-129-5p expression had a higher ratio of vascular invasion ($P = 0.021$), intrahepatic metastasis ($P = 0.035$), and higher recurrence rate ($P = 0.031$) than patients with high miR-129-5p expression (Table 1). Furthermore, patients in the low miR-129-5p expression group had a significantly poorer prognosis than those in the high miR-129-5p expression group ($P = 0.0254$; Fig. 1). Multivariate survival analysis demonstrated that vascular invasion

Table 1
Correlation between miR-129-5p expression and the clinicopathological data of the HCC patients.

Characteristics	miR-129-5p expression		*P*
	Low	High	
Age			0.314
<50	30	25	
≥50	56	62	
Sex			0.376
Male	68	53	
Female	18	34	
Etiology			0.227
Non-infection	8	12	
HepatitisB	69	64	
Hepatitis C	9	11	
Liver cirrhosis			0.138
Absence	20	30	
Presence	66	57	
Tumor size (cm)			0.412
≤ 5	27	22	
> 5	59	65	
Serum AFP (μg/L)			0.315
≤ 20	24	35	
>20	62	52	
Vascular invasion			**0.021**
Absence	47	62	
Presence	39	25	
Intra-hepatic metastasis			**0.035**
Absence	36	61	
Presence	50	26	
Recurrence			**0.031**
No	40	53	
Yes	46	34	

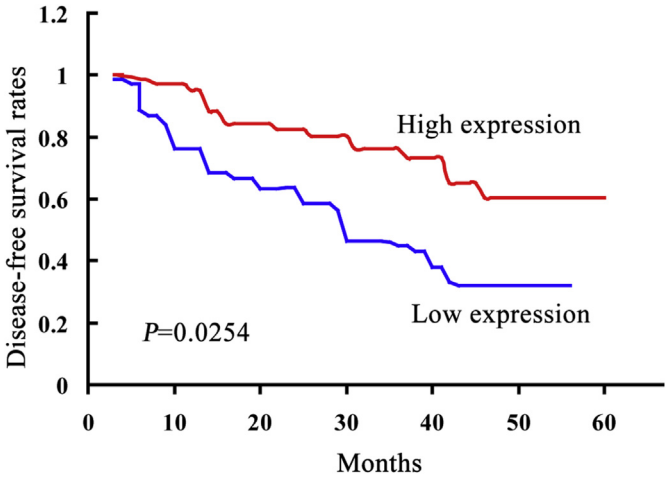

Fig. 1. The correlation between miR-129-5p expression, detected by real-time reverse transcription polymerase chain reaction, and the disease-free survival of 173 hepatocellular carcinoma (HCC) patients was analyzed using the Kaplan–Meier survival curve.

(Hazard ratio, HR = 2.649, $P = 0.038$), intrahepatic metastasis (HR = 2.161, $P = 0.029$), and low miR-129-5p expression (HR = 2.436, $P = 0.017$) were independent prognostic markers indicating poor survival for HCC patients (Table 2).

3.2. Effects of miR-129-5p on HCC cell migration and invasion

To explore the potential impact of miR-129-5p upon HCC cell migration and invasion we analyzed the expression of miR-129-5p in different HCC cell lines and the hepatic immortalized cell line, LO2. Our results showed that expression of miR-129-5p was lowest in high-invasive MHCC-97H cells. Whereas low-invasive SMMC-7721 cells had the highest miR-129-5p levels (Fig. 2A). We therefore performed subsequent invasion assays with MHCC-97H cells transfected with pre-miR-129-5p or a negative control (NC) and SMMC-7721 cells transfected with anti-miR-129-5p inhibitor or anti-miR-129-5p control (Fig. 2B). In the migration and invasion assays, ectopic expression of miR-129-5p attenuated the migration and invasion capabilities of MHCC-97H cells in comparison to levels with the negative controls (Fig. 2B). In contrast, introduction of anti-miR-129-5p inhibitor into SMMC-7721 cells downregulated miR-129-5p expression and increased cell migration capabilities (Fig. 2B). Transwell analysis showed that HCC cells transfected with anti-miR-129-5p inhibitor presented with greater invasion capacity than cells transfected with anti-NC (Fig. 2C). These results suggest suppressive effects of miR-129-5p on HCC metastasis.

Table 2
Cox regression analysis of prognosis factors associated with disease-free survival rates in HCC patients.

Factors	HR (95%CI)	*p*
Age (≥50/<50)	0.976 (0.942–1.132)	0.421
Sex (Female/Male)	1.102 (0.981–1.343)	0.093
HBV (±)	0.721 (0.453–1.463)	0.418
Liver cirrhosis (yes/no)	0.879 (0.573–4.97)	0.167
Tumor size (>5 cm/≤ 5 cm)	1.346 (0.678–3.162)	0.312
Serum AFP (>20 ug/L/≤ 20 ug/L)	0.794 (0.508–6.012)	0.297
Vascular invasion (yes/no)	2.649 (1.156–6.483)	0.038
Intra-hepatic metastasis (yes/no)	2.161 (0.912–4.136)	0.029
miR-129-5p expression (low/high group)	2.436 (1.724–4.378)	0.017

*$P < 0.05$ was considered significant; HBV: hepatitis B virus; AFP:Alpha fetoprotein; HR:Hazard ratio; 95% CI: 95% confidence interval.

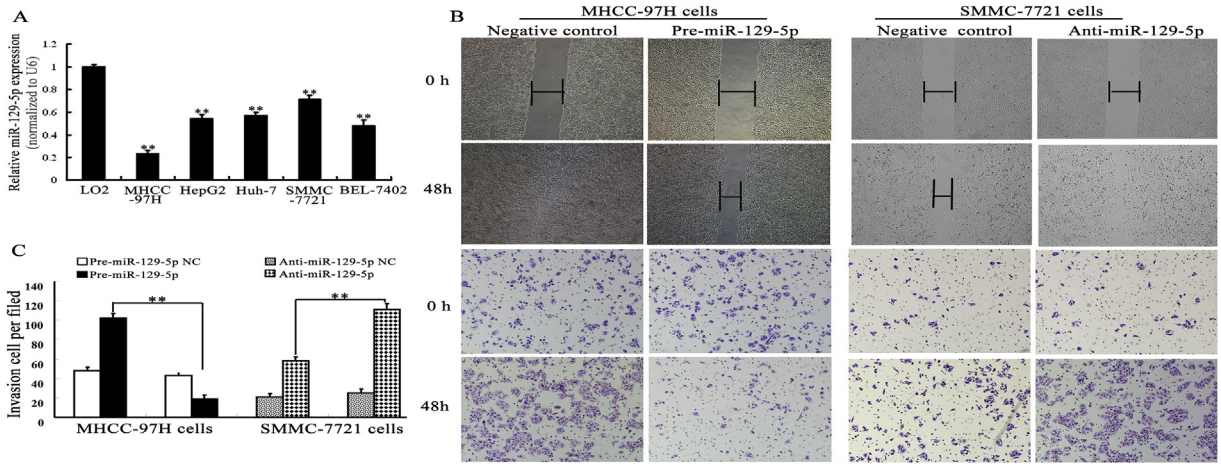

Fig. 2. Effects of miR-129-5p on hepatocellular cancer (HCC) cell invasion. (A) Expression levels of miR-129-5p in normal liver cells and different HCC cell lines (MHCC-97H, SMMC-7721, Huh-7, HepG2 and BEL-7402). (B, C) Invasion analysis of MHCC-97H and SMMC-7721 cells using transwell assays after transfection with pre- or anti-miR- miR-129-5p, respectively. (Original magnification, × 100). **P < 0.01.

3.3. MiR-129-5p directly targets ETS1 via 2 essential target sites

To further investigate the target genes regulated by miR-129-5p that may contribute to its biological function, we chose to focus on ETS1 due to its importance in cell motility and invasion, as indicated in the TargetScan database [17]. We performed a luciferase reporter assay to examine whether miR-129-5p directly targeted ETS1. We found lower ETS1 3′-UTR luciferase activity in the miR-129-5p group compared with the control, suggesting that miR-129-5p could directly target the 3′-UTR of ETS1. There were 2 predicted miR-129-5p target sites in the 3′-UTR of ETS1 mRNA (Fig. 3A). The 3 mutant reporter vectors were transfected into the MHCC-97H cell lines together with pre-miR-129-5p. Luciferase expression was no longer regulated by miR-129-5p after the deletion of the 30–437 and/or 3443–3450 nucleotide locations in the 3′-UTR (Fig. 3B). This suggested that both target sites in the 3′-UTR of ETS1 mRNA were essential for the regulation by miR-129-5p. Moreover, miR-129-5p overexpression significantly reduced expression of ETS1 mRNA and protein in MHCC-97H cells, whereas anti-miR-338-3p inhibitor induced their expressions in SMMC-7721 cells (Fig. 3C).

3.4. MiR-129-5p represses HCC cell migration and invasion by direct targeting of ETS1

To demonstrate that miR-129-5p effects on invasion are exerted through ETS1, we performed a rescue experiment. SMMC-7721 cells were transfected with ETS1 siRNA in the absence or presence of anti-miR-129-5p. Upon siRNA mediated upon ETS1 knockdown, the increased cell invasion and metastasis caused by anti-miR-129-5p was reversed (Fig. 4A, B). The co-transfection of anti-miR-129-5p with ETS1 siRNA significantly reversed anti-miR-129-5p-induced ETS1 protein expression (Fig. 4C). In addition, knockdown of Ets1 in SMMC-7721 cells resulted in decreased cellular invasion capacity (Fig. 4A, B, C). These results suggested that knockdown of Ets1 phenocopied the effects of miR-129-5p overexpression in inhibiting the invasion of MHCC-97H cells. Therefore, further

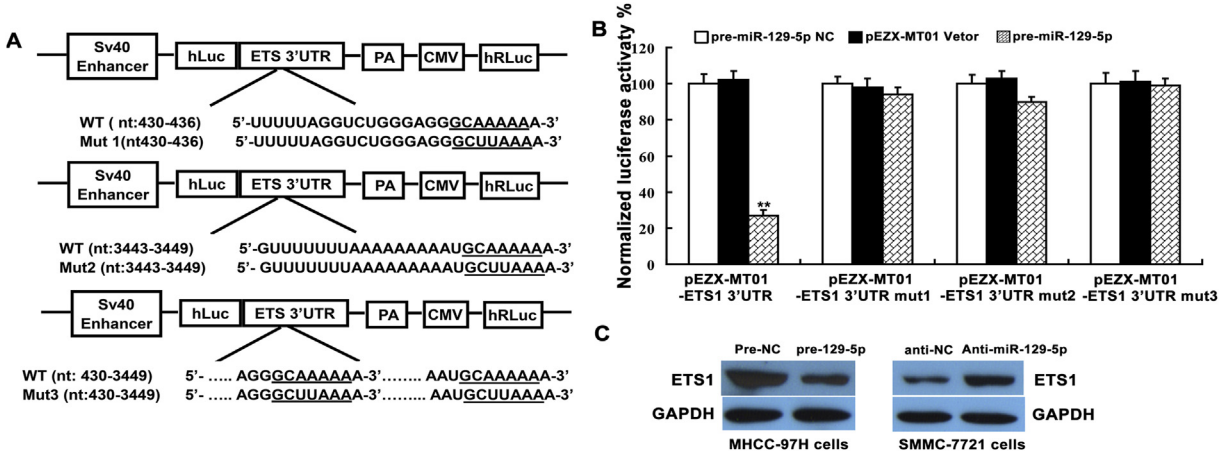

Fig. 3. MiR-129-5p could directly regulate the expression of ETS1. (A) Bioinformatic analysis of miR-129-5p allowed binding site prediction in the ETS1 3′ untranslated region (-UTR). There were 2 putative miR-129-5p target sites located in this region (nucleotides 430–437 and 3443–3450). Three mutant reporter vectors were constructed by the deletion of 2 target sites individually (PEZX-MT01-ETS1-3′-UTR m1, PEZX-MT01-ETS1-3′-UTR m2) or both (PEZX-MT01-ETS1-3′-UTR m3). (B) The luciferase reporter assay showed that miR-129-5p could significantly suppress the luciferase activity of PEZX-MT01-ETS1-3′-UTR in MHCC-97H cells, while having no effect on the 3 mutant reporter vectors. Data are representative of 3 independent experiments. (C) Overexpression of miR-129-5p and knockdown of endogenous miR-129-5p by siRNA reduced and enhanced protein levels of ETS1, respectively. **P < 0.01.

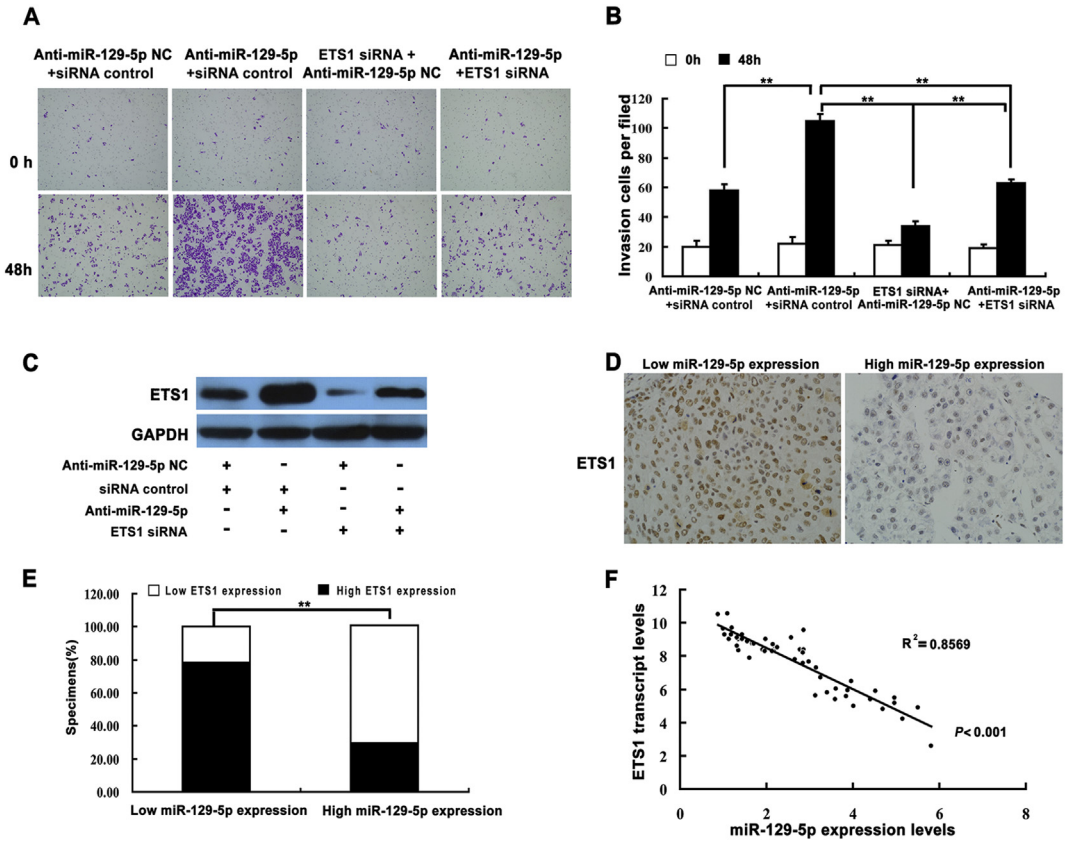

Fig. 4. miR-129-5p suppresses hepatocellular carcinoma (HCC) cell invasion through inhibition of ETS1. SMCC-7721 cells were transfected with ETS1 siRNA or/and anti-miR-129-5p. Cell invasiveness in a transwell invasion chamber was evaluated (A, B). (C) The expression of ETS1 was detected by western blot analysis in SMCC-7721 cells. (D) The ETS1 levels were detected by immunohistochemical analysis in HCC tissues with low or high miR-129-5p expression. (E) Percentage of tissue specimens indicating low or high miR-129-5p expression and their association with the expression levels of ETS1 in clinical HCC specimens. (F) MiR-129-5p levels were inversely correlated with ETS1 expression in HCC tissue. $^{**}P < 0.01$.

demonstrating that miR-129-5p inhibited HCC metastasis by downregulating ETS1.

To explore the association between miR-129-5p and ETS1 expression in HCC tissues, we performed real-time PCR and immunohistochemical analysis. Tumors presenting with high miR-129-5p levels were found to have low levels of ETS1 (Fig. 4D, E), whereas tumors demonstrating a low level of miR-129-5p showed high ETS1 levels (Fig. 4D, E). Moreover, we demonstrated miR-129-5p expression to be inversely associated with ETS1 expression in these HCC tissue specimens (Fig. 4F). These results were consistent with our *in vitro*.

4. Discussion

It has been previously reported that miR-129-5p was deregulated in several tumor types, including neuroendocrine tumors [18], gastric cancer [19], medullary thyroid carcinoma [20], and HCC [15]. MiR-129-5p has also been suggested to be upregulated in laryngeal squamous cell carcinoma [21] and act as an oncogene. In the present study, we confirmed that low miR-129-5p expression was strongly correlated with portal vein invasion, recurrence, and poor survival. We found that miR-129-5p overexpression induced suppression of MHCC-97H cell invasion and, its inhibition significantly increases SMMC-7721 cell invasion. Moreover, we identified ETS1 as a novel target of miR-129-5p. Our results showed that miR-129-5p downregulation was correlated with ETS1 upregulation in HCC tissues. MiR-129-5p inhibited cell migration and invasion via targeting ETS1 in HCC cells. Therefore, miR-129-5p may serve as a novel prognostic marker in HCC patients.

MiR-129-5p is located in a fragile site in chromosome 7q32 [22]. Previous research has shown that miR-129-5p is the functional form of miR-129, consistent with our results [23]. The observations in our experiment showed that the other members of the miR-129 family (miR-129-1-3p and miR-129-2-3p) did not play an important role in HCC cell activity (data not shown). Yu Liu et al. found that the expression of miR-129-5p could increase apoptosis and reduce migration of HCC cells by suppressing the expression of VCP [15]. However, the exact function and downstream targets of miR-129-5p in HCC remain unknown.

The transcription factor ETS1 binds to specific DNA sequences containing a GGAA/T core motif. To date, growing evidence indicates that overexpression of Ets1 is highly associated with some types of cancer [24,25]. Previous studies have indicated that Ets1 expression is also correlated with the clinicopathological features of HCC, including tumor histological differentiation, invasion, and metastasis [26,27]. Recent studies have shown ETS1 to be regulated by miRNAs. For example, overexpression of miR-193b significantly suppresses the proliferation, migration, and invasion of HCC cells via targeting Ets1 and cyclin D1 [28]. In addition, overexpression of miR-1 and miR-499 inhibits Ets1 expression through directly targeting the 3′-UTR, resulting in decreased invasion and migration of HCC cells [27]. In our study, we predicted ETS1 to be a novel target gene of miR-129-5p. We confirmed this hypothesis by demonstrating that miR-129-5p represses the expression of ETS1 through direct interaction with 2 sites located at its 3′-UTR, thus inhibiting

the invasion and metastasis of HCC cells. Moreover, we showed that ETS1 knockdown could partially abrogate the invasion ability of SMMC-7721 cells induced by anti-miR-129-5p. Furthermore, this knockdown partially phenocopied the effects of miR-129-5p overexpression in HCC cell lines. These results collectively verified the concept that ETS1 is a functional target of miR-129-5p.

To the best of our knowledge, our study is the first to show that downregulation of miR-129-5p expression is significantly associated with vascular invasion, intrahepatic metastasis, and poor patient survival. Furthermore, we have revealed that miR-129-5p inhibits the invasion and metastasis of HCC cells through directly targeting the ETS1 3′-UTR. Additionally, we reported a negative correlation between miR-129-5p expression levels and ETS1 levels in HCC tissues. These results suggest that miR-129-5p could be a useful prognostic marker and/or therapeutic target in HCC.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.04.075.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.04.075.

References

[1] C.M. Wong, I.O. Ng, Molecular pathogenesis of hepatocellular carcinoma, Liver Int. 28 (2008) 160e174.
[2] K. Okuda, Early recognition of hepatocellular carcinoma, Hepatology 6 (1986) 729–738.
[3] The Liver Cancer Study Group in Japan, Primary liver cancer in Japan: clinicopathologic features and results of surgical treatment, Ann. Surg. 211 (1990) 277–287.
[4] M.V. Iorio, C.M. Croce, MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review, EMBO Mol. Med. 4 (2012) 143–159.
[5] A. Budhu, H.L. Jia, M. Forgues, C.G. Liu, D. Goldstein, A. Lam, K.A. Zanetti, Q.H. Ye, L.X. Qin, C.M. Croce, Z.Y. Tang, X.W. Wang, Identification of metastasisrelated microRNAs in hepatocellular carcinoma, Hepatology 47 (2008) 897–907.
[6] T. Li, J. Xie, C. Shen, D. Cheng, Y. Shi, Z. Wu, Q. Zhan, X. Deng, H. Chen, B. Shen, C. Peng, H. Li, Z. Zhu, miR-150–5p inhibits hepatoma cell migration and invasion by targeting MMP14, PLoS One 9 (2014) e115577.
[7] Y. Li, D. Xu, C. Bao, Y. Zhang, D. Chen, F. Zhao, J. Ding, L. Liang, Q. Wang, L. Liu, J. Li, M. Yao, S. Huang, X. He, microRNA-135b,a HSF1 target,promotes tumor invasion and metastasis by regulating RECK and EVI5 in hepatocellular carcinoma, Oncotarget 6 (2015) 2421–2433.
[8] H. Kan, W. Guo, Y. Huang, D. Liu, MicroRNA-520g induces epithelial-mesenchymal transition and promotes metastas hepatocellular carcinoma by targeting SMAD7, FEBS Lett. 589 (2015) 102–109.
[9] X.H. Huang, J.S. Chen, Q. Wang, X.L. Chen, L. Wen, L.Z. Chen, J. Bi, L.J. Zhang, Q. Su, W.T. Zeng, miR-338-3p suppresses invasion of liver cancer cell by targeting smoothened, J. Pathol. 225 (2011) 463–472.
[10] X.W. Yang, L.J. Zhang, X.H. Huang, L.Z. Chen, Q. Su, W.T. Zeng, W. Li, Q. Wang, miR-145 suppresses cell invasion in hepatocellular carcinoma cells: miR-145 targets ADAM17, Hepatol. Res. 44 (2014) 551–559.
[11] X.H. Huang, Q. Wang, J.S. Chen, X.H. Fu, X.L. Chen, L.Z. Chen, W. Li, J. Bi, L.J. Zhang, Q. Fu, W.T. Zeng, L.Q. Cao, H.X. Tan, Q. Su, Bead-based microarray analysis of microRNA expression in hepatocellular carcinoma: miR-338 is downregulated, Hepatol. Res. 39 (2009) 786–794.
[12] R. Shen, S. Pan, S. Qi, X. Lin, S. Cheng, Epigenetic repression of microRNA-129-2 leads to overexpression of SOX4 in gastric cancer, Biochem. Biophys. Res. Commun. 394 (2010) 1047–1052.
[13] L. Dyrskjøt, M.S. Ostenfeld, J.B. Bramsen, A.N. Silahtaroglu, P. Lamy, R. Ramanathan, N. Fristrup, J.L. Jensen, C.L. Andersen, K. Zieger, S. Kauppinen, B.P. Ulhøi, J. Kjems, M. Borre, T.F. Orntoft, Genomic profiling of microRNAs in bladder cancer: miR-129 is associated with poor outcome and promotes cell death in vitro, Cancer Res. 69 (2009) 4851–4860.
[14] E. Bandres, X. Agirre, N. Bitarte, N. Ramirez, R. Zarate, J. Roman-Gomez, F. Prosper, J. Garcia-Foncillas, Epigenetic regulation of microRNA expression in colorectal cancer, Int. J. Cancer 125 (2009) 2737–2743.
[15] Y. Liu, Y. Hei, Q. Shu, J. Dong, Y. Gao, H. Fu, X. Zheng, G. Yang, VCP/p97, down-regulated by microRNA-129-5p,could regulate the progression of hepatocellular carcinoma, PLoS One 7 (2012) e35800.
[16] J.S. Chen, X.H. Huang, Q. Wang, X.L. Chen, X.H. Fu, H.X. Tan, L.J. Zhang, W. Li, J. Bi, FAK is involved in invasion and metastasis of hepatocellular carcinoma, Clin. Exp. Metastasis 27 (2010) 71–82.
[17] Z. Shaikhibrahim, N. Wernert, ETS transcription factors and prostate cancer: the role of the family prototype ETS-1, Int. J. Oncol. 40 (2012) 1748–1754.
[18] Kristina B.V. Døssing, Tina Binderup, Bogumil Kaczkowski, Anders Jacobsen, Maria Rossing, Ole Winther, Birgitte Federspiel, Ulrich Knigge, Andreas Kjær, Lennart Friis-Hansen, Down-regulation of miR-129-5p and the let-7 family in neuroendocrine tumors and metastases leads to up-regulation of their targets Egr1, G3bp1, Hmga2 and Bach1, Genes. (Basel) 6 (2014) 1–21.
[19] X. Yu, H. Song, T. Xia, S. Han, B. Xiao, L. Luo, Y. Xi, J. Guo, Growth inhibitory effects of three miR-129 family members on gastric cancer, Gene 532 (2013) 87–93.
[20] L. Duan, X. Hao, Z. Liu, Y. Zhang, G. Zhang, MiR-129-5p is down-regulated and involved in the growth, apoptosis and migration of medullary thyroid carcinoma cells through targeting RET, FEBS Lett. 588 (2014) 1644–1651.
[21] M. Li, L. Tian, L. Wang, H. Yao, J. Zhang, J. Lu, Y. Sun, X. Gao, H. Xiao, M. Liu, Down-regulation of miR-129-5p inhibits growth and induces apoptosis in laryngeal squamous cell carcinoma by targeting APC, PLoS One 8 (2013) e77829.
[22] R. Ogawa, H. Ishiguro, Y. Kuwabara, M. Kimura, A. Mitsui, T. Katada, K. Harata, T. Tanaka, Y. Fujii, Expression profiling of micro-RNAs in human esophageal squamous cell carcinoma using RT-PCR, Med. Mol. Morphol. 42 (2009) 102–109.
[23] P. Brest, S. Lassalle, V. Hofman, O. Bordone, V. Gavric Tanga, C. Bonnetaud, C. Moreilhon, G. Rios, J. Santini, P. Barbry, C. Svanborg, B. Mograbi, B. Mari, P. Hofman, MiR-129-5p is required for histone deacetylase inhibitor-induced cell death in thyroid cancer cells, Endocr. Relat. Cancer 18 (2011) 711–719.
[24] J.C. Hahne, A.F. Okuducu, A. Sahin, V. Fafeur, S. Kiriakidis, N. Wernert, The transcription factor ETS-1: its role in tumour development and strategies for its inhibition, Mini Rev. Med. Chem. 8 (2008) 1095–1105.
[25] A. Furlan, C. Vercamer, F. Bouali, I. Damour, A. Chotteau-Lelievre, N. Wernert, X. Desbiens, A. Pourtier, Ets-1 controls breast cancer cell balance between invasion and growth, Int. J. Cancer 135 (2014) 2317–2328.
[26] K. Kanda, T. Nakayama, S. Onizuka, T. Tomioka, T. Kanematsu, Expression of the Ets-1 proto-oncogene is linked to cell differentiation of human hepatocellular carcinoma, Hepatogastroenterology 49 (2002) 747–751.
[27] W. Wei, Z. Hu, H. Fu, Y. Tie, H. Zhang, Y. Wu, X. Zheng, MicroRNA-1 and microRNA-499 downregulate the expression of the ets1 proto-oncogene in HepG2 cells, Oncol. Rep. 28 (2012) 701–706.
[28] C. Xu, S. Liu, H. Fu, S. Li, Y. Tie, J. Zhu, R. Xing, Y. Jin, Z. Sun, X. Zheng, MicroRNA-193b regulates proliferation, migration and invasion in human hepatocellular carcinoma cells, Eur. J. Cancer 46 (2010) 2828–2836.