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Repression of miR-17-5p with elevated expression of E2F-1 and c-MYC in non-metastatic hepatocellular carcinoma and enhancement of cell growth upon reversing this expression pattern

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ABSTRACT

E2F-1, c-MYC, and miR-17-5p is a triad of two regulatory loops: a negative and a positive loop, where c-MYC induces the expression of E2F-1 that induces the expression of miR-17-5p which in turn reverses the expression of E2F-1 to close the loop. In this study, we investigated this triad for the first time in hepatocellular carcinoma (HCC), where miR-17-5p showed a significant down-regulation in 23 non-metastatic HCC biopsies compared to 10 healthy tissues; however, E2F-1 and c-MYC transcripts were markedly elevated. Forced over-expression of miR-17-5p in HuH-7 cells resulted in enhanced cell proliferation, growth, migration and clonogenicity with concomitant inhibition of E2F-1 and c-MYC transcripts expressions, while antagomirs of miR-17-5p reversed these events. In conclusion, this study revealed a unique pattern of expression for miR-17-5p in non-metastatic HCC patients in contrast to metastatic HCC patients. In addition we show that miR-17-5p is the key player among the triad that tumor growth and spread.

1. Introduction

In the context of tumor biology, hyper-proliferation and invasion are two distinct hallmarks of cancer. They are also important determinants of progression and consequently metastasis in vivo [1]. miR-17-5p is an upstream regulator of E2F-1; it constitutes an autoregulatory triad with E2F-1 and c-MYC oncoproteins [2]. This interesting regulatory loop has been recently discovered between these three key players where c-Myc/E2F/miR-17-92 network is composed of two feedback loops: a positive self-feedback loop for protein module (c-Myc/E2F) and a negative loop between the miRNA and the proteins [3]. The first positive self-feedback loop is through the concomitant induction of expression between E2F-1 and c-MYC proteins where each transcription factor has been proved to induce the expression of the other [4,5], as *E2F1* promoter contains c-MYC responsive element through which E2F-1 protein expression is induced [6]. The second loop, negative loop, is through the interaction between miR-17-5p and its downstream target, where E2F-1 expression is inhibited by the miR-17-92 cluster member. In return, E2F-1 and c-Myc induce the transcription of miR-17-5p, thus forming a negative feedback loop in the interaction network [2,7]. This triad and the non-stop interaction between the three key players have been proved in secondary cell lines only, nevertheless, has never been investigated in human cancers.

Interestingly, each one of the triad component plays a role in cancer especially in hepatocellular carcinoma (HCC). miR-17-5p is an important member of miR-17-92 cluster that was described as oncogenic microRNA oncomiR. miR-17-5p was described as a key regulator of the G1/S phase cell cycle transition [8]. Elevated expression of miR-17-5p was evidenced in several cancers where its over-expression promoted cell cycle progression, proliferation, inhibited apoptosis. It also contributed to advanced tumor progression as in gastric, lung cancer, chronic myeloid leukemias and B-cell mantel cell lymphomas [9–12]. In HCC, miR-17-5p was found to be generally over-expressed [13–15], where it showed a significant up-regulation in metastatic HCC and contributed to metastatic activity in HuH-7 through p38 mitogen activated kinase-heat shock protein 27 (p38 MAPK-HSP27) pathway. However it has never been evaluated in non-metastatic HCC tissues [13].

Similarly, in HCC, genetic analyses have revealed c-Myc genomic amplification that was present in up to 70% of viral and alcohol-related HCC [16]. Furthermore, the presence of c-Myc amplification portends a more advanced and aggressive pheno-

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type, indicating that c-Myc plays a critical role in pathogenesis of HCC [17,18]. c-Myc has been also reported to contribute in metastasis and invasion during hepatocarcinogenesis through interaction with HIF-1α, which is up-regulated during hypoxia and induces angiogenesis. HIF-1α cooperates with c-Myc to enhance the expression of vascular-endothelial growth factor-A (VEGFA), a critical gene for angiogenesis [19]. On the other hand, E2F-1 mRNA and protein over-expression has been frequently evidenced in HCC with strong association with tumor progression and proliferation [20,21], and the deregulation of E2F1 is implicated in hepatocarcinogensies [22,23]. The importance of both E2F-1 and c-MYC is in their capacity to trigger proliferation by driving quiescent cells into S phase in the absence of other mitogenic stimuli [24–28] and in sensitizing cells to apoptosis either via p53-dependent or p53-independent mechanisms [29–31].

To date, c-MYC/E2F-1/miR-17-5p triad has never been investigated in HCC, and there is no clue which of the players is responsible for tumor progression and metastasis. Therefore, this study aims at investigating the expression of c-MYC/E2F/miR-17-5p network in non-metastatic HCC patients, in addition to highlighting the leading player for the transformation of liver tumors from localized non-metastatic tumor into metastatic HCC.

Table 1
Characteristic features of non-metastatic hepatocellular carcinoma (HCC) patients and healthy controls.

HCC and cirrhotic patients	Age	49 ± 13.5
	Sex: male/female	22/1
	Ethanol abuse	None
	Aspartate aminotransferase (AST) (U/L)	100.5 ± 65.8
	Alanine aminotransferase (ALT) (U/L)	85.6 ± 95.6
	Alkaline phosphatase (U/L)	110.2 ± 60.7
	Serum albumin (g/dL)	4.6 ± 1.5
	Serum AFP (ng/mL)	155.7 ± 22.3
	HCV Ab	100% (23 HCC patients)
	HBV Ab	17.3% (4/23 HCC patients)
Healthy controls (liver donors)	Age	21–42 years old
	Sex	70% males and 30% females
	Ethanol abuse	None
	HCV Ab	None
	HBV Ab	None

2. Materials and methods

2.1. Study patients

This study included 23 HCC patients who underwent liver transplant surgery in the Kasr El Einy Hospital, Cairo University, Egypt. Nine Cirrhotic tissues were taken from some of the same HCC patients with HCC focal lesions. Ten healthy liver biopsies were obtained from the healthy donors. Healthy donors were non-diabetic, non-hypertensive and negative to hepatitis B and C viruses Table 1. The study was approved by the Cairo University's ethical review committee. All participants gave their written informed consent. All patients were non-metastatic with no extrahepatic manifestations and no vascular invasion. Most of the patients (65.5%) had more than one focal lesion as indicated in the pathology report and were subjected to clinical assessment as shown in Table 2

Table 2
Number/sizes of focal lesions according to Milan criteria.

Patients	Number of focal lesions	Size of focal lesions
Patient 1	3 focal lesions	1.5 cm, 1 cm and 1 cm
Patient 2	Unifocal	2.5 cm
Patient 3	3 focal lesions	2 cm, 2.5 cm and 3 cm
Patient 4	3 focal lesions	2 cm, 2 cm and 3.5 cm
Patient 5	Unifocal	1.5 × 2 cm
Patient 6	3 focal lesions	3 × 4 cm, 1 cm and 1 cm
Patient 7	Unifocal	4 cm
Patient 8	3 focal lesions	4 cm, 1 cm and 1 cm
Patient 9	3 focal lesions	1 cm, 1 cm and 1.5 cm
Patient 10	Unifocal	2.5 cm
Patient 11	2 focal lesions	1 cm and 1.7 cm
Patient 12	3 focal lesions	1 cm each
Patient 13	Unifocal	3 cm
Patient 14	3 focal lesions	3 cm, 1.5 cm and 2 cm
Patient 15	3 focal lesions	1 cm, 1 cm and 4 cm
Patient 16	2 focal lesions	3 cm and 1.5 cm
Patient 17	2 focal lesions	1.5 cm and 3 cm
Patient 18	3 focal lesions	2.5 cm, 2.5 cm, 1.5 cm
Patient 19	3 focal lesions	1.5 cm. 1 cm and 1 cm
Patient 20	Unifocal	2 cm
Patient 21	Unifocal	1.5 cm
Patient 22	3 focal lesion	3 cm, 2.5 cm and 1 cm
Patient 23	Unifocal	3 cm

2.2. Cell cultures

HuH-7 cells were maintained in Dulbecco's modified Eagle's medium(DMEM) (Lonza, Switzerland) supplemented with 4.5 g/L glucose, 4 mmol/L L-glutamine, 10% fetal bovine serum and Mycozap (1:500, Lonza, Switzerland) at 37 °C in 5% CO2 atmosphere.

2.3. Transfection of microRNA oligonucleotides

HuH-7 cell lines were transfected with mimics and inhibitors of miR-17-5p and scrambled microRNAs (Qiagen, Germany) to examin the effect of miR-17-5p on E2F-1 and c-MYC transcripts expression and for functional analysis experiments. All transfection experiments were carried out in triplicates using HiPerfect Transfection Reagent (Qiagen, Germany), according to the manufacture's protocol; the experiments were repeated three times. Cells that were only exposed to transfection reagent were designated as Mock cells, while cells transfected with scrambled microRNAs were designated as Scr-miR cells. Cells transfected with miR-17-5p were designated as miR-17-5p cells; cells transfected with miR-17-5p inhibitor were designated as Anti-miR-17-5p cells.

2.4. mRNA and microRNA extraction from liver biopsies and HCC cell lines

mRNAs and microRNAs were extracted from liver biopsies and HCC cell lines. Fresh liver samples (HCC, Cirrhotic and healthy tissues) were collected during surgery and were immediately snap-frozen in liquid nitrogen. The specimens were manually pulverized in liquid nitrogen, and about 100 mg of tissues powder were used for large and small RNA extraction using mirVana miRNA Isolation Kit (Ambion, USA), according to the manufacture's protocol. HCC cell lines were harvested 48 h after transfection according to HiPerfect Transfection Reagent protocol: 150 ng oligonucleotides were used for HuH-7 cells transfection in 6-well plate. RNA yield was quantified with a spectrophotometer, and RNA integrity was tested by 18S rRNA bands detection on 1% agarose gel electrophoresis. RNA samples with Optical Density 260/280 more than two were excluded from the study.

2.5. Bioinformatics

Using bioinformatics algorithms microrna.org (<http://www.microrna.org>), miRDB (<http://www.mirdb.org/miRDB/>), DIANA Lab (<http://www.diana.cslab.ece.ntua.gr/>), and Target Scan (<http://www.targetscan.org/>), miR-17-5p downstream targets have been predicted.

2.6. miRNA and mRNA quantification

The extracted microRNAs were reverse transcribed into single stranded complementary DNA (cDNA) using TaqMan® MicroRNA Reverse Transcription Kit (ABI, USA) and specific primers for hsa-miR-17-5p and RNU6B. mRNA was reverse transcribed into cDNA using the high-capacity cDNA reverse transcription kit (ABI, USA), according to the manufacturer's instruction. Relative expression of miR-17-5p and RNU6B (for normalization) as well as E2F-1, c-MYC and beta-2 microglobulin (as housekeeping gene for normalization) was quantified using TaqMan Real-Time Q-PCR (ABI Assay IDs: 002308, 001093, Hs00153451_m1, Hs00153408_m1 and Hs00984230_m1, respectively) using StepOne™ Systems (ABI, USA). Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method. All PCR reactions including controls were run in duplicate reactions.

2.7. Cell proliferation and viability assays (BrdU and MTT)

For BrdU incorporation assay, HuH-7 cells were seeded 24 h prior to transfection in black 96-well plates and transfected with 12.5 ng oligonucleotides (according to HiPerfect protocol) with initial constant cell count 5×10^4 cells/well. Forty-eight hours after oligonucleotides transfection, cells were labeled with BrdU labeling reagent for 4 h (with final concentration 100 μM) using the Cell Proliferation ELISA kit (Roche Applied Biosystems, Germany). Cells were then fixed using FixDenate for 30 min then incubated with Anti-BrdU POD (with final concentration 10 μM) for 90 min. For cell viability experiments, using MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, 10,000 HuH-7 cells were seeded in 200 μl media per well in a 96 well plate and incubated 24 h prior to transfection with 12.5 ng of miR-17-5p mimics or inhibitors (according to HiPerfect protocol). Forty-eight hours post-transfection, 20 μl MTT solution (5 mg/ml MTT in PBS) was added to each well. After incubation for 5 h, formazan (MTT metabolic product) was resuspended in 200 μl DMSO. Colorimetric measurements and absorbance were performed using Wallac 1420 Victor2 Multilabel Counter (Perkin Elmer, USA). All cell proliferation experiments were done in triplicates and repeated three times.

2.8. Growth assays

2.8.1. Colony-forming assay

HuH-7 cells were seeded with initial count of 1000 cells/well and left to adhere overnight. Cells were then transfected with miR-17-5p mimics and inhibitors. Twenty-four hours post transfection; cells were detached by trypsinization to be imbedded in on-soft agarose bottom layer with 0.76% and top layer with 0.36%. Cells were incubated at 37 °C to colonize for two weeks. Colonies were observed under light microscope and counted per well. All colony forming assays were done in duplicates (two wells/test) and repeated three times as previously described [32].

2.8.2. Cell scratch wound healing assay

HuH-7 cells were left for 80–90% confluence in 6-well plates. Forty-eight hours post-transfection; five scratches/well were made in each plate with 200-μl pipette tip. Detached cells were washed out using serum-free medium. Medium was then added, and culture plates were incubated at 37 °C. Forty-eight hours post-scratching, migration was documented, and wound closure was quantified with *Image J* software (<http://rsbweb.nih.gov/ij/download.html>) by measuring the surface area covered by the cells. All Scratch assays were done in duplicates (two wells/test which represents 10 scratches/test) and repeated three times as previously described [32].

3. Results

3.1. Screening of miR-17-5p, E2F-1 and c-MYC in non-metastatic HCC tissues and Huh-7cell lines

miR-17-5p showed a significant down-regulation in non-metastatic HCC compared to healthy tissues ($p = 0.008$), as shown in Fig. 1a. On the other hand, E2F-1 and c-MYC transcripts expression showed a significant over-expression in non-metastatic HCC tissues compared to healthy liver biopsies ($p < 0.0001$ and $p = 0.0038$, respectively). All experiments were done in duplicates.

3.2. Correlation analysis between miR-17-5p and E2F-1and c-MYC mRNA expressions in non-metastatic HCC tissues

Using Spearman statistical method of correlation, relative quantification (RQ) values showed a significant inverse correlation between miR-17-5p and both E2F-1 and c-MYC mRNA in 23 non-metastatic HCC patients with correlation coefficient of Spearman $r = -0.9077$ ($p < 0.0001$) and Spearman $r = -0.6663$ ($p = 0.0005$), respectively (Fig. 1b).

3.3. Bioinformatic

miR-17-5p accession number and mature sequences were retrieved using miRBase database (<http://www.mirbase.org/>). In-silico predictions were carried out using four different softwares, E2F-1 was predicted to be a downstream target to miR-17-5p and surprisingly c-MYC was also found to be targeted by miR-17-5p.

3.4. Manipulating miR-17-5p expression and its downstream targets

Mimicked HuH-7 cells with miR-17-5p mimics showed up-regulation up to 208 ($p = 0.0005$) fold increase compared to mock cells (Supplementary Data 1). Mimicking of miR-17-5p in HuH-7 cells resulted in a significant repression of E2F-1, as well as c-MYC transcripts expression ($p = 0.001$ and $p = 0.0005$, respectively) compared to mock cells. While inhibitors of miR-17-5p significantly increased E2F-1 and c-MYC transcripts expression in HuH-7 cells ($p = 0.007$ and $p = 0.0004$, respectively) compared to mimicked cells (Fig. 2a and b).

3.5. Impact of manipulating miR-17-5p and its targets E2F-1 and c-MYC on cellular proliferation and viability

BrdU incorporation assay was used for investigating cell proliferation and growth. Mimicking of miR-17-5p in HuH-7 cells showed a significant increase in cellular proliferation ($p = 0.0017$) compared to negative control and mock cells. Recovery experiments using miR-17-5p antagomirs showed significant recovery of the proliferative effect of miR-17-5p on HuH-7 cells ($p < 0.0001$) (Fig. 3a).

MTT assay was used for investigating cell viability and growth. miR-17-5p over-expression promoted HuH-7 cells survival significantly ($p = 0.0034$) compared to negative control and mock cells.

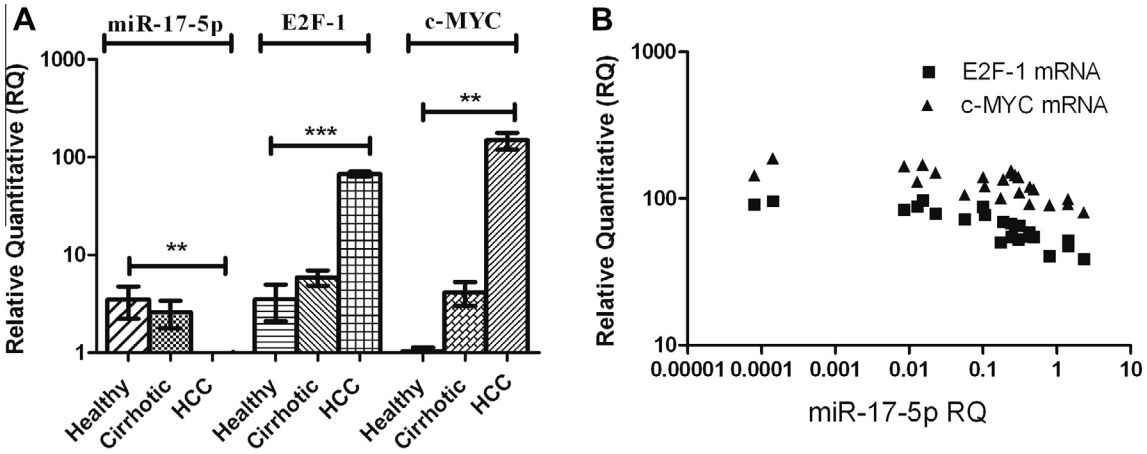

Fig. 1. miR-17-5p, E2F-1 and c-MYC screening in liver tissues and their correlation analysis; (A) miR-17-5p expression was down-regulated in non-metastatic HCC patients with higher expression in cirrhotic tissues compared to healthy liver tissues. E2F-1 and c-MYC mRNA expression showed a significant higher expression in HCC tissues compared to both cirrhotic and healthy tissues. One-way analysis of variance (ANOVA) was performed. (B) RQ values of miR-17-5p and E2F-1 and c-MYC mRNA in HCC tissues were analyzed using Spearman method of correlation. A significant inverse correlation was found between miR-17-5p and E2F-1 and c-MYC with correlation coefficient of Spearman $r = -0.9077$ and -0.66($p < 0.0001$ and $p = 0.0005$), respectively.

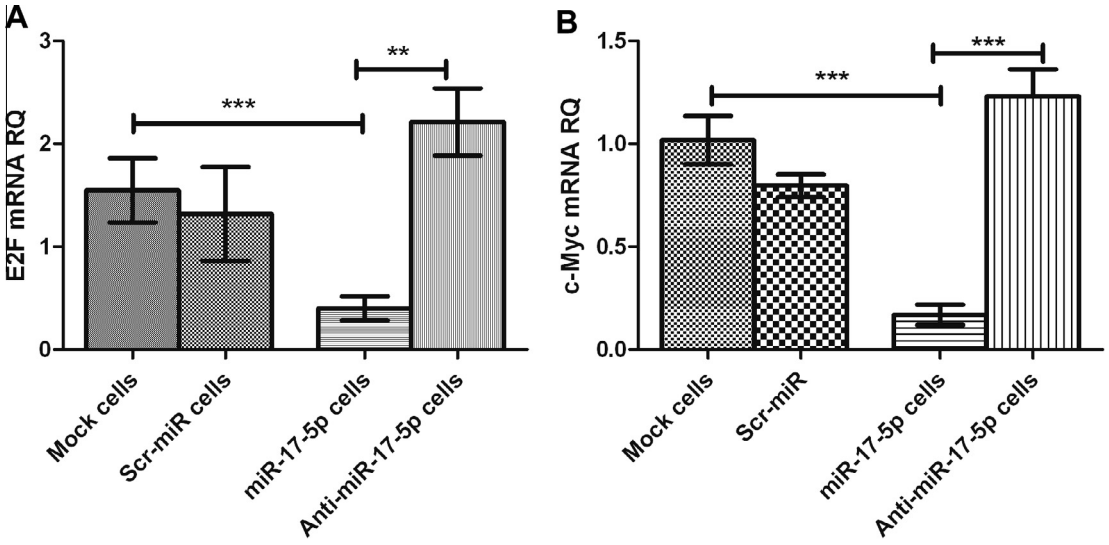

Fig. 2. Impact of miR-17–5p on E2F-1 and c-MYC transcript expression; (A and B) Mimicking of miR-17-5p in HuH-7 cells resulted in a significant down-regulation of E2F-1 and c-MYC mRNAs levels ($p = 0.0001$ and $p = 0.0005$, respectively) compared to controls. Inhibitors of miR-17-5p in HuH-7 cells resulted in a marked increase in E2F-1 and c-MYC mRNA levels compared to miR-17-5p mimicked cells ($p = 0.007$ and $p = 0.0004$, respectively).

While recovery experiments using antgomirs for miR-17-5p showed significant decrease in the proliferative effect of miR-17-5p on HuH-7 cells ($p = 0.0256$), as shown in Fig. 3b.

3.6. Impact of manipulating miR-17-5p and its targets E2F-1 and c-MYC on cellular anchorage-independent growth

miR-17-5p intended over-expression induced high colongenicity (colony number = 119.3 ± 8.9) in HuH-7 cells compared to mock cells (colony number = 44 ± 3.2) ($p = 0.0002$). Inhibition of miR-17-5p suppressed its tumorgenic effect by significantly decreasing the colony forming property of HuH-7 cells (colony number = 36.3 ± 3.2) compared to mock cells ($p = 0.0001$) as shown in Fig. 4a.

3.7. Impact of manipulating miR-17-5p, E2F-1 and c-MYC on cellular migration

Images for two-dimensional scratch-migration assay were documented at 5-fold magnification [33]. Transfection of miR-17-5p mimics led to significant enhancement in tumor cell migration in HuH-7 cells covering 89% of the original scratch compared to mock and Scr-miR with original scratch coverage of (80.7% and 81%, respectively) ($p = 0.0003$). Migration promotion by miR-17-5p was reversed using miR-17-5p anatgomirs in HuH-7 cells compared to mimicked cells ($p = 0.0014$) (Fig. 4b).

4. Discussion

miR-17-5p, E2F-1 and c-MYC, each separately, was shown to promote HCC progression and metastasis; the three of them showed up-regulation in HCC cell lines as well as metastatic HCC [13,34], but the status of expression of each of them in non-metastatic HCC has never been highlighted. Therefore, this study was set in an approach to answer two questions: the first is to investigate the status of the c-MYC/E2F-1/miR-17-5p network in non-metastatic HCC tissues, and the second is to clarify which of them contributes mostly in HCC progression. The interaction between the three network members was clear in other *in vitro* studies

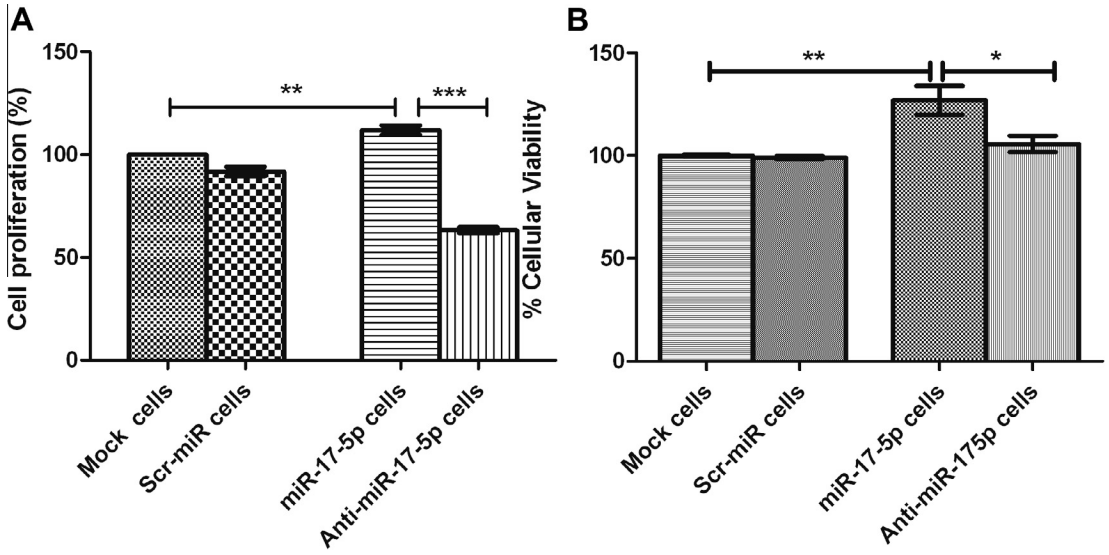

Fig. 3. Impact of miR-17-5p on cellular proliferation and viability; (A) BrdU assay; mimics of miR-17-5p significantly increased cellular proliferation in HuH-7 cells compared to controls. Inhibitors of miR-17-5p significantly inhibited cellular proliferation compared to miR-17-5p mimicked cells. (B) MTT assay; mimics of miR-17-5p significantly increased cellular viability in HuH-7 cells compared to controls. Inhibitors of miR-17-5p significantly suppressed cellular viability compared to miR-17-5p mimicked cells. One-way analysis of variance (ANOVA) was performed for statistical analysis.

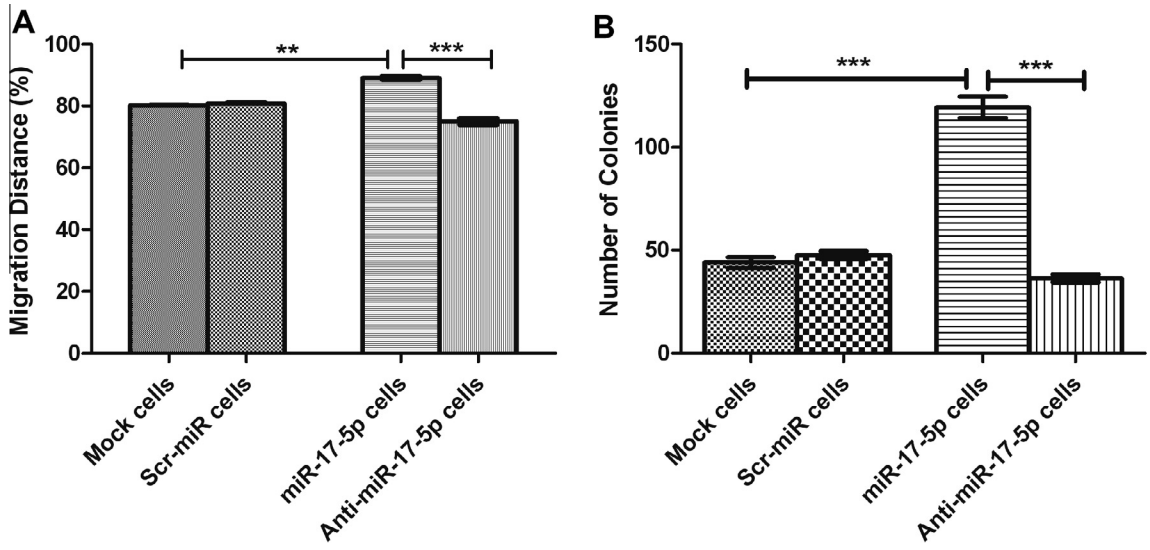

Fig. 4. Impact of miR-17-5p on cellular migration and anchorage-independent growth; (A) miR-17-5p mimics significantly promoted cellular migration in HuH-7 cells compared to the original scratch. Inhibitors of miR-17-5p significantly repressed cellular migration compared to miR-17-5p mimicked cells. (B) Colony forming assay; miR-17-5p mimics markedly increased anchorage-independent growth of HuH-7 cells reflected increased colonies count compared to control cells. Inhibitors of miR-17-5p repressed cellular anchorage-independent growth compared to miR-17-5p mimicked cells reflected in a fewer number of colonies compared to mimicked cells. One-way analysis of variance (ANOVA) was performed for statistical analysis.

where E2F-1 and c-MYC proteins have been proven to induce the expression of one another. Therefore, over-expression of one of them will lead to the elevation of the other, positive self-feedback loop [4,5]. Moreover, both proteins were found to induce miR-17-5p expression [2,7]. On the other hand, miR-17-5p itself was found to repress the expression of E2F-1 protein forming the negative self-feedback loop [2,3]. This established an unusual structured network in which c-MYC activates the transcription of E2F-1 while simultaneously inhibiting its translation, so there is an expression-balance between the three players in cancers.

In our study, we have shown for the first time a unique expression signature of the triad in non-metastatic HCC patients. We showed that miR-17-5p expression is significantly down-regulated in non-metastatic HCC patients compared to healthy liver tissues (Fig. 1a). Our data contradicts with previous studies, where miR-17-5p was over-expressed in metastatic HCC patients and was concomitantly up-regulated with the metastatic heat shock protein 27 (HSP27) [13]. This might indicate that miR-17-5p is only elevated in metastatic HCC.

We further screened the rest of the triad components, where E2F-1 and c-MYC have shown an over-expression of their transcripts in the same pool of non-metastatic HCC patients compared to healthy controls (Fig. 1a). This might be explained by previous data where E2F-1 and c-MYC mRNA has been frequently reported

to be over-expressed in HCC without specifying the tumor stage [16–18,20,35]. For further confirmation, a correlation analysis of miR-17-5p with E2F-1 and c-MYC mRNA expressions showed a significant inverse correlation in all of the HCC patients investigated (Fig. 1b).

So our results have raised a tempting question: which of the triad members is mainly responsible for triggering the HCC progression and metastasis especially that miR-17-5p is down-regulated in non-metastatic HCC, while E2F-1 and c-MYC are up-regulated in both metastatic and non-metastatic HCC. To answer this question, forced and antagonized expression of miR-17-5p in HuH-7 cells was performed followed by E2F-1 and c-MYC expression analysis. E2F-1 and c-MYC mRNA expression showed a significant inhibition upon miR-17-5p intended over-expression which was recovered upon antagonizing miR-17-5p expression in HuH-7 cells (Fig. 2). The E2F-1 down-regulation goes along with previous data that showed down-regulation of E2F-1 proteins upon miR-17-5p over-expression [2,3,13], but the down-regulation of c-MYC upon miR-17-5p mimicking might be either due to an induced effect of E2F-1-c-MYC regulatory loop [4,5] or due to direct effect of miR-17-5p on c-MYC that was surprisingly revealed by our bioinformatics results which has to be further confirmed.

To analyze the impact of miR-17-5p mimicking with the concomitant inhibition of E2F-1 and c-MYC expression on cell behavior, and to investigate which of the three players is responsible for tumor progression, cell proliferation, viability, migration and anchorage-independent growth experiments were carried out as an indicator for HCC progression and metastasis. Using BrdU incorporation and MTT assays, the over-expression of miR-17-5p with the repression of E2F-1 and c-MYC have shown increased cellular viability as well as proliferation. On the other hand, antagonizing miR-17-5p expression have shown increased E2F-1 and c-MYC, but at the same time cell viability and proliferation were suppressed (Fig. 3). This would show that HCC cellular viability and proliferation is more dependent on miR-17-5p over-expression rather than E2F-1 and c-MYC. That goes along with the previous data stating that the oncogenic behavior of miR-17-5p in HCC cells is reflected in increased cellular proliferation [13]. On the other hand, our findings might support the hypothesis that both transcription factors, E2F-1 and c-MYC, can act as regulators of apoptosis and tumor suppressors as well [29,30,36]. Furthermore, miR-17-5p forced over-expression and consequently E2F-1 and c-MYC expression inhibition resulted in a marked increase of HuH-7 clonogenicity and migration compared to mock cells (Fig. 4). This confirms that the metastatic behavior of HCC cells is mainly dependent on miR-17-5p rather than E2F-1 and c-MYC. This agrees with previous findings that showed the close relation between miR-17-5p and HSP 27 protein which enhances cellular migration [13].

Our results show an unusual pattern of expression of miR-17-5p/E2F-1/c-MYC triad in non-metastatic HCC. They also show that the HCC progression is mainly dependent on the increased expression of miR-17-5p rather than E2F-1 and c-MYC.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.04.003.

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