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miR-455-5p suppresses hepatocellular carcinoma cell growth and invasion via IGF-1R/AKT/GLUT1 pathway by targeting IGF-1R

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ABSTRACT

Hepatocellular carcinoma (HCC) is among the most frequently observed forms of cancer. MicroRNAs (miRNAs) are increasingly thought to play a key role in regulating the onset and progression of a wide range of cancer types. In the present report, we found that miR-455-5p expression was significantly decreased in both HCC patient tumor tissues and cell lines, and that this reduction in expression was linked to poorer patient outcomes. When we overexpressed miR-455-5p in HCC cell lines (Huh7 and HepG2), this was linked with impaired proliferation, colony formation, migration, and invasion. We further found that this miRNA was able to directly bind the insulin growth factor receptor (IGF-1R) 3′-untranslated region, thereby suppressing IGF-1R expression in HCC cells. Consistent with this, miR-455-5p overexpression was associated with reduced glucose transporter (GLUT) 1 expression, which in turn inhibited HCC cell uptake of glucose, production of lactate, and generation of ATP. Together these results thus indicate that mIR-455-5p is able to suppress tumor functionality via impairing glycolysis in HCC cells, highlighting this miRNA as a potential target for anti-cancer therapeutic interventions.

1. Introduction

Hepatocellular carcinoma (HCC) is among the most frequently observed forms of cancer, and it has high mortality rates globally [1–3]. As there are few effective biomarkers of HCC, early detection of this disease is challenging and as such most patients are only diagnosed when the disease is advanced and complicated by distant metastases [4]. While HCC patient outcomes have been improved by liver transplantation and chemotherapy, long-term survival is still relatively poor [4]. As such, it is vital that research better clarify the molecular mechanisms governing HCC progression.

MicroRNAs (miRNAs) are small, evolutionarily conserved RNA molecules that lack coding potential [5,6]. These miRNAs play broad regulatory roles in the context of cellular proliferation, stress responses, and differentiation [6,7]. Dysregulated miRNA activity is associated with many types of disease, including cancer [8–11]. Multiple miRNAs have been found to be expressed at altered levels in HCC, and these alterations impact HCC development and progression [12,13]. The specific role of miR-455-5p in HCC to date, however, has not been assessed.

Aerobic glycolysis is a common feature evident in many types of cancer cells, with tumor cells glycolytically processing glucose even when sufficient oxygen is present to support mitochondrial respiration [14,15]. In order to sustain rapid tumor cell growth, key glycolysis-associated proteins including glycolytic enzymes and glucose transporters (GLUTS) are frequently expressed at elevated levels. Insulin growth factor receptor (IGF-1R) is able to phosphorylate AKT in response to insulin, leading to GLUT upregulation [16–18]. As such, miRNAS targeting this IGF-1R-AKT-GLUT1 axis may represent promising therapeutic agents well-suited to disrupting cancer progression. Indeed, in this report we determined that HCC tumors exhibit decreased miR-455-5p expression that was associated with poor patient outcomes. Importantly, we found that when overexpressed, miR-455-5p was able to impair HCC cell malignancy via suppressing IGF-1R expression and thereby disrupting glycolysis.

2. Materials and methods

2.1. Clinical samples

83 pairs of HCC patient tumor tissues and normal control tissues were collected surgically at Wenzhou People's Hospital l from January

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Table 1
Correlation between clinicopathologic characteristics and miR-455-5p expression in hepatocellular carcinoma.

Characteristics		$n = 83$	miR-455-5p		P
			High expression (n = 42)	Low expression (n = 41)	
Age (y)	< 50	35	14	21	0.099
	≥50	48	28	20	
Sex	Male	69	33	36	0.261
	Female	14	9	5	
HBV	Absent	24	15	9	0.167
	Present	59	27	32	
Serum AFP level (ng/mL)	< 20	23	14	9	0.247
	≥20	60	28	32	
Tumor size (cm)	< 5	31	21	10	0.016*
	≥5	52	21	31	
No. of tumor nodules	1	69	36	33	0.525
	≥2	14	6	8	
Cirrhosis	Absent	34	19	15	0.423
	Present	49	23	26	
Venous infiltration	Absent	50	29	21	0.097
	Present	33	13	20	
Edmondson-Steiner grading	I + II	63	34	29	0.276
	III	20	8	12	
TNM tumor stage	I + II	65	38	27	0.007*
	III	18	4	14	

HBV hepatitis B virus, AFP alpha-fetoprotein, TNM tumor-node-metastasis.
* Statistically significant.

2010 and November 2014, with no patients having undergone treatment prior to surgery. Samples were snap frozen prior to analysis. All patients had provided written informed consent, and the Ethical Committee of the Wenzhou People's Hospital approved this study, which was consistent with the Declaration of Helsinki. Patient characteristics are summarized in Table 1.

2.2. Cell culture and transfection

The HepG2, Hep3B, Bel-7404, and Huh7 HCC cell lines were from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All cells were grown at 37℃ in a 5% CO_2 incubator in DMEM (Gibco, NY, USA) containing 10% FBS (Gibco). miR-455-5p mimics and miR-455-5p inhibitor were purchased from Sigma-Aldrich. IGF1R siRNA was obtained from Life Technologies. Lipofectamine 3000 (Thermo Fisher Scientific, USA) was used for transfecting cells with miRNA constructs at a 20 nM concentration as in previous reports [19,20]. IGF1R siRNA was transfected into cells for 24 h using Lipofectamine RNAiMAX Reagent (Invitrogen).

2.3. Cell proliferation measurements

HepG2 and Huh7 cell proliferation was assessed via Cell Counting Kit-8 (CCK-8) (Beyotime, Shanghai, China) based on provided instructions. Briefly, cells that had been transfected with appropriate miRNA constructs were plated in 96-well plates (1000 cells/well) for 24 h, after which 10 μl CCK-8 reagent was per well for 3 h at 37∘C, after which a microplate reader (Bio-Rad Laboratories, CA, USA) was used to measure absorbance at 450 nm.

Table 2
Primers for RT-qPCR.

Primer sequence	
MiR-455-5p forward	GCGATGTCGGTCAACACT
MiR-455-5p Reverse	TGGTGCAGTCAAGCAGGC
U6 Forward	CTCGCTTCGGCAGCACA
U6 Reverse	AACGCTTCACGAATTTGCGT

2.4. Colony formation assay

Following transfection with appropriate miRNA mimic or control constructs, HepG2 and Huh7 cells were plated into 35-mm plates (3000 cells/plate) in normal growth media. Cells were incubated for 2 weeks, after which 4% paraformaldehyde was used to fix colonies, which were then stained for 15 min using 1% Crystal Violet at room temperature. Numbers of colonies were then counted with a microscope.

2.5. RT-qPCR

A miRcute miRNA isolation kit (Tiangen, Beijing, China) was used to extract total mIRNAs based on provided directions, and then RT-qPCR was conducted as in previous reports [19,20]. PCR conditions were as below: 95 ˚C for 5 min; 40 cycles at 95 ˚C for 10 s and 60 ˚C for 1 min. Expression of U6 RNA was detected for normalization.U6 RNA was used for normalization purposes, with the $2^{-\triangle\triangle CT}$ method used to quantify relative miR-455-5p expression Primers specific formiR-455-5p and U6 are shown in Table 2.

2.6. Western blotting

At 48 h post-transfection with appropriate control or mIR-455-5p mimic constructs, HepG2 and Huh7 cells were lysed in cold RIPA buffer (Beyotime) for 10 min, after which western blotting was performed as in previous reports [19,20]. Blots were probed with the following primary antibodies: anti-IGF-1R (1:1000, 9750S), anti-pAKT (1:1000, Ser473, 9271S), and anti-AKT (1:1000, 9272S) from Cell Signaling Technology (MA, USA); anti-GLUT1 (1:1000, sc-377228) from Santa Cruz Biotechnology (TX, USA). An Enhanced ECL detection kit (Millipore, MA, USA) was used for protein detection.

2.7. Luciferase reporter assay

The psiCHECK-2 luciferase reporter was transformed so as to contain WT or mutant versions of the 3′-UTR miR-455-5p binding site from IGF-1R. The WT or mutant reporter was then co-transfected into HepG2 or Huh7 cells in 24-well plates along with miR-455-5p mimics or control miRNA. Cells were then allowed to incubate for 48 h, after which the Dual-GLO Luciferase Assay System (Promega, WI, USA) was used to assess luciferase activity, with Renilla luciferase used for normalization purposes.

2.8. Glucose uptake assay

The Glucose Uptake Colorimetric Assay kit (BioVision, CA, USA) was used to assess glucose uptake based on provided instructions, as in previous reports [21].

2.9. Statistical analysis

Data are means ± standard deviation of three independent experiments. One way ANOVAs and Student's t-tests were used to compare groups, with SPSS 17.0 (CA, USA) used for all analyses. $P < 0.05$ was the significance threshold.

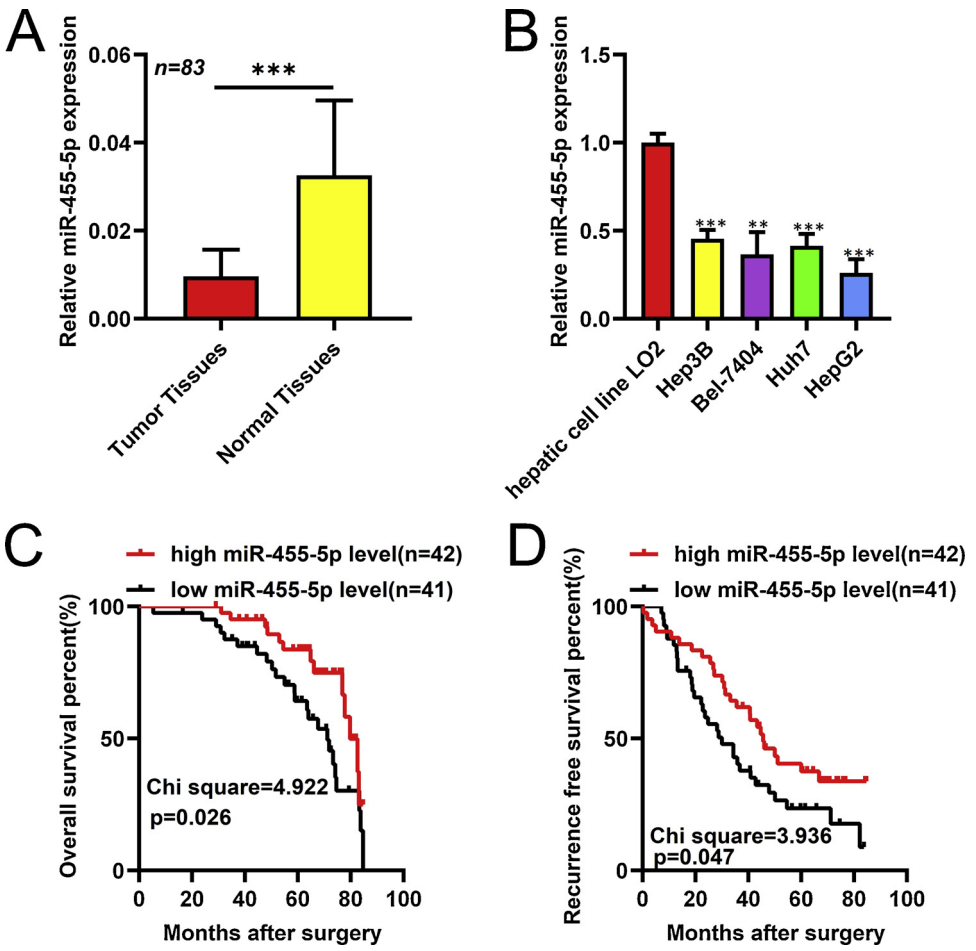

Fig. 1. HCC tissues and cells exhibit reduced miR-455-5p expression.
(A) Levels of miR-455-5p expression were assessed in 83 pairs of HCC tumor samples and adjacent non-tumor (ANT) liver tissues. (B) miR-455-5p levels in the Hep3B, Bel-7404, Huh7, and HepG2 HCC cell lines and the control LO2 line were assessed via RT-PCR. (C and D) Correlations between miR-455-5p and HCC patient recurrence-free and overall survival, as compared via log rank test. **P < 0.01; ***P < 0.001.

3. Results

3.1. HCC tissues exhibit reduced miR-455-5p expression

We began by assessing the expression of miR-455-5p in human HCC patient samples via RT-qPCR, using 83 pairs of tumor and adjacent normal tissues. We found a significant reduction in miR-455-5p levels in HCC tissues relative to those in matched normal control samples (Fig. 1A). To further extend these findings, we additionally assessed miR-455-5p expression in both HCC cell lines and LO2 control cells, revealing a significant reduction in miR-455-5p expression in HepG2, Hep3B, Bel-7404, and Huh7 cells relative to LO2 controls (Fig. 1B). To assess the clinical relevance of miR-455-5p expression in HCC, we divided these 83 HCC patients into miR-455-5p-high and -low groups based upon median miR-455-5p expression, revealing that reduced miR-455-5p expression correlates significantly with both larger tumor size ($p = 0.016$) and more advanced stage ($p = 0.007$) (Table 1). To further explore the prognostic relevance of miR-455-5p expression, we conducted a log-rank test aimed at assessing the relationship between HCC patient survival and miR-455-5p levels. This analysis revealed that lower levels of miR-455-5p expression were correlated with a significantly poorer patient prognosis (Fig. 1C and D). This thus strongly suggested that miR-455-5p is linked with HCC progression.

3.2. When overexpressed, miR-455-5p disrupts HCC cell proliferation and invasion

We next explored the functional relevance of miR-455-5p in HCC via overexpressing this miRNA in Huh7 and HepG2 by transfecting them with a miR-455-5p mimic, with increased expression confirmed via RT-qPCR relative to transfection with a miRNA control construct (Fig. 2A). CCK-8 assessment revealed that overexpression of miR-455-5p markedly suppressed HCC proliferation (Fig. 2B and C) and colony formation for both cell lines tested (Fig. 2D). We further found miR-455-5p overexpression to be associated with a marked drop in the migratory and invasive activity of both HepG2 and Huh7 cells (Fig. 2E and F). We next decreased miR-455-5p in HCC in Huh7 and HepG2 by transfecting them with a miR-455-5p mimic, with increased expression confirmed via RT-qPCR relative to transfection with a miRNA control construct (Fig. 2A). CCK-8 assessment revealed that overexpression of miR-455-5p markedly suppressed HCC proliferation (Fig. 2B and C) and colony formation for both cell lines tested (Fig. 2D). We further found miR-455-5p overexpression to be associated with a marked drop in the migratory and invasive activity of both HepG2 and Huh7 cells (Fig. 2E and F). This thus revealed that increased miR-455-5p expression impairs HCC cell proliferative and invasive capabilities.

3.3. miR-455-5p directly targets IGF-1R in HCC

In order to establish how miR-455-5p may influence HCC

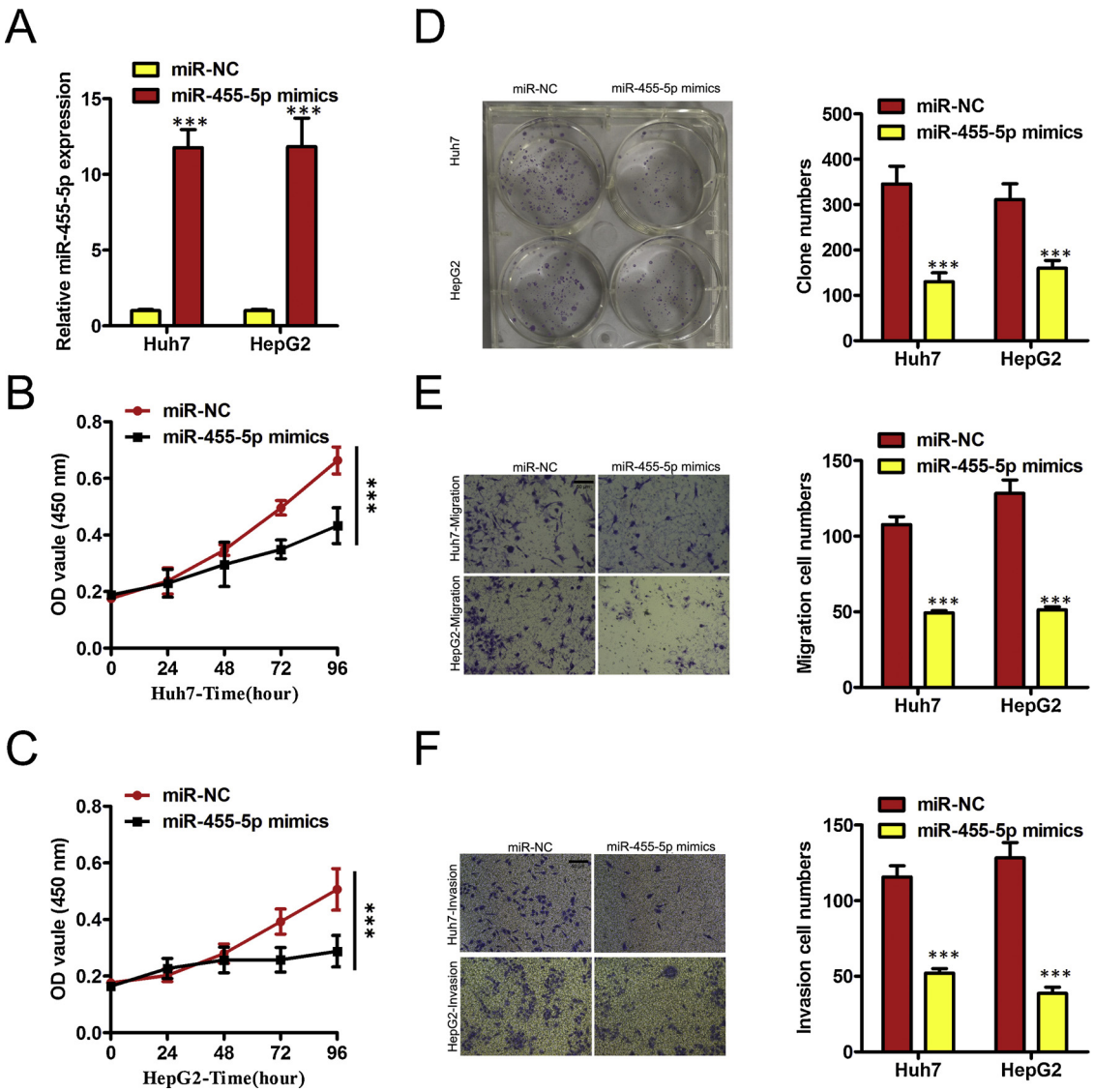

Fig. 2. miR-455-5p overexpression inhibits HCC cell growth.
(**A**) Expression of miR-455-5p was assessed in HepG2 and Huh7 transfected using either control miRNAs or miR-455-5p mimics via RT-qPCR, with U6 used for normalization purposes. (**B**) The proliferation of HepG2 and (**C**) Huh7 cells was assessed following miR-455-5p mimic transfection, revealing a marked decline in proliferation relative to control cells. (**D**)HCC cell colony formation was markedly reduced by miR-455-5p overexpression, with representative images shown on the left and quantification of colony numbers shown on the right. (**E** and **F**). Transwell assays were used to gauge the migration and invasion of cells expressing varying levels of miR-455-5p. *P < 0.05; **P < 0.01; ***P < 0.001.

proliferation and invasivity, we used the Targetscan database to identify potential target mRNAs for this miRNA, revealing IGF-1R as one such predicted target (Fig. 3A). In order to validate this prediction, we co-transfected a luciferase reporter bearing a WT or mutated version of the candidate miR-455-5p binding site in the IGF-1R 3′-UTR region into Huh7 and HepG2 cells along with miR-455-5p or control. When we assessed luciferase activity after 48 h, we found miR-455-5p overexpression to markedly decrease luciferase activity of the WT reporter, whereas the mutated reporter activity was not affected (Fig. 3B and C). This is consistent with mIR-455-5p directly binding the IGF-1R 3′-UTR. To confirm the functional outcomes of such binding, we assessed IGF-1R expression in HCC cells that had been transfected with either control or miR-455-5p mimic, revealing that overexpression of this miRNA was associated with a significant drop in IGF-1R expression in Huh7 and HepG2 cells (Fig. 3D). Western blotting further confirmed that miR-455-5p mimic transfection was linked to a significant drop in IGF-1R protein levels (Fig. 3E). This thus suggested that miR-455-5p can directly bind IGF-1R mRNA, thereby suppressing its expression within HCC cells. We further assessed IGF-1R expression in paired HCC patient tumor and normal tissue samples, revealing significantly higher IGF-1R expression in HCC tumor samples relative to paired normal tissue controls (Fig. 3F). Furthermore, there was a strong negative correlation between the expression of IGF-1R and miR-455-5p in these HCC tissue samples (Fig. 3G). Together, these findings strongly suggested that miR-455-5p can directly target IGF-1R in HCC.

3.4. MiR-455-5p controls IGF-1R/AKT/GLUT1 pathway activity

IGF-1R has previously been shown to activate AKT and thereby upregulate GLUT1, thereby inducing cancer cell glycolysis. Given the observed suppression of IGF-1R by miR-455-5p, we next assessed levels of AKT and GLUT1 via western blotting. miR-455-5p overexpression was associated with a marked reduction in both AKT phosphorylation (S473) and GLUT1 expression in Huh7 and HepG2 cells (Fig. 4A). We further sought to confirm this finding via transfecting HCC cells with a miR-455-5p antagomir, which was able to significantly decrease

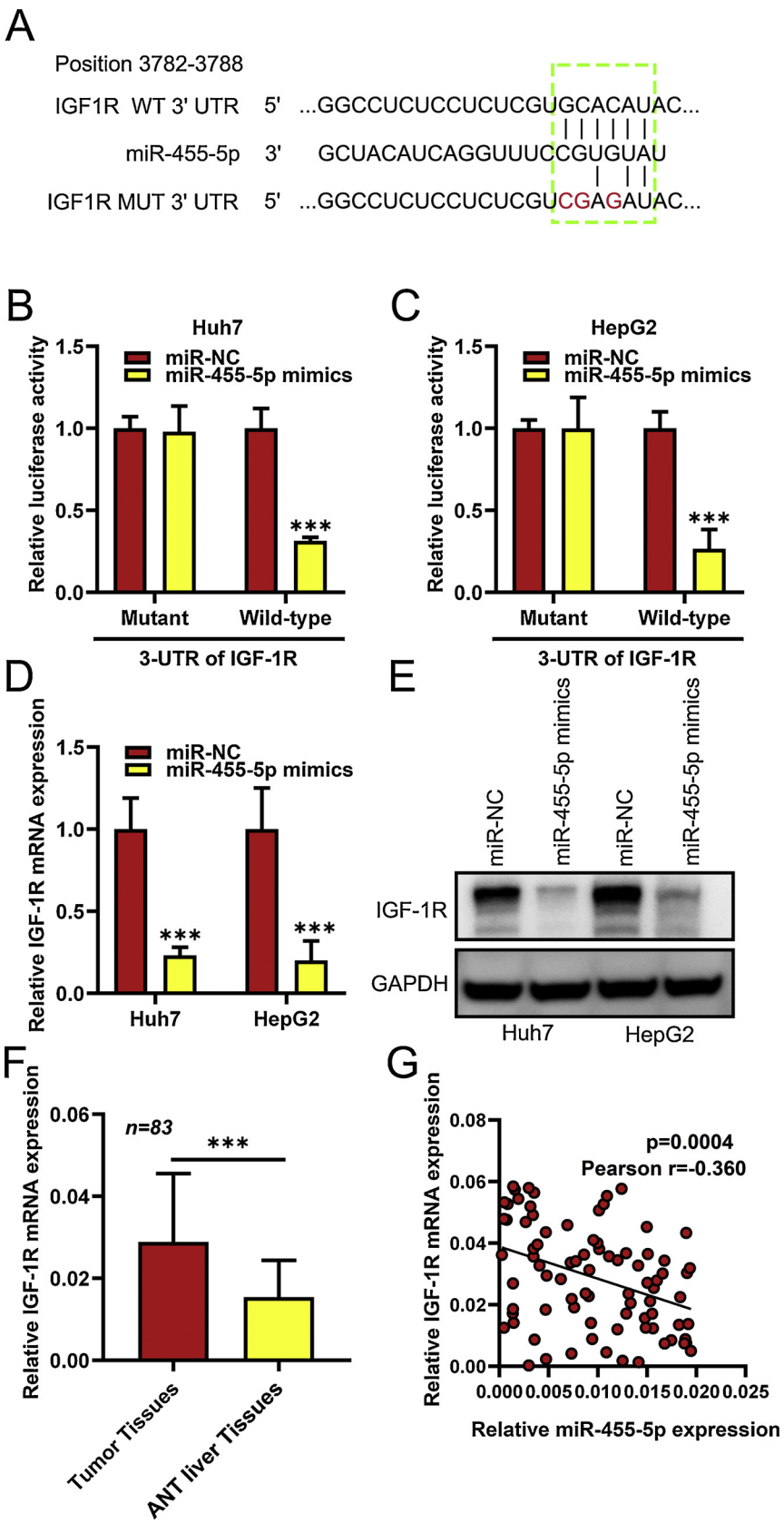

Fig. 3. miR-455-5p directly targets IGF-1R in HCC cells. (**A**) The putative miR-455-5p binding site in the IGF-1R 3′-UTR is shown. (**B**, **C**) A luciferase reporter vector encoding either a WT or mutant version of this binding site was transfected into HepG2 and Huh7 cells, with or without miR-455-5p co-transfection, and relative luciferase activity was quantified. (**D** and **E**) Levels of IGF-1R protein and mRNA levels in HCC cells transfected with miR-455-5p mimic or control constructs. (**F**) IGF-1R expression in pairs of HCC patient tumor and adjacent non-tumor (ANT) liver tissues as measured via RT-qPCR. (**G**) Spearman's correlation test was used to assess the correlation between miR-455-5p expression and IGF-1R. *P < 0.05; **P < 0.01; ***P < 0.001.

endogenous expression of miR-455-5p in both tested cell lines (Fig. 4B). Western blotting further demonstrated that downregulating miR-455-5p significantly enhanced IGF-1R and GLUT1 expression, and enhanced AKT phosphorylation (Fig. 4C). This thus showed that miR-455-5p is able to negatively regulate the IGF-1R-AKT-GLUT1 axis in HCC cells.

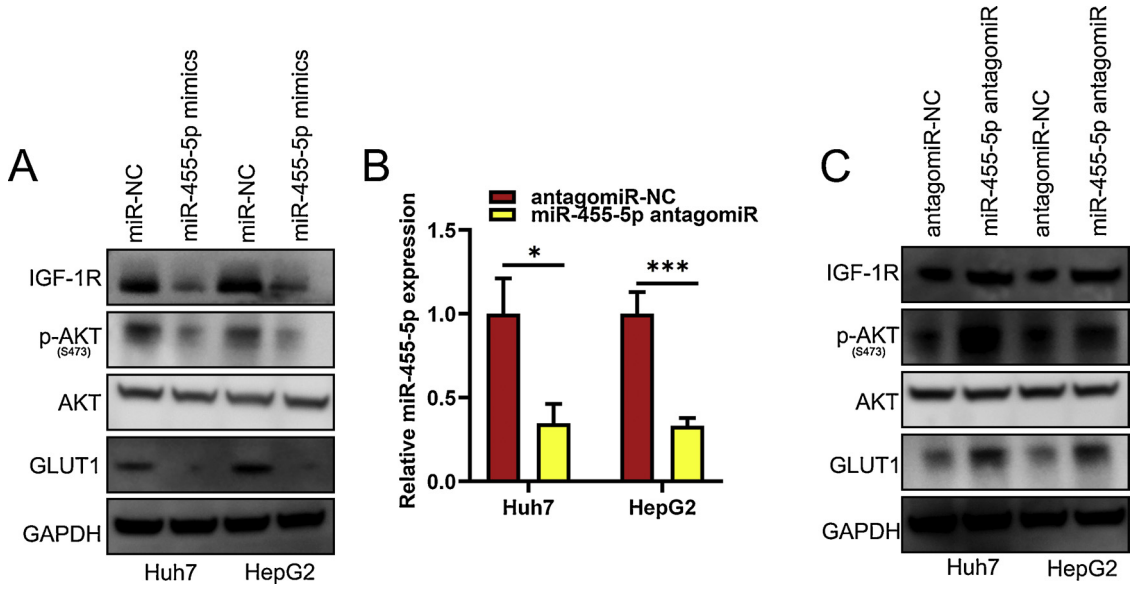

Fig. 4. miR-455-5p suppresses the IGF-1R/AKT/GLUT1 pathway.
Levels of AKT and GLUT1 protein were assessed in HepG2 and Huh7 cells that had been transfected using miR-455-5p mimics or control constructs. (**B**) A miR-455-5p antagomir was transduced into HCC cells to knock down endogenous miR-455-5p, with RT-qPCR used to confirm knockdown. (**C**) IGF-1R, AKT and GLUT1 protein levels were measured in HCC cells following miR-455-5p depletion. *P < 0.05; **P < 0.01; ***P < 0.001.

3.5. MiR-455-5p controls HCC cell glycolysis

GLUT1 expression levels are closely linked to cellular glucose uptake, and as miR-455-5p was able to suppress cellular GLUT1 expression, we next assessed the effect of this miRNA on HCC cell glycolytic activity. miR-455-5p overexpression was associated with a marked drop in HepG2 and Huh7 cellular glucose uptake (Fig. 5A). Consistent with this, we observed significantly lower levels of lactate in HCC cells that overexpressed miR-455-5p relative to control cells (Fig. 5B). Metabolic processing of glucose is the main source of ATP in cells, and so we next assessed the impact of miR-455-5p on ATP levels, revealing that overexpression of this miRNA was associated with reduced ATP levels in both tested cell lines (Fig. 5C), consistent with the observed reduction in glucose uptake and lactate production. Similar results are also shown in HepG2 and Huh7 with IGF-1R knockdown (Fig. 5D–F). We further assessed these same parameters in cells following transfection of a miR-455-5p antagomir, revealing that depleting miR-455-5p was linked with significantly increased glucose uptake and lactate generation in both cell lines (Fig. 5G and H). Similarly, knockdown of miR-455-5p was associated with increased ATP levels (Fig. 5I). Together, these findings thus indicate that miR-455-5p represents a novel negative regulator of glycolytic metabolism in HCC cells.

4. Discussion

HCC is an exceedingly common and yet deadly cancers globally, with the continued identification of novel diagnostic and prognostic indicators being vial to the detection and treatment of this disease. There is increasing evidence suggesting that miRNA dysregulation plays a role in HCC progression [22,23]. Accumulating reports indicated miR-455-5p played as the oncogenic role in many cancers. For example, it has been reported that miR-455-5p promoted melanoma metastasis by inhibiting CPEB1 [24]. Meanwhile, in non-small cell lung cancer, miR-455-5p could promote cell growth and invasion by targeting SOCO3 [25]. In the present report, we observed marked miR-455-5p downregulation in HCC samples and cells, and lower expression of this miRNA was associated with indicators of more advanced HCC such as a larger tumor size and a more advanced stage. This revealed that miR-455-5p might play a key role in HCC progression.

In the current study, miR-455-5p was decreased in HCC tissues and correlated with the advanced progression of HCC patients. Molecular studies indicated that miR-455-5p targeted and negatively regulated the expression of IGF-1R in HCC cells. IGF-1R was overexpressed in HCC tissues and negatively correlated with that of miR-455-5p. It is widely accepted that miRNAs usually target many different mRNAs in cells. Previous studies have identified some targets of miR-455-5p including RAB18 and RAF1 [26,27]. It might be necessary to clarify whether other targets, such as RAB18 and RAF1, are involved in the suppressive function of miR-455-5p in HCC.

Aerobic glycolysis is used far more extensively in cancer cells than in normal cells, with metabolic reprogramming being an essential process that provides these cells with the large amounts of energy needed for their rapid proliferation [28]. As such, suppression of glycolysis represents a potentially highlight effective means of inhibiting tumor progression. Several miRNAs have been found to target genes in the aerobic glycolysis pathway including GLUT1, G6PD, and LDHA [29,22]. IGF-1R is a transmembrane tyrosine kinase protein in the insulin receptor family [23–35]. IGF-1R is upregulated in multiple cancer types including HCC and renal cell carcinoma [36,37]. IGF-1R is able to bind with its ligand IGF-1 to stimulate PI3K/AKT pathway activation [38–40]. AKT phosphorylation at S473 controls GLUT1 activity and localization within cells, thereby controlling the ability of cancer cells to take up glucose. IKKβ and NF-κB are able to regulate lymphoma cell survival through control of GLUT1 membrane trafficking by way of AKT regulation [41]. Several reports have been reports that many miRNAs may repress IGF axis in HCC, such as miRNA-486-5p enhances HCC suppression by targeting IGF-1R and its downstream genes [42], and miR-615-5p depresses natural killer cells cytotoxicity by repressing IGF-1R in HCC [43]. In this report, we found that overexpressing miR-455-5p led to decreased IGF-1R expression in HCC cells, thereby suppressing AKT phosphorylation and GLUT1 expression. As a result, glucose uptake, lactate production, and ATP generation were all markedly impaired when this miRNA was overexpressed, thus highlighting miR-455-5p as a novel negative regulator of glycolytic metabolism.

In conclusion, our findings highlight the downregulation of miR-455-5p in HCC, and conclusively demonstrate that overexpressing this miRNA can suppress HCC cell proliferation and invasive potential. We found that miR-455-5p is able to directly suppress IGF-1R expression,

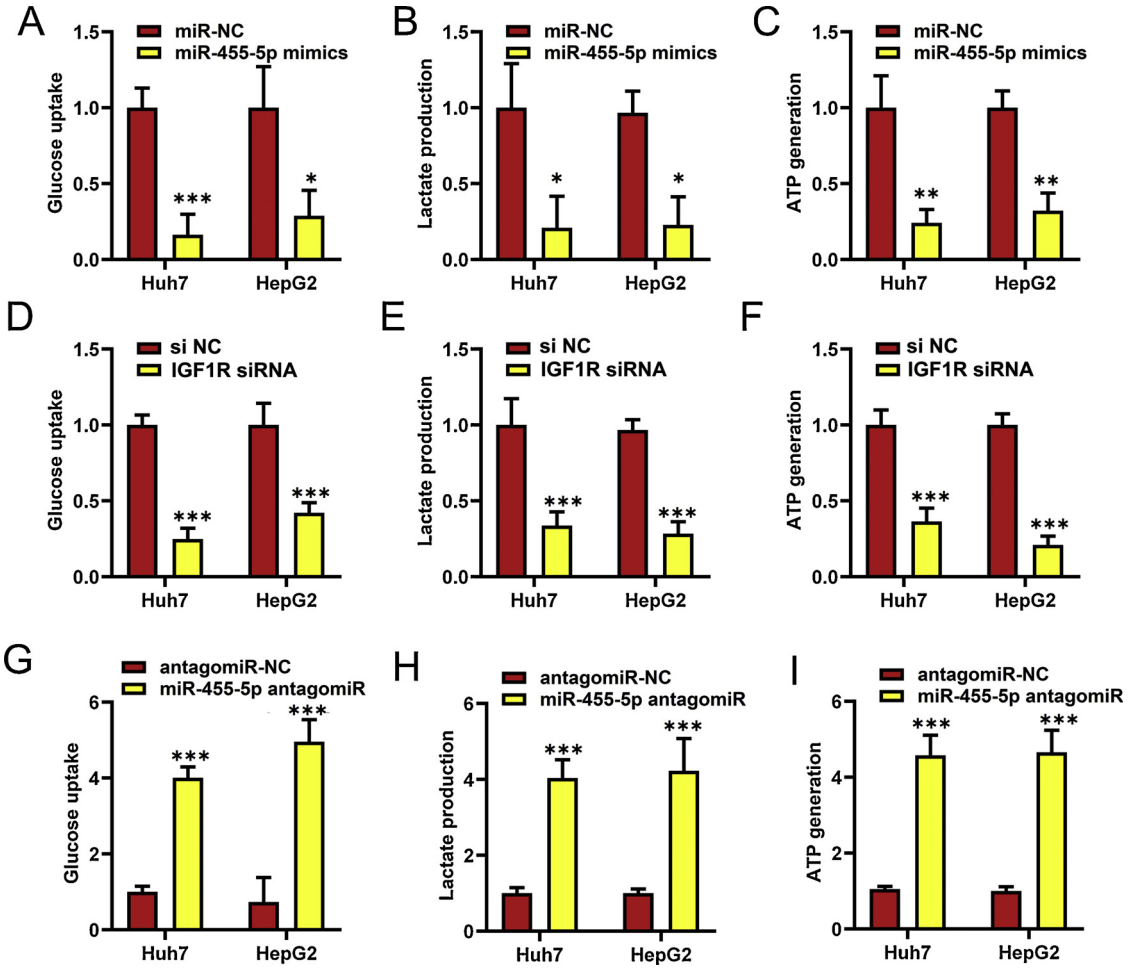

Fig. 5. MiR-455-5p controls HCC cell glycolysis.
(**A**, **B**) Rates of glucose uptake and lactate production were assessed in HepG2 and Huh7 cells that had undergone transfection using miR-455-5p mimics or control constructs. (**C**) Levels of ATP were measured in cells treated with the indicated miRNA constructs. (**D**, **E**) Rates of glucose uptake and lactate production were assessed in HepG2 and Huh7 cells that had undergone transfection using IGF1R siRNA or control constructs. (**F**) Levels of ATP were measured in cells treated with the indicated IGF1R constructs. (**G**, **H**) Glucose uptake and lactate production were measured in HCC cells in which endogenous miR-455-5p had been knocked down. (**I**) Following miR-455-5p knockdown, levels of ATP were assessed in HCC cells. *P < 0.05; **P < 0.01; ***P < 0.001.

thereby inhibiting activation of the AKT/GLUT1 pathway to suppress HCC cell glycolytic activity. Together these results thus highlight a novel role for miR-455-5p in HCC, suggesting that targeting this miRNA may represent a novel therapeutic strategy in affected individuals.

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Declaration of Competing Interest

None declared.

References

[1] D. da Motta Girardi, T.S. Correa, M. Crosara Teixeira, G. Dos Santos Fernandes, Hepatocellular carcinoma: review of targeted and immune therapies, J. Gastrointest. Cancer 49 (3) (2018) 227–236.
[2] S. Daher, M. Massarwa, A.A. Benson, T. Khoury, Current and future treatment of hepatocellular carcinoma: an updated comprehensive review, J. Clin. Transl. Hepatol. 6 (1) (2018) 69–78.
[3] L. Kulik, J.K. Heimbach, F. Zaiem, J. Almasri, L.J. Prokop, Z. Wang, M.H. Murad, K. Mohammed, Therapies for patients with hepatocellular carcinoma awaiting liver transplantation: a systematic review and meta-analysis, Hepatology 67 (1) (2018) 381–400.
[4] M. Schwartz, S. Roayaie, M. Konstadoulakis, Strategies for the management of hepatocellular carcinoma, Nat. Clin. Pract. Oncol. 4 (7) (2007) 424–432.
[5] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2) (2004) 281–297.
[6] A.M. Mohr, J.L. Mott, Overview of microRNA biology, Semin. Liver Dis. 35 (1) (2015) 3–11.
[7] V. Ambros, The functions of animal microRNAs, Nature 431 (7006) (2004) 350–355.
[8] E. Gentilin, E. Degli Uberti, M.C. Zatelli, Strategies to use microRNAs as therapeutic targets, Best Pract. Res. Clin. Endocrinol. Metab. 30 (5) (2016) 629–639.
[9] H. Qu, W. Xu, Y. Huang, S. Yang, Circulating miRNAs: promising biomarkers of human cancer, Asian Pac. J. Cancer Prev. 12 (5) (2011) 1117–1125.
[10] P.B. Kwak, S. Iwasaki, Y. Tomari, The microRNA pathway and cancer, Cancer Sci. 101 (11) (2010) 2309–2315.
[11] T.A. Farazi, J.I. Hoell, P. Morozov, T. Tuschl, MicroRNAs in human cancer, Adv. Exp. Med. Biol. 774 (2013) 1–20.
[12] W. Zhang, S. Qian, G. Yang, L. Zhu, B. Zhou, J. Wang, R. Liu, Z. Yan, X. Qu, MicroRNA-199 suppresses cell proliferation, migration and invasion by down-regulating RGS17 in hepatocellular carcinoma, Gene 659 (2018) 22–28.
[13] Z. Liu, Y. Wang, C. Dou, L. Sun, Q. Li, L. Wang, Q. Xu, W. Yang, Q. Liu, K. Tu, MicroRNA-1468 promotes tumor progression by activating PPAR-γ-mediated AKT signaling in human hepatocellular carcinoma, J. Exp. Clin. Cancer Res. 37 (1) (2018) 49.
[14] M. Akram, Mini-review on glycolysis and cancer, J. Cancer Educ. 28 (3) (2013) 454–457.
[15] J. Zheng, Energy metabolism of cancer: glycolysis versus oxidative phosphorylation (Review), Oncol. Lett. 4 (6) (2012) 1151–1157.
[16] G. Lenz, A. Hamilton, S. Geng, T. Hong, M. Kalkum, J. Momand, S.E. Kane, J.M. Huss, t-Darpp activates IGF-1R signaling to regulate glucose metabolism in trastuzumab-resistant breast cancer cells, Clin. Cancer Res. 24 (5) (2018) 1216–1226.

[17] Y. Wang, X. Zhao, D. Shi, P. Chen, Y. Yu, L. Yang, L. Xie, Overexpression of SIRT1 promotes high glucose-attenuated corneal epithelial wound healing via p53 regulation of the IGFBP3/IGF-1R/AKT pathway, Invest. Ophthalmol. Vis. Sci. 54 (5) (2013) 3806–3814.
[18] N. Garg, S. Thakur, C.A. McMahan, M.L. Adamo, High fat diet induced insulin resistance and glucose intolerance are gender-specific in IGF-1R heterozygous mice, Biochem. Biophys. Res. Commun. 413 (3) (2011) 476–480.
[19] T. Zhou, S. Chen, X. Mao, miR-145-5p affects the differentiation of gastric cancer by targeting KLF5 directly, J. Cell. Physiol. 234 (5) (2019) 7634–7644.
[20] Y. Feng, T. Sun, Y. Yu, Y. Gao, X. Wang, Z. Chen, MicroRNA-370 inhibits the proliferation, invasion and EMT of gastric cancer cells by directly targeting PAQR4, J. Pharmacol. Sci. 138 (2) (2018) 96–106.
[21] L. Ren, Y. Yao, Y. Wang, S. Wang, MiR-505 suppressed the growth of hepatocellular carcinoma cells via targeting IGF-1R, Biosci. Rep. 39 (7) (2019).
[22] A. Salvi, E. Abeni, N. Portolani, S. Barlati, G. De Petro, Human hepatocellular carcinoma cell-specific miRNAs reveal the differential expression of miR-24 and miR-27a in cirrhotic/non-cirrhotic HCC, Int. J. Oncol. 42 (2) (2013) 391–402.
[23] R.H. Xu, L.Y. Zheng, D.L. He, J. Meng, L.P. Xia, X.B. Hao, Z.Z. Zhang, Profiling of differentially expressed microRNAs (miRNAs) during differentiation of rat hepatic oval cells (HOCs) into hepatocellular carcinoma (HCC) cells, Clin. Transl. Oncol. 17 (3) (2015) 230–237.
[24] E. Shoshan, A.K. Mobley, R.R. Braeuer, T. Kamiya, L. Huang, M.E. Vasquez, A. Salameh, H.J. Lee, S.J. Kim, C. Ivan, G. Velazquez-Torres, K.M. Nip, K. Zhu, et al., Reduced adenosine-to-inosine miR-455-5p editing promotes melanoma growth and metastasis, Nat. Cell Biol. 17 (2015) 311–321.
[25] J. Wang, Y. Wang, D. Sun, J. Bu, F. Ren, B. Liu, S. Zhang, Z. Xu, S. Pang, Xu S, miR-455-5p promotes cell growth and invasion by targeting SOCO3 in non-small cell lung cancer, Oncotarget 8 (2017) 114956–114965.
[26] J. Liu, J. Zhang, Y. Li, L. Wang, B. Sui, D. Dai, MiR-455-5p acts as a novel tumor suppressor in gastric cancer by down-regulating RAB18, Gene 591 (2) (2016) 308–315, https://doi.org/10.1016/j.gene.2016.07.034.
[27] J. Chai, S. Wang, D. Han, W. Dong, C. Xie, H. Guo, MicroRNA-455 inhibits proliferation and invasion of colorectal cancer by targeting RAF proto-oncogene serine/threonine-protein kinase, Tumor Biol. 36 (2) (2015) 1313–1321, https://doi.org/10.1007/s13277-014-2766-3.
[28] X.B. Li, J.D. Gu, Q.H. Zhou, Review of aerobic glycolysis and its key enzymes – new targets for lung cancer therapy, Thorac. Cancer 6 (1) (2015) 17–24.
[29] J.Y. Fan, Y. Yang, J.Y. Xie, Y.L. Lu, K. Shi, Y.Q. Huang, MicroRNA-144 mediates metabolic shift in ovarian cancer cells by directly targeting Glut1, Tumour Biol. 37 (5) (2016) 6855–6860.
[30] M. Liu, J. Gao, Q. Huang, Y. Jin, Z. Wei, Downregulating microRNA-144 mediates a metabolic shift in lung cancer cells by regulating GLUT1 expression, Oncol. Lett. 11 (6) (2016) 3772–3776.
[31] P. Li, X. Yang, Y. Cheng, X. Zhang, C. Yang, X. Deng, P. Li, J. Tao, H. Yang, J. Wei, J. Tang, W. Yuan, Q. Lu, X. Xu, M. Gu, MicroRNA-218 increases the sensitivity of bladder cancer to cisplatin by targeting Glut1, Cell. Physiol. Biochem. 41 (3) (2017) 921–932.
[32] X. Zhao, C. Lu, W. Chu, B. Zhang, Q. Zhen, R. Wang, Y. Zhang, Z. Li, B. Lv, H. Li, J. Liu, MicroRNA-124 suppresses proliferation and glycolysis in non-small cell lung cancer cells by targeting AKT-GLUT1/HKII, Tumour Biol. 39 (5) (2017) 1010428317706215.
[33] Y.L. Kong, Y. Shen, J. Ni, D.C. Shao, N.J. Miao, J.L. Xu, L. Zhou, H. Xue, W. Zhang, X.X. Wang, L.M. Lu, Insulin deficiency induces rat renal mesangial cell dysfunction via activation of IGF-1/IGF-1R pathway, Acta Pharmacol. Sin. 37 (2) (2016) 217–227.
[34] B. Davaadelger, L. Duan, R.E. Perez, S. Gitelis, C.G. Maki, Crosstalk between the IGF-1R/AKT/mTORC1 pathway and the tumor suppressors p53 and p27 determines cisplatin sensitivity and limits the effectiveness of an IGF-1R pathway inhibitor, Oncotarget 7 (19) (2016) 27511–27526.
[35] G. Chen, T. Fang, Z. Huang, Y. Qi, S. Du, T. Di, Z. Lei, X. Zhang, W. Yan, MicroRNA-133a inhibits osteosarcoma cells proliferation and invasion via targeting IGF-1R, Cell. Physiol. Biochem. 38 (2) (2016) 598–608.
[36] T.M. Cardillo, P. Trisal, R. Arrojo, D.M. Goldenberg, C.H. Chang, Targeting both IGF-1R and mTOR synergistically inhibits growth of renal cell carcinoma in vitro, BMC Cancer 13 (2013) 170.
[37] M.G. Refolo, R. D'Alessandro, C. Lippolis, N. Carella, A. Cavallini, C. Messa, B.I. Carr, IGF-1R tyrosine kinase inhibitors and Vitamin K1 enhance the antitumor effects of Regorafenib in HCC cell lines, Oncotarget 8 (61) (2017) 103465–103476.
[38] Q. Wang, H. Wang, Y. Jia, H. Ding, L. Zhang, H. Pan, Luteolin reduces migration of human glioblastoma cell lines via inhibition of the p-IGF-1R/PI3K/AKT/mTOR signaling pathway, Oncol. Lett. 14 (3) (2017) 3545–3551.
[39] N. Chapuis, J. Tamburini, P. Cornillet-Lefebvre, L. Gillot, V. Bardet, L. Willems, S. Park, A.S. Green, N. Ifrah, F. Dreyfus, P. Mayeux, C. Lacombe, D. Bouscary, Autocrine IGF-1/IGF-1R signaling is responsible for constitutive PI3K/Akt activation in acute myeloid leukemia: therapeutic value of neutralizing anti-IGF-1R antibody, Haematologica 95 (3) (2010) 415–423.
[40] C. Chen, Y. Xu, Y. Song, IGF-1 gene-modified muscle-derived stem cells are resistant to oxidative stress via enhanced activation of IGF-1R/PI3K/AKT signaling and secretion of VEGF, Mol. Cell. Biochem. 386 (1–2) (2014) 167–175.
[41] T.G. Sommermann, K. O'Neill, D.R. Plas, E. Cahir-McFarland, IKKβ and NF-κB transcription govern lymphoma cell survival through AKT-induced plasma membrane trafficking of GLUT1, Cancer Res. 71 (23) (2011) 7291–7300.
[42] R.A. Youness, H.M. El-Tayebi, R.A. Assal, K. Hosny, G. Esmat, A.I. Abdelaziz, MicroRNA-486-5p enhances hepatocellular carcinoma tumor suppression through repression of IGF-1R and its downstream mTOR, STAT3 and c-Myc, Oncol. Lett. 12 (2016) 2567–2573.
[43] M.A. Rahmoon, R.A. Youness, A.I. Gomaa, M.T. Hamza, I. Waked, H.M. El Tayebi, A.I. Abdelaziz, MiR-615-5p depresses natural killer cells cytotoxicity through repressing IGF-1R in hepatocellular carcinoma patients, Growth Factors 35 (2017) 76–87.