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Effects of Irisin Combined with Gefitinib on Expression Levels of miRNAs in the HepG2 Cell Line

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This study aims to investigate the effects of the combination of irisin and gefitinib on the expression of AKT1, FNCD5, VEGF, Bcl-2, EGFR, PI3K, CDK1, and ERBB2 genes, which play critical roles in the pathogenesis of hepatocellular carcinoma, beside the expression of miRNAs targeting these genes in the HepG2 cell line. MTT assay was applied to evaluate the cytotoxicity induced by irisin or gefitinib. Expressions of miR- 664b-5p, miR-3922-5p, miR-6843-3p, miR-8085, miR-381-5p, miR-1275, miR-1304, and miR-6801-5p, and the expression of target genes of these miRNAs were determined by RT-PCR. The most effective time for the combination of irisin and gefitinib in HepG2 cells was 48 hours, and the dose was 3.125 µM for irisin and 10.34 µM for gefitinib. PI3K, Akt, EGF, and ErbB2 expressions were significantly decreased in irisin groups compared to the control group. We found that irisin alone and in combination with gefitinib increased miR-664b-5p, miR-381-5p, miR-3922-5p, miR-1275, and miR-1304 gene expressions, whereas the combined treatment of irisin with gefitinib decreased miR-8085 expression. Our study shows that irisin can enhance the anticancer effects of gefitinib in the HepG2 cell line and dysregulated miRNA expressions contribute to HCC pathogenesis by decreasing cell proliferation and inducing apoptosis.

Keywords: Hepatocellular Carcinoma, irisin, gefitinib, miRNA

Introduction

One of the most common primary cancer types of the liver is Hepatocellular carcinoma (HCC)[1]. While the worldwide incidence of liver cancer is 4.7%, it ranks 2nd among cancer-related deaths[1,2]. HCC accounts for approximately 90% of liver cancer cases and it is estimated that more than one million cases will be reported by 2025[3]. Unfortunately, more than 50% of HCC patients are diagnosed at an advanced stage of the disease[4]. The most preferred treatment modality in HCC is surgical intervention. In cases where surgery is inappropriate, other treatment modalities such as chemotherapy, radiotherapy, and ablation are preferred. Since resistance to anticancer drugs is observed in most patients with HCC, new methods are being investigated in the treatment of HCC[5]. This situation reveals the necessity of alternative chemotherapeutic options. EGF receptor (EGFR) has been reported to be expressed in many solid tumors. EGFR has become a therapeutic target because its overexpression or amplification confers the ability of tumor cells to survive. Therefore, the small molecule tyrosine kinase inhibitor gefitinib was developed[6]. Overexpression of EGFR and ErbB family receptor tyrosine kinases was seen in 68% and 84% of HCCs, respectively[7]. Schiffer *et. al.*[8] reported that gefitinib-blocking EGFR activity has been reported to have antitumoral effects on the development of HCC in rats exposed to diethylnitrosamine. However, in large early clinical trials, hepatocytotoxic effects of gefitinib were reported in patients treated with standard doses of gefitinib[9]. Therefore, combination therapies with other molecules or biological agents are often applied to reduce the cytotoxic effects of anti-cancer agents and increase their anti-tumor efficacy.

Irisin is a myokine formed by cleavage of the transmembrane-bound protein FNDC5 and secreted from skeletal muscle during exercise[10]. It has been reported that irisin inhibits cancer cell proliferation, migration, and invasion via the PI3K/AKT pathway in cells[11, 12]. Zhang and Ke *et al.*[13] showed that irisin application decreased the number of HCC cells. miRNAs are small non-coding RNA molecules that play an important role in regulating tumor development[14]. Cheng *et al.*[15] reported that irisin dose-dependently inhibited the survival, migration, and invasion of osteosarcoma cells. They reported that miR-214-3p

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promoted U2OS cell migration, EMT, and invasion by inhibiting irisin/FNDC5 expression.

This study aimed to determine the cytotoxic effects of irisin combined with gefitinib on the HepG2 cell line. In addition, to investigate the effects on the expressions of AKT1, FNCD5, VEGF, Bcl-2, EGFR, PI3K, CDK1 and ERBB2 genes, which play an important role in the pathogenesis of HCC, and miRNAs targeting these genes (miR-1304, miR-1275, miR-381-5p, miR-3922-5p, miR-664b-5p, miR-6801-5p, miR-6843-3p, miR-8085) by screening miRWalk2.0 and Targetscan databases.

Material and Methods

Cell culture and treatments

HepG2 and AML12 cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and cultured in accordance with the manufacturer's instructions. Cells were cultured in DMEM-F12 HAM (Sigma-Aldrich, USA) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 U/mL streptomycin. The cells were transferred into culture flasks and maintained at 37 °C in a humidified atmosphere with 5% CO_2. Control cells were observed daily using an inverted microscope (Olympus), and new passages were initiated when the cells reached approximately 90% confluence.

Cell viability

HepG2 cells ($5x10^3$ cells/well) were cultured in 96-well plates with DMEM (10% FBS) and subsequently treated with varying concentrations of irisin (Cayman Chemical, Ann Arbor, MI, USA) (0.81, 1.625, 3.125, 6.25, 12.5, 25, 50, 100 µM) for 24 and 48 hours. Additionally, cells were exposed to gefitinib (0.81, 1.625, 3.125, 6.25, 12.5, 25, 50, 100 µM) for 24 and 48 hours. Untreated HepG2 cells were considered as controls. Cells were treated with gefitinib (0.81-100µM) combined with 50% inhibitive concentration (IC_{50}) of (3.125 µM) irisin for 48 h. The cells were incubated at 37°C for 4 hours after adding 20 µl of MTT solution (5 mg/mL) to each well of the 96-well plate. The formazan salt crystals formed in the wells were checked under a microscope and then 100 µL of DMSO was applied to dissolve these crystals. Finally, the 96-well plate was measured at 570 nm absorbance with a microplate reader (Thermo Scientific Multiskan GO, USA). The average of the absorbance values determined in the control wells was taken and this value was determined as 100% live cells. The absorbance values read from the irisin, gefitinib and combined treated wells were proportional to the control absorbance value and accepted as percent viability. GraphPad Prism 5.0 software was used to analyze the IC_{50}.

qReal-Time PCR

Total RNA was extracted from the control and treated HepG2 cells using an RNA isolation kit (Column Pure RNA Miniprep Kit, ABM Good, Canada). The quality and quantity of the obtained RNAs were assessed using a nanodrop (OPTIZEN NanoQ, South Korea). Subsequently, complementary DNA synthesis was performed by cDNA synthesis kit (OneScript Plus cDNA Synthesis Kit, ABM Good), and the synthesized cDNA was used as a template for real-time PCR reactions conducted on the QuantStudio 6 Flex Real-Time PCR system (Applied Biosystems, USA). GAPDH was used as a housekeeping gene in the study. Each target genes expression level was normalized to the endogenous GAPDH (ΔCt) gene. Gene expression values were then calculated according to the formula $2^{-\Delta\Delta ct}$. The sequences of specific primers are shown in Table 1.

miRNA isolation and cDNA synthesis

The miRNAs in the control and treatment groups were isolated using the HighPure miRNA Isolation Kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions and stored at -80 °C for further processing. The purity and concentration of the extracted miRNA were confirmed by nanodrop UV-spectrophotometer (OPTIZEN NanoQ) based on A260/A280 absorbance ratios. c-DNA synthesis from isolated RNAs was performed using miRNA All-In-One cDNA Synthesis Kit (Cat. No. G899, ABM good company, US).

Table 1 — Primers used in real-time PCR

Gene	Primers
PI3K	F: TTGTCTGTCACACTTCTGTAGTT R: AACAGTTCCCATTGGATTCAACA
Akt	F: ATGAGCGACGTGGCTATTGTGAAT R: GAGGCCGTCAGCCACAGTCTGGATG
ErbB2	F: CCTCTGACGTCCATCATCTC R: ATCTTCTGCTGCCGTCGCTT
Bcl-2	F: ATGGACGGGTCCGGGGAG R: TCAGCCCATCTTCTTCCA
EGF	F: GAATTCAGTTGCCCTGACTCTACCGC R: GAATTCGCAGACAGCCACCATGAT
VEGF	F: AGGAGGGCAGAATCATCACG R: CAAGGCCCACAGGGATTTTCT
CycD1	F: GATCAAGTGTGACCCGGACT R: TCCTCCTCTTCCTCCTCCTC
GAPDH	F: CCTGCCAAGTATGATGACATCAA R: AGCCCAGGATGCCCTTTAGT

Determination of Gene Expressions of miRNAs by Real Time PCR

Synthesized cDNAs were quantified using the BrightGreen miRNA qPCR MasterMix (Cat. MasterMix-Ms, ABM Good) kit and the RT-PCR method (StepOne Real Time PCR, Applied Biosystem). RNU6B was used for normalization. Fold changes compared to the control were calculated with the formula $2^{-\Delta\Delta Ct}$.

Statistical analysis

Statistics were performed using Prism® 5 software (GraphPad Software, Inc., La Jolla, CA, USA). The data are expressed as the mean ± standard deviation (SD). Comparisons between the groups were conducted using a one-way ANOVA analysis of variance followed by the Bonferroni multiple comparison test. GraphPad Prism 5.0 software was used to analyze the IC_{50}. This study calculated the IC_{50} dose of irisin using the Regression Probit (IBM SPSS Statistics 22) application. Statistical significance was considered significant at $p<0.05$.

Results

Cell Viability

We found that the viability of HepG2 cells decreased in a gefitinib dose-dependent manner for 24 and 48h (Fig. 1A). Additionally, the cytotoxic IC50 dose of irisin for HepG2 cells was determined as 3.125 µM for 48h (Fig1 A). We did not observe any significant change in HepG2 cell viability following 24 h of irisin application. However, we determined that 48h application reduced cell proliferation by approximately 20% (Fig. 1B). The results demonstrated the dose-dependent cytotoxicity of gefitinib on HepG2 with obtained IC50 values of 16.58 µM for 24 h, or 10.34 µM for 48h, respectively (Fig 1. B). To assess the cytotoxicity of gefitinib combined with irisin, HepG2 cells were incubated with eight concentrations of gefitinib ranging from 0.81 to 100 µM, combined with IC50 concentrations (3.125 µM) of irisin for 24 and 48 h (Fig. 1C). We observed that the most effective time for the combined application of irisin and gefitinib for HepG2 cells was 48 h, with application doses of 3.125 µM for irisin and 10.34 µM for gefitinib. It was found that the application of Irisin had no effect on the viability of AML12 cells (Fig. 1D).

Effects of irisin, gefitinib, and irisin-gefitinib combination on gene expressions

In HepG2 cells, the expressions of PI3K, Akt, EGF, and ErbB2 were significantly downregulated in the irisin groups compared to the control group ($p<0.05$), but this decrease was not significant for VEGF, CycD1, and Bcl-2. The gene expression of CycD1 was

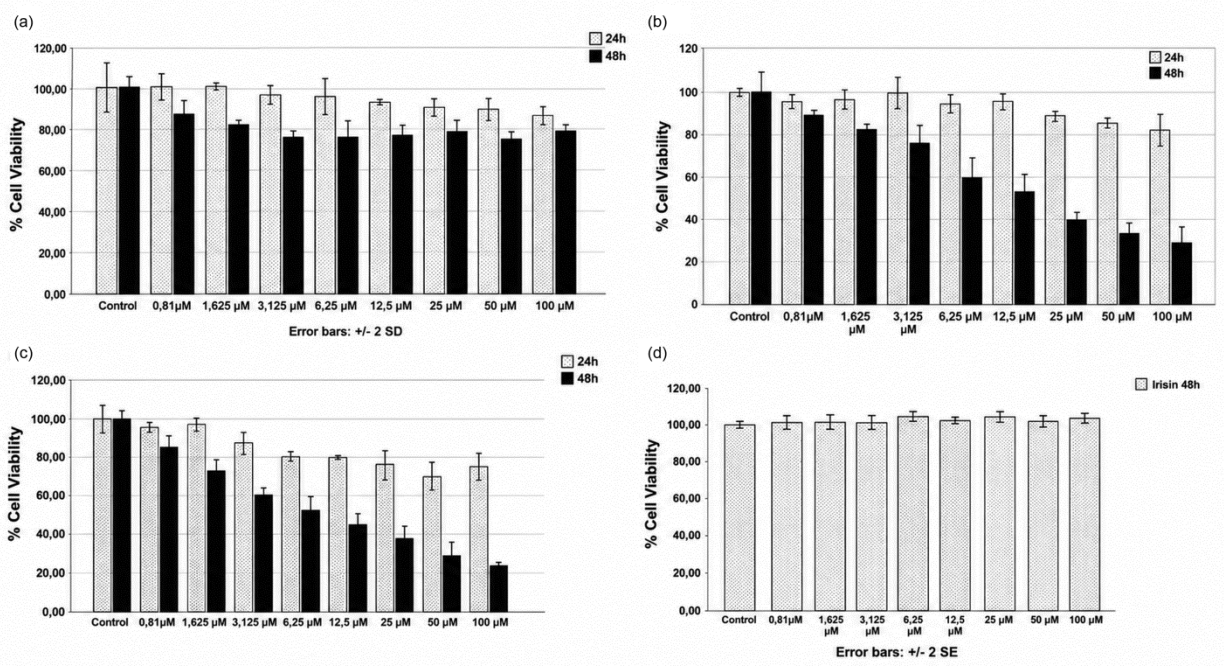

Fig. 1 — The effect of different concentrations of irisin and gefitinib on HepG2 cell viability for 24 and 48 h; A) irisin (0.81-100 µM), B) gefitinib (0.81-100 µM), C) Cells were treated with different concentrations of gefitinib (0.81-100 µM) combined with 3.125 µM of irisin for 24 and 48 h, D) The effect of irisin on AML12 cell viability. Cell viabilities were then measured by MTT assay

Table 2 — The changes in gene expression levels (fold change) in HepG2 cells

Genes	Irisin (3.125 μM)	Gefitinib (10.34 μM)	Irisin- Gefitinib
PI3K	0.37*± 0.16	0.74± 0.12	0.37*± 0.20
Akt	0.48*± 0.16	0.46*± 0.07	0.46*± 0.02
Bcl-2	0.97± 1.1	0.50± 0.20	0.26*± 0.15
ErbB2	0.06*± 0.02	1.05± 0.18	0.64± 0.11
EGF	0.18*± 0.09	0.61± 0.08	0.18*± 0.04
VEGF	0.52± 0.06	1.10± 0.27	0.67± 0.07
CycD1	0.33± 0.03	12.03*± 1.37	5.18*± 1.72

*p<0,05 compared with control

Table 3 — The changes in miRNA (fold change) expression levels of HepG2 cells treated with irisin alone, gefitinib alone, and combination irisin-gefitinib for 48 h

	İrisin		Gefitinib		İrisin+Gefitinib	
miRNA	FC	P	FC	P	FC	P
miR- 664b-5p	4,9	0,001	1,83	0,271	9,40	0,015
miR-3922-5p	2,4	0,043	-1,28	0,578	4,22	0,021
miR-6843-3p	1,5	0,815	-1,45	0,456	1,32	0,708
miR-8085	-1,1	0,775	1,27	0,399	-2,97	0,004
miR-381-5p	1,7	0,674	1,26	0,160	2,27	0,040
miR-1275	12,2	0,001	4,09	0,017	7,14	0,018
miR-1304	6,0	0,036	2,64	0,036	4,98	0,025
miR-6801-5p	-1,2	0,290	-1,42	0,271	1,2	0,821

significantly increased in the groups treated with the gefitinib and irisin-gefitinib combination compared to the control group. Furthermore, the irisin-gefitinib combination induced the gene expressions of PI3K, Akt, EGF and Bcl-2genes (Table 2).

Result of miRNA expression

miR-664b-5p, miR-3922-5p, miR-1275 and miR-1304 expressions were found to be significantly up-regulated only in irisin and irisin-gefitinib combination treated HepG2 cells compared to the control group ($p<0.05$, Table 2). In addition, miR-6843-3p and miR-381-5p levels were increased in irisin-treated groups, but this increase was not significant compared to the control group ($p>0.05$). We found that miR-8085 and miR-6801-5p were down-regulated in the irisin treated group compared to the control group ($p>0.05$). In HepG2 cells treated with irisin and gefitinib combined, miR-8085 expression was down-regulated and miR-381-5p expression was up-regulated ($p<0.05$). miR-6843-3p and miR-6801-5p expressions were not statistically significant. A significant increase in miR-1275 and miR-1304 expression levels was detected only in cells treated with gefitinib compared to the control group ($p<0.05$, Table 3).

Discussion

Dysregulation of miRNAs plays a pivotal role in HCC pathogenesis by post-transcriptionally modulating gene expressions involved in tumor progression and biomarker identification[16]. While irisin has been shown to suppress proliferation and induce apoptosis in various cancer models, its combinatorial effect with gefitinib on HepG2 cell proliferation and miRNA expression profiles remains unexplored[17]. To address this gap, the present study investigated the effects of irisin and gefitinib combination on the expression profiles of critical pathogenic genes and their targeting miRNAs in the HepG2 cell line.

In a study, miR-1275 was found to be severely under-expressed in HCC and cirrhotic liver tissues of patients with HCV-induced liver cancer[18]. Another study observed that miR-1275 was down-regulated in human HCC tissue samples and suppressed tumor growth by targeting IGF2BP1 and IGF2BP3[19]. In the study conducted in HCC cells, it was found that miR-1275 was expressed at low levels in HCC by qRT-PCR and transcriptome sequencing. In the same study, miR-1275, which was upregulated after genistein treatment, was found to reduce EMT in HCC cells through inhibition of the EIF5A2/PI3K/Akt signaling pathway[20]. Consistent with these studies, we found that irisin-gefitinib combination therapy significantly increased miR-1275 and suppressed PI3K/Akt expression compared to controls. Bioinformatic tools (miRWalk, TargetScan, and miRDB) also identified PI3K and AKT as direct targets of miR-1275. In conclusion, we propose that

irisin-gefitinib combination therapy suppresses the PI3K/Akt pathway by increasing miR-1275 expression, thereby potentially attenuating HCC progression and metastasis.

In a study on nasopharyngeal carcinoma, it was reported that miR-8085 expression increased in serum-derived exosomes and this increase supported cell proliferation and migration[21]. In our study, we found that irisin and gefitinib combination treatment decreased miR-8085 expression levels. Furthermore, in silico target screening identified the FNDC5 gene among the targets of miR-8085. Based on these findings, it is suggested that the reduction of miR-8085 may lead to an increase in FNCD5 expression, which may contribute to cancer regression by promoting irisin synthesis.

The tumor-suppressive character of miR-664b-5p observed in our study aligns with previous reports in the literature. Specifically, Li *et al.*[22] demonstrated its down regulation in HCC tissues and HepG2 and SUN-475 cell lines, noting that its over expression impaired cell viability, migration, and invasion. This suppressive role is further supported by findings in cervical cancer, where miR-664 down regulation correlates with altered apoptotic signaling, and its mimics significantly decrease Bcl-2 levels[23].Consistent with these mechanism models, we demonstrated that both irisin alone and irisin-gefitinib combination treatments unregulated miR-664b-5p while concurrently decreasing Bcl-2 levels, suggesting that miR-664b-5p over expression may induce HCC apoptosis via Bcl-2 suppression. Crucially, since the combination therapy increased miR-664b-5p expression significantly more than gefitinib monotherapy, our findings highlight a potential clinical benefit where irisin could mitigate gefitinib-induced hepatotoxicity.

Overexpression of miR-1304 has been reported to significantly reduce the colony formation and viability of NSCLC cells, as well as induce cell apoptosis and G0/G1 phase cell cycle arrest[24]. Othman *et al.*[25] found that the miR-1304 expression level was decreased in A549 and SK-LU1 human lung adenocarcinoma cells, while it increased in NP-69 normal cells. Furthermore, bioinformatics analysis revealed that miR-1304 was associated with MAPK/ERK, WNT, PI3K/AKT, TGF-β, and various apoptotic signaling pathways. In our study, miR-1304 was upregulated after gefitinib and irisin treatments, which may contribute to treatment by inducing apoptosis in HCC cells.

As a result of our screening of the miRWalk database, we found that miR-381-5p is among the ERB2 gene targets. In our study, the expression of miR-381-5p, which is thought to target the ErbB2 gene, was significantly increased after gefitinib and gefitinib-irisin treatments, while ErbB2 expression was significantly decreased after irisin treatment. These findings suggest that overexpression of miR-381-5p may contribute to the reduction of cell proliferation by decreasing ErbB2 levels.

miR-3922-5p has emerged as a crucial tumor suppressor that is tightly regulated by upstream non-coding RNA networks across various malignancies. Specifically, Shi *et al.*[26] demonstrated in breast cancer models that lncRNA HOXC-AS3 directly binds and negatively regulates miR-3922-5p, thereby driving tumor migration and invasion. Su *et al.*[27] reported both in vitro and in vivo that overexpression of HOXC-AS3 significantly increased HCC cell proliferation, while HOXC-AS3 interference significantly decreased HCC cell proliferation, suggesting that HOXC-AS3 promotes HCC cell proliferation. In our study, we suggest that irisin and combination therapies may contribute to the pathogenesis of HCC by decreasing cell invasion and proliferation through increasing miR-3922-5p expression.

Conclusion

Our study indicates that irisin treatment has the potential to enhance the anticancer effects of gefitinib in HepG2 cell line. Moreover, the synergistic effect of irisin and gefitinib combined treatment may significantly contribute to HCC pathogenesis by regulating dysregulated miRNA expressions, which subsequently suppress cell proliferation and promote apoptosis. These findings suggest that the combination of irisin and gefitinib may be a promising treatment approach for HCC.

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Declaration of interest statement

No authors have any financial/conflicting interests to disclose.

References

1 Cai Z & Liu Q, Understanding the Global Cancer Statistics 2018: implications for cancer control. *Sci China Life Sci*, 64 (2021) 1017.

2 Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA & Jemal A, Global cancer statistics. GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers. *CA Cancer J Clin,* 68 (2018) 394.

3 Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, Lencioni R, Koike K, Zucman-Rossi J & Finn RS, Hepatocellular carcinoma. *Nat Rev Dis Primers*, 7 (2021) 6.

4 Tang W, Chen Z, Zhang W, Cheng Y, Zhang B, Wu F, Wang Q, Wang S, Rong D, Reiter FP, De Toni EN & Wang X, The mechanisms of sorafenib resistance in hepatocellular carcinoma: theoretical basis and therapeutic aspects. *Signal Transduct Target Ther,* 5 (2020) 87.

5 Pathil A, Armeanu S, Venturelli S, Mascagni P, Weiss TS, Gregor M, Lauer UM & Bitzer M, HDAC inhibiton treatment of hepatoma cells induces both TRAIL-independent apoptosis and restoration of sensitivity to TRAIL. *Hepatology,* 43 (2006) 425.

6 Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, Gemma A, Harada M, Yoshizawa H, Kinoshita I, Fujita Y, Okinaga S, Hirano H, Yoshimori K, Harada T, Ogura T, Ando M, Miyazawa H, Tanaka T, Saijo Y, Hagiwara K, Morita S & Nukiwa T. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med,* 362 (2010) 2380.

7 Farazi PA & DePinho RA. Hepatocellular carcinoma pathogenesis: from genes to environment. *Nature,* 6 (2006) 674.

8 Schiffer E, Housset C, Cacheux W, Wendum D, Desbois-Mouthon C, Rey C, Clergue F, Poupon R, Barbu V & Rosmorduc O, Gefitinib, an EGFR inhibitor, prevents hepatocellular carcinoma development in the rat liver with cirrhosis. *Hepatology,* 41 (2005) 307.

9 Takeda M, Okamoto I, Fukuoka M & Nakagawa K, Successful treatment with erlotinib after gefitinib-related severe hepatotoxicity. *J Clin Oncol*, 28 (2010) 273.

10 Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Boström EA, Choi JH, Long JZ, Kajimura S, Zingaretti MC, Vind BF, Tu H, Cinti S, Højlund K, Gygi SP & Spiegelman BM, A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature,* 481(2012) 463.

11 Shao L, Li H, Chen J, Song H, Zhang Y, Wu F, Wang W, Zhang W, Wang F, Li H & Tang D, Irisin suppresses the migration, proliferation, and invasion of lung cancer cells via inhibition of epithelial-to-mesenchymal transition. *Biochem Biophys Res Commun,* 485 (2017) 598.

12 Zhang D, Zhang P, Li L, Tang N, Huang F, Kong X, Tan X & Shi G, Irisin functions to inhibit malignant growth of human pancreatic cancer cells via downregulation of the PI3K/AKT signaling pathway. *Onco Targets Ther,* 12 (2019) 7243.

13 Zhang J, Ke M, Ren Y, Bi J, Du Z, Zhang M, Wang Y, Zhang L, Wu Z, Lv Y & Wu R, Serum Irisin Predicts Posthepatectomy Complications in Patients with Hepatocellular Carcinoma. *Dis Markers,* 28 (2019) 9850191. https://doi.org/10.1155/2019/9850191

14 Cai H, Miao M & Wang Z. miR-214-3p promotes the proliferation, migration and invasion of osteosarcoma cells by targeting CADM1. *Oncol Lett,* 16 (2018) 2620.

15 Cheng G, Xu D, Chu K, Cao Z, Sun X & Yang Y, The Effects of MiR-214-3p and Irisin/FNDC5 on the Biological Behavior of Osteosarcoma Cells. *Cancer Biother Radiopharm*, 35 (2020) 92.

16 Callegari E, Elamin BK, Sabbioni S, Gramantieri L & Negrini M. Role of microRNAs in hepatocellular carcinoma: A clinical perspective. *Onco Targets Ther*, 6 (2013) 1167.

17 Roy B, Ghose S & Biswas S. Therapeutic strategies for miRNA delivery to reduce hepatocellular carcinoma. *Semin Cell Dev Biol*. 124 (2022) 134.

18 Fawzy IO, Hamza MT, Hosny KA, Esmat G, El Tayebi HM & Abdelaziz AI, MiR-1275: A single microRNA that targets the three IGF2-mRNA-binding proteins hindering tumor growth in hepatocellular carcinoma. *FEBS Lett,* 589 (2015) 2257.

19 Yang J, Qin T, Liu S, Tang H, Liu M & Wang Q, Interaction analysis of miR-1275/IGF2BP1/IGF2BP3 with the susceptibility to hepatocellular carcinoma. *Biomark Med*, 1 (2020) 283.

20 Yang X, Jiang W, Kong X, Zhou X, Zhu D & Kong L, Genistein Restricts the Epithelial Mesenchymal Transformation (EMT) and Stemness of Hepatocellular Carcinoma via Upregulating miR-1275 to inhibit the EIF5A2/PI3K/Akt Pathway. *Biology (Basel),* 11 (2022) 10.

21 Zhou XJ, Xu HM, Huang GS & Lin BR. Nasopharyngeal carcinoma derived exosomes regulate the proliferation and migration of nasopharyngeal carcinoma cells by mediating the miR-99a-5p BAZ2A axis. *Braz J Otorhinolaryngo*, 90 (2024) 101343.

22 Li H, Guo D, Zhang Y, Yang S & Zhang R. MiR-664b-5p inhibits hepatocellular cancer cell proliferation through targeting oncogene AKT2. *Cancer Biother Radiopharm,* 35(2020) 605.

23 Lv M, Ou R, Zhang Q, Lin F, Li X, Wang K & Xu Y, MicroRNA-664 suppresses the growth of cervical cancer cells via targeting c-Kit. *Drug Des Devel Ther,* 13 (2019) 2371.

24 Li CG, Pu MF, Li CZ, Gao M, Liu MX, Yu CZ, Yan H, Peng C, Zhao Y, Li Y, Ma ZL, Qi XM, Wang YZ, Miao LL & Ren J, MicroRNA-1304 suppresses human non-small cell lung cancer cell growth in vitro by targeting heme oxygenase-1. *Acta Pharmacol Sin*, 38 (2017) 110.

25 Othman N, In LL, Harikrishna JA & Hasima N, Bcl-xL silencing induces alterations in hsa-miR-608 expression and subsequent cell death in A549 and SK-LU1 human lung adenocarcinoma cells. *PLoS One*, 8 (2013) e81735.

26 Shi SH, Jiang J, Zhang W, Sun L, Li XJ, Li C, Ge QD & Zhuang ZG, A Novel lncRNA HOXC-AS3 Acts as a miR-3922-5p Sponge to Promote Breast Cancer Metastasis. *Cancer Invest,* 2 (2020) 1.

27 Su C, Wang W, Mo J, Liu F, Zhang H, Liu Y, Chen X, Liao Z, Zhang B & Zhu P, Long noncoding RNA HOXC-AS3 interacts with CDK2 to promote proliferation in hepatocellular carcinoma. *Biomark Res,* 28 (2022) 65.