Original Paper

The p53/miR-34a/SIRT1 Positive Feedback Loop in Quercetin-Induced Apoptosis

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Key Words

Quercetin • P53 • MiR-34a • HCC • SIRT1

Abstract

Background: The anti-tumor effects of quercetin have been reported, but the underlying molecular mechanisms remain to be elucidated. The aim of present study was to explore the role of miRNA in the anticancer effects of quercetin. *Methods:* The differential miRNAs expression between the HepG2 and Huh7 cells treated by quercetin were detected by microarray. The xCELLigence, Flow cytometry, RT-PCR and Western blot were used to analyze the cell proliferation, cell apoptosis, cell cycle arrest, anti-tumor genes, and protein expression. *Results:* miR-34a was up-regulated in HepG2 cells treated by quercetin exhibiting wild-type p53. When inhibiting the miR-34a, the sensitivity of the cells to quercetin decreased and the expression of the SIRT1 was up-regulated, but the acetylation of p53 and the expression of some genes related to p53 down-regulated. *Conclusion:* miR-34a plays an important role in the anti-tumor effects of quercin in HCC, miR-34a may be a tiemolecule between the p53 and SIRT1 and is composed of a p53/miR-34a/SIRT1 signal feedback loop, which could enhance apoptosis signal and significantly promote cell apoptosis.

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Introduction

Hepatocellular carcinoma (HCC) is the sixth most common type of cancer and the third most common cause of cancer deaths worldwide. Because conventional therapies, including surgical resection, chemotherapy and radiation, are often inadequate in treating this disease,

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new treatment options are critically needed. Despite the emergence of novel targeted agents and the use of various therapeutic combinations, no treatment options are available that are curative in patients with advanced cancer [1]. The magnitude of this problem mandates the need for novel therapeutic agents, specifically the use of agents for chemoprevention [2].

Quercetin is a flavonoid possessing potential chemopreventive properties [3]. It is a functionally pleiotropic molecule, possessing multiple intracellular targets, affecting different cell signalling processes usually altered in cancer cells, with limited toxicity on normal cells [4]. Several studies have indicated that a treatment with quercetin triggered numerous cellular events such as p53 activation, cell cycle arrest and induction of caspase-mediated apoptosis in HCC cells [5, 6]. However, more research work should be performed to elucidate the anticancer mechanisms of quercetin.

Recently, microRNAs (miRNAs), which are endogenous noncoding RNAs of ~22 nucleotides length, have been found to play roles in diverse biological processes, including cellular differentiation, proliferation and death [7-10]. It has emerged as an important gene regulators being critically involved in carcinogenesis and cancer chemoprevention [11-14]. miR-34a is a star molecule and has been found to participate in the regulation of p53 pathways as well as the tumour suppressor activity [15-17], miR-34a becomes more and more important and could be a good clue to discover some unknown field. The aim of present study was to investigate the role of miR-34a in the anticancer effects of quercetin.

Materials and Methods

Chemicals

Quercetin dehydrate was purchased from Sigma Chemical Company, St. Louis, MO, USA. Stock solution of quercetin (100mM) was prepared by dissolving in 0.5% DMSO and was diluted with Dulbecco's modified Eagles medium (DMEM) prior to use for the desired concentration. The final concentration of quercetin in DMSO was 0.1% (v/v).

Cell culture

HepG2 and Huh7 cells used in the study were purchased from the American Type Culture Collection (ATCC, Manassas, VA). These cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Thermo Electron; Waltham, MA, USA) supplemented with 10% FBS, and were cultured at 37 ° C in a humidified incubator in an atmosphere containing 5% CO_2.

Cell Proliferation Analysis (Real Time Cellular Analysis)

The xCELLigence DP device from Roche Diagnostics (Mannheim, Germany) was used to monitor cell proliferation in real-time. HepG2 cells (5×10^3) and Huh7 cells (5×10^3) were seeded in 96 well electronic microtiter plates (E-Plate™; Roche Diagnostic), and were cultured with DMEM contained 0μM, 2μM, 4μM, 8μM, 16μM, 32μM or 0mM, 0.0625mM, 0.125mM, 0.25mM, 0.5mM, 1mM of quercetin, respectively. All cells were measured for 96 h with the xCELLigence system according to the instructions in the user's manual. Cell density measurements were performed in quadruplicate with a programmed signal detection every 20 min. Data acquisition and analyses were performed with the RTCA software (version 1.2, Roche Diagnostics). The rate of cell proliferation was used as index [18].

Cell cycle analysis

The cell suspension at a density of 5×10^5 per well was seeded in six-well plates. The cells were incubated at 37°C for 24 h and treated with 31.25 μM quercetin for 48 h. Wells containing culture medium with 0.1% DMSO were used as controls. The cells were harvested and centrifuged at 1500 g for 5 min, washed with PBS, and centrifuged again. Then, adherent and floating cell populations were combined and fixed in ethanol (70% in PBS), washed with PBS, and then stained with 50 μg/ml PI and 50 μg/ml RNase A in PBS at room temperature in the dark for 30 min. Cell distribution in the different phases of the cell cycle was analyzed by flow cytometry using the ModFit LT V3.0 software (Verity Software House, Topsham, USA).

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MiRNA Microarray Profiling

HepG2 and Huh7 cells were treated with 31.25 μM quecetin for 12 h respectively, the cells treated with vehicle served as controls. The total miRNA was isolated by mirVana™ miRNA Isolation Kit (Invitrogen, USA). Expression profiling of circulating miRNAs was performed using TaqMan human miRNA arrays and assays in accordance with the manufacturer's instructions (Taqman Low Density Array Human microRNA, Applied Biosystems, Foster City, CA, USA). In brief, total RNA was reverse transcribed using Megaplex primer pool A (Applied Biosystems) which contained sequence-specific primers for 381 specific miRNAs plus 3 controls (pool A). Real-time quantitative PCR was performed for 381 miRNAs, using A microfluidic cards, each containing primers and probes for 381 specific miRNAs plus 3 controls and thermal-cycled on an Applied Biosystems 7900 HT instrument.

Flow cytometry analysis

The number of apoptotic cells induced by quercetin was measured by flow cytometry using Annexin V assay kit from BD (CA, USA). Briefly, HepG2 or Huh7 cells ($5×10^5$) were seeded in the 6 well plates, and were treated with quercetin (31.25μM) for 48h, the cells treated with vehicle served as controls. Then the cells were harvested and washed with cold PBS, and the cell pellet was resuspended in 1× binding buffer at a concentration of $2×10^5$ cells/ml. Five μl of Annexin V-FITC and 5 μl of PI were added per 100 μl of cell suspension and vortexed gently. The stained samples were incubated for 15 min at room temperature in the darkness. An additional 400μl of 1× binding buffer was added to each tube. Samples were analyzed by C6 Accuri® flow cytometer (Accuri Cytometers, Ann Arbor, MI, USA) within 1 h. A total of $1×10^4$ events were acquired using green channel FL1 for Annexin V-FITC and the red channel FL2 for PI. Data are presented as mean ± SE of duplicate experiments measured in triplicate, and data acquisition and analyses were performed with the CFlow software (version 1.0.264.25, Accuri cytometers, Inc).

RNA interference

siRNA against human p53 (Gene pharma, China), miRNA-34a inhibitor (Ribobio Biotech, China) and control scrambled siRNA (Gene pharma, China) were pre-designed and synthesized. HepG2 cells ($3×10^5$) were transfected with 100nM p53 siRNA, miR-34a inhibitor or the scrambled duplex using Lipofectamine RNAiMAX (Invitrogen, USA). After incubation for 24 h, the cells were incubated with fresh medium and subsequently incubated for 48 h. Then the cells were harvested by centrifugation at 110 g for 5 min for the further analysis.

Quantitative real-time PCR

For quantitative expression analysis of miRNAs and mRNA, total RNAs were isolated by mirVana™ miRNA Isolation Kit (Invitrogen, USA) from HepG2 and Huh-7 cells transfected with miR-34a inhibitor, miR-34a mimics or control scrambled siRNA according to the manufacturer's instructions. cDNA was synthesized using PrimeScript® RT reagent Kit (Takara, Japan) with specific miR-34a RT primers (RiboBio, China) or random mRNA primers according to the manufacturer's instructions. Relative quantitative real-time PCR was performed using the SYBR Premix Ex Taq kit (TAKARA, Japan) in the ABI Prism 7900 (Applied Bio systems, USA) with the primers of miRNA-34a (RiboBio, China). The comparative cycle threshold (Ct) method was applied to quantify the expression levels of miRNAs. Relative expression levels were calculated by the $2^{-\Delta\Delta Ct}$ method. *U6* small nuclear RNA was used as an internal standard for miRNA.

Western Blot Analysis

HepG2 or Huh7 cells ($5×10^5$) were seeded in the 6 well plates and treated with quercetin (31.25μM), cells treated by vehicle was used as controls. Cells were harvested at 6, 12, 24, 48h after quercetin treatment, respectively. The cell pellets were washed with cold phosphate-buffered saline (PBS) and lysed in radio immunoprecipitation assay (RIPA) buffer containing 150mM NaCl, 10mM Tris (pH 7.2), 0.1% sodium dodecyl sulfate (SDS), 1% Triton X-100, 1% deoxycholate, 5mM EDTA and protease/phosphatase inhibitors. Protein concentration was determined by the Pierce BCA Protein assay kit (Thermo Fisher Scientific Inc., Rockford, IL, USA). Equivalent amounts of protein (40μg) were separated by 10% SDS–PAGE and transferred to a polyvinylidene difluoride membrane (Millipore, Billerica, MA, USA). The membrane was blocked in TBST containing 5% nonfat milk and incubated with anti-SIRT1, anti-ace-p53, anti-p21, anti-Caspase 3, anti-CD38, anti-Cyclin B1, anti-GAPDH, specific rabbit antibody or anti-p53 specific mouse antibody at 4

Fig. 1. The cell proliferation inhibition and Cyclin B1 expression in HepG2 and Huh7 cells treated by quercetin. (A) The cell proliferation inhibition curves in HepG2 exposed to 0-256μM quercetin and Huh7 cells exposed to 0-1000μM quercetin showed that the GI50% and GI99% concentrations of quercetin were 13.16 ±1.29 μM and 31.25±2.05 μM in HepG2 cells and 151.32±5.23 μM and 321.25 ±20.43 μM in Huh7 cells respectively, there were significant differences between HepG2 and Huh7 cells (*P*<0.01). (B) Cyclin B1 expression was down regulated in the HepG2 and Huh7 cells exposed to 31.25 μM quercetin, as compared with control group (*P*<0.01).
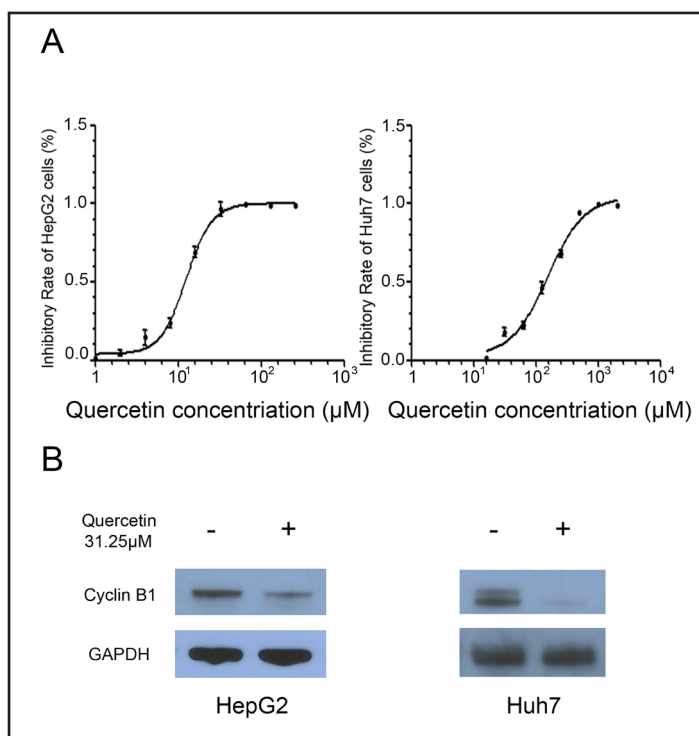
°C overnight, respectively. The membrane was then incubated with horseradish peroxidase conjugated anti-rabbit or mouse immunoglobulin G (Southern Biotechnology Associates, Inc., Birmingham, AL, USA) for 1 h at room temperature and visualized with enhanced chemiluminescence reagents (Thermo Fisher Sci-entific Inc.) following exposure to X-ray films. The intensity of the bands was scanned by a Gel Doc 2000 system, and the densitometric assay was performed using Quantity One software (Bio-Rad, Inc., Berkeley, CA, USA).

Statistical analysis

Differences between groups were analyzed using conventional Student's t test or ANOVA. Each experiment was repeated at least three times, and the data are presented as mean ± SD. A P value <0.05 and <0.01 were considered as significant differences. Statistical calculations were executed using SPSS version 13.0 (SPSS, Inc.).

Table 1. Thirteen differential expression miRNAs between the HepG2 and Huh7 cells treated with quercetin for 24h were detected by microarray

Detector	Huh7 Quercetin VS Vehicle	HepG2 Quercetin VS Vehicle
hsa-let-7b	0.49	2.75
hsa-let-7e	0.69	4.04
hsa-miR-185	1.10	2.69
hsa-miR-192	0.91	2.24
hsa-miR-194	1.68	5.89
hsa-miR-212	0.52	3.34
hsa-miR-34a	0.70	2.95
hsa-miR-362-5p	0.42	2.89
hsa-miR-424	0.50	5.48
hsa-miR-491-5p	0.81	2.70
hsa-miR-339-5p	0.74	0.36
hsa-miR-125a-5p	0.99	0.21
hsa-miR-652	0.99	0.40

Results

The cell proliferation inhibition and apoptosis in HepG2 and Huh7 cells treated by quercetin
Fig. 1A showed the growth inhibition curves in HepG2 exposed to 0-256 μM quercetin and Huh7 cells exposed t 0-1000 μM quercetin. The quercetin could induce growth inhibition of the HepG2 and Huh7 cells, the GI50% and GI99% concentrations of quercetin were 13.16 μM and 31.25 μM in HepG2 cells and 151.32μM and 321.25 μM in Huh7 cells respectively, there were significant differences between HepG2 and Huh7 cells (*P*<0.01).

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Table 2. Cell cycle, cyclin B1 expression and apoptosis rate in HepG2 cells and Huh7 cells exposed to 31.25 µM quercetin for 24 h (mean ±SD). Note: a: as compared with vehicle group, $P<0.01$; b: as compared with Huh7 cells, $P<0.01$; C: as compared with vehicle group, $P<0.05$; d: as compared with Huh7 cells, $P<0.05$

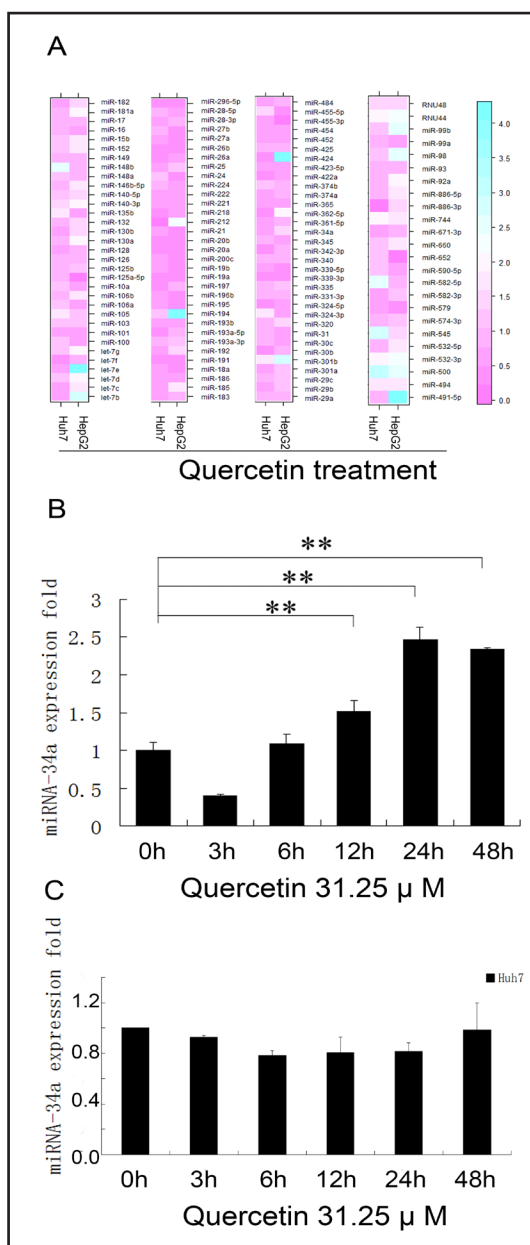| group | HepG2 cells | | | | | |
	Cell cycle (%)			Cyclin B1	Apoptosis (%)	Necrosis (%)
	G1	S	G2/M			
quercetin	47.07±1.03	19.77±2.28	33.17±2.13[a]	0.26±0.01[a]	22.55±0.6[ab]	7.75±0.41[cd]
Vehicle	59.37±0.81	19.2±1.68	21.43±0.87	0.58±0.04	4.67±0.97	3.20±0.94
	Huh7 cells					
	Cell cycle (%)			Cyclin B1	Apoptosis (%)	Necrosis (%)
	G1	S	G2/M			
quercetin	43.00±1.35	28.63±1.56	28.37±1.01[a]	0.14±0.02[a]	6.97±1.45	4.43±0.25
Vehicle	58.03±1.37	21.22±2.38	20.77±1.37	0.64±0.02	7.20±1.20	4.83±0.55

Fig. 2 The expression level of miR-34a in the HepG2 and Huh7 cells treated by 31.25µM quercetin. (A) miRNAs with differential expression between the HepG2 and Huh7 cells treated with quercetin for 24h were detected by microarray. (B) The results of qRT-PCR indicated that miRNA-34a expression levels were up-regulated with time-dependent manner in HepG2 cell treated with 31.25 quercetin, as compared with control group**: $P<0.01$. (C) The results of qRT-PCR indicated that miRNA-34a expression level was changed slightly in Huh7 cell treated with quercetin, as compared with control group.

The cell cycle analysis demonstrated that both of the HepG2 and Huh7 cells underwent the G2/M cell cycle arrest at a dose of 31.25 µM of quercetin (Table 2), and the cycle arrest related protein (cyclinB1) levels were down-regulated (Fig. 1B), as compared with controls ($P<0.01$).

The apoptosis assay (Table 2) indicated that quercetin could result in cellular apoptosis and HepG2 cells were more sensitive to the quercetin than Huh7 cells at a dose of 31.25 µM of quercetin ($P<0.01$).

miR-34a is involved in the antitumor effects of Quercetin

Differential expressed miRNAs between the HepG2 and Huh7 cells treated with quercetin were detected by microarray (Fig. 2A), there were 10 up-regulated miRNA (>2 fold) and 3 down-regulated miRNA (<2 fold) in HepG2 cells as compared to those in Huh7 cells (Table 1). The results (Fig. 2B) of qRT-PCR indicated that miRNA-34a expression
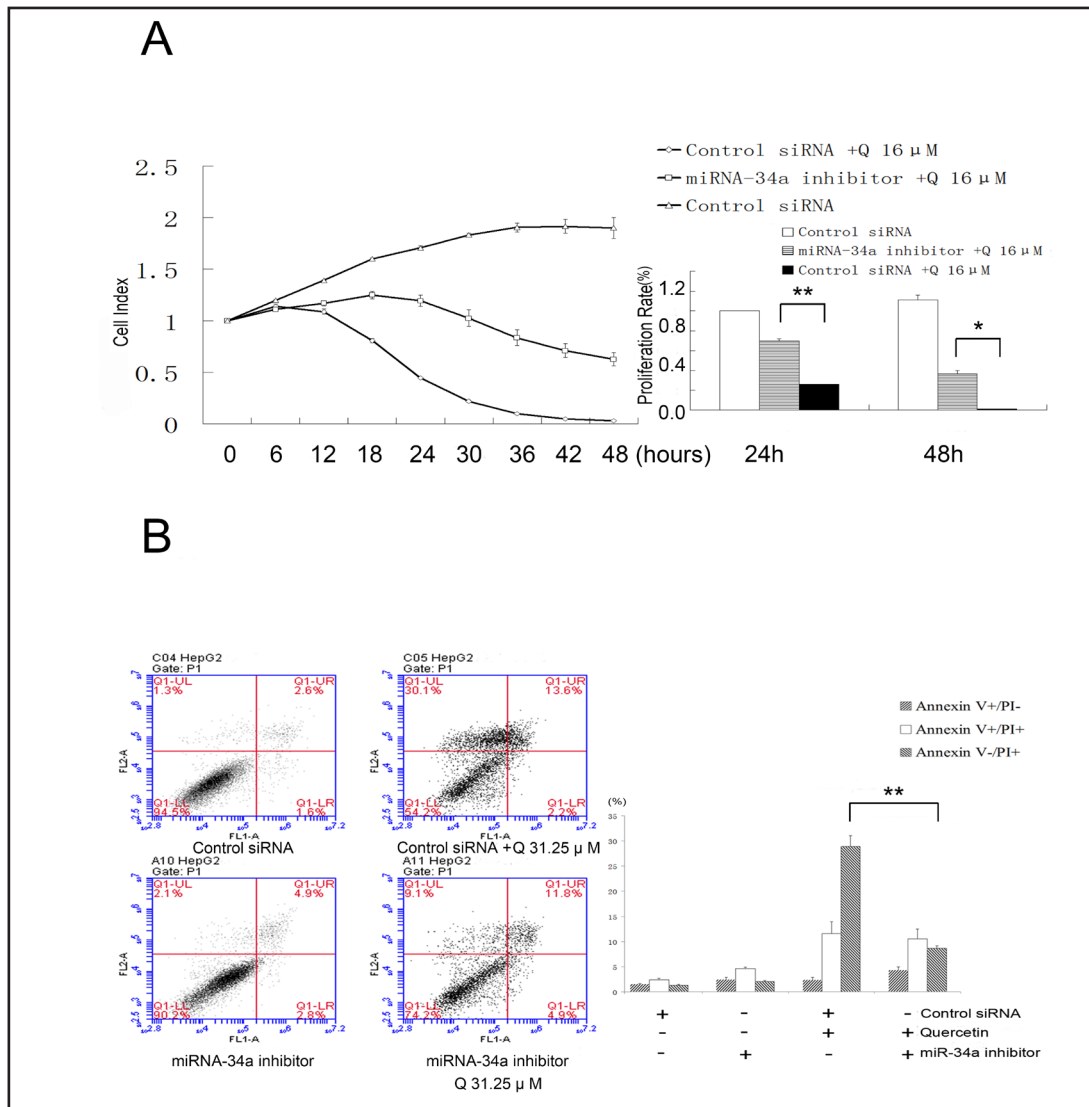
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Fig. 3. The proliferation inhibition and apoptosis induced by 16μM quercetin in HepG2 cells by interfering the miR-34a expression. (A) Real Time Cellular Analysis experiment indicated that the proliferation rates in miR-34a inhibitor+16μM quercetin group were significantly higher than those in control siRNA+16μM quercetin group at 24h**$P<0.01$ and at 48h *$P<0.05$. (B) The apoptosis assay showed that the apoptosis rate in miR-34a inhibitor+16μM quercetin group was significantly lower than that in control siRNA+16μM quercetin group, **$P<0.01$.

Table 3. The protein expression levels of p53 and p21 in HepG2 cells treated with 31.25μM quercetin. (mean ±SD). Note: a: as compared with 0h group, $P<0.01$; b: as compared with 6h group, $P<0.01$

group	Exposure to 31.25μM quercetin			
	0h	6h	12h	24h
p53	1±0.09	2.41±0.06[a]	4.40±0.41[ab]	4.51±0.34[ab]
p21	1±0.04	1.46±0.04[a]	2.30±0.06[ab]	2.55±0.02[ab]

levels were up-regulated with time-dependent manner in HepG2 cell treated with 31.25 μM quercetin, as compared with control group ($P<0.01$). The results (Fig. 2C) of qRT-PCR indicated that miRNA-34a expression level was changed slightly in Huh7 cell treated with 31.25 μM quercetin, as compared with control group. Those results demonstrated that the sensitive of HepG2 cells to quercetin was significantly higher, as compared with Huh7 cells.

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Table 4. SIRT1 protein expression in HepG2 cells exposed to quercetin at different doses and times (mean ±SD). Note: a: as compared with control group, $P<0.01$; b: as compared with 16μM group, $P<0.01$; c: as compared with 32μM group, $P<0.01$; d: as compared with 6 h group, $P<0.01$; e: as compared with 24 h group, $P<0.01$; f: as compared with control group, $P<0.05$

group	Exposure to quercetin for 24 h				Exposure to 31.25μM quercetin			
	0μM	16μM	32μM	64μM	0h	6h	24h	48h
SIRT1	1.00±0.07	1.2±0.07	0.44±0.07[ab]	0.09±0.02[abc]	1±0.03	0.79±0.03[f]	0.48±0.02[ad]	0.04±0.01[ade]

Fig. 4. Quercetin treatment induces miR-34a expression of HepG2 cells via p53. (A) p53 and p21 expression levels were up-regulated with time-dependent in HepG2 cells treated with 31.25μM quercetin. (B) The expression level of miR-34a after transfecting with siRNA at the 24th h was not significantly different from that at the 0th in the HepG2 cells exposed to 31.25μM quercetin.

The proliferation inhibition and apoptosis induced by quercetin in HepG2 cells by interfering the miR-34a expression.

In Fig. 3A, the real Time Cellular Analysis experiment indicated that the proliferation rates in miR-34a inhibitor+16μM quercetin group were significantly higher than those in control siRNA+16μM quercetin group at 24h ($P<0.01$) and at 48h ($P<0.05$). In Fig. 3B, the apoptosis assay showed that the apoptosis rate in miR-34a inhibitor+16μM quercetin group was significantly lower than that in control siRNA+16μM quercetin group ($P<0.01$).

The above data suggested that miR-34a plays a key role in quercetin induced proliferation inhibition and cell apoptosis.

Quercetin treatment induces miR-34a expression of HepG2 cells via p53

Fig. 4A and Table3 showed that p53 and p21 expression levels were up-regulated with time-dependent in HepG2 cells treated with 31.25μM quercetin. Also Fig. 4B exhibited that the expression level of miR-34a after transfecting with siRNA at the 24th h was not significantly different from that at the 0th in the HepG2 cells exposed to 31.25μM quercetin. These results suggested that quercetin-induced miR-34a expression in hepatoma cells is largely dependent on p53.

SIRT1 is part of a miR-34a-mediated antitumor network in quercetin

Table 4 and Fig. 5A and B displayed that SIRT1 protein expression in HepG2 cells exposed to quercetin at different doses and times. The results showed that SIRT1 protein expression increased with dose and time of exposure to quercetin, when the doses and times of exposure to quercetin were 32μM, 64μM and 24 h or 48h, SIRT1 protein expression enhanced significantly, as compared with control group ($P<0.01$ or $P<0.05$).

Table 5 and Fig. 5C determined that SIRT1, acylation p53, p21 and Caspase 3 cleavage expression in HepG2 cells exposed to 31.25 μM quercetin for 24h. The results indicated that
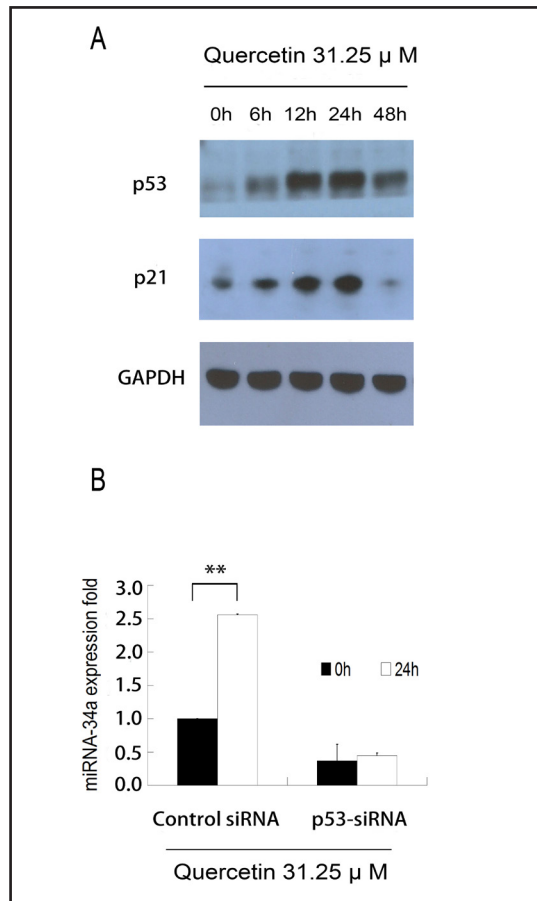
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Table 5. SIRT1, acylation p53, p21 and Caspase 3 cleavage expression in HepG2 cells exposed to31.25 µM quercetin for 24h (mean ±SD). Note: a: as compared with control group, *P*<0.01; b: as compared with control group, *P*<0.05; c: as compared with Quercetin group, *P*<0.01; d: as compared with Quercetin group, *P*<0.05; e: as compared with Quercetin+ NC inhibitor group, *P*<0.01; f: as compared with Quercetin+ NC inhibitor group, *P*<0.05

| Group	SIRT1	acylation p53	p21	Caspase 3
Control	1.58±0.04	0.28±0.03	1.05±0.04	0.29±0.01
Quercetin	0.48±0.09[a]	0.86±0.01[a]	1.38±0.01[b]	0.43±0.01[b]
Quercetin + NC inhibitor	0.14±0.04[a d]	1.09±0.10[ad]	1.22±0.03[b d]	1.04±0.01[ac]
Quercetin + miRNA-34a inhibitor	2.46±0.22[bce]	0.03±0.01[c e]	0.84±0.01[df]	0.03±0.02[bce]

Fig. 5. (A and B) SIRT1 protein expression in HepG2 cells exposed to quercetin at different doses and times. (C) SIRT1, acylation p53, p21 and Caspase 3 cleavage expression in HepG2 cells exposed to 31.25 µM quercetin for 24h.

SIRT1 expression significantly reduced, and acylation p53, p21 and Caspase 3 cleavage expression significantly enhanced in Quercetin + NC inhibitor group, as compared with control group (*P*<0.01 or *P*<0.05). However, SIRT1 expression significantly elevated, and acylation p53, p21 and Caspase 3 cleavage expression significantly declined in Quercetin + miRNA-34a inhibitor group, as compared with control group, Quercetin group and Quercetin + NC inhibitor group (*P*<0.01 or *P*<0.05). According to above results, it was supposed that miR-34a may enhance the cell proliferation inhibition and cell apoptosis related to p53 through inhibiting SIRT1 in HepG2 cells treated with quercetin.

Discussion

Quercetin exhibits anticancer activities in many types of cancers [19-21], and is rich in food and has low cytotoxicity to the normal cells, so quercetin has a great value in medicine. Therefore, understanding thoroughly its anti-tumor mechanisms is very important. Some literatures demonstrated that the proliferation inhibition effects of quercetin were associated with the induction of cell cycle arrest and apoptosis [22]. In our study, it was confirmed that quercetin could induce G2/M cell cycle arrest and apoptosis.
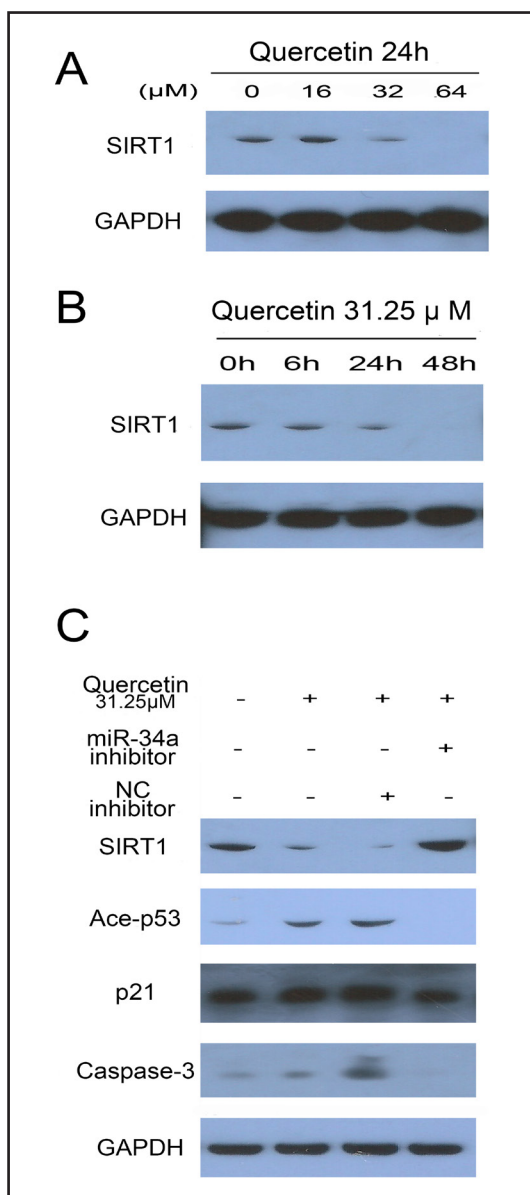
Moreover, the results of miRNA array demonstrated that there were 10 up-regulated miRNAs and 3 down-regulated miRNAs in HepG2 cells, as compared with Huh7 cells. The significant difference between the HepG2 and Huh7 is the status of p53 (HepG2 is wt and Huh7 is mutant), so the miR-34a became the focus of study. miR-34a is directly regulated by p53, and can silence the mRNA expression of SIRT1 as a feed back loop to acetylate the p53 and therefore enhance the apoptosis signal.

As for the anti-tumor mechanism, p53 is the important factor. Its mutant or stability greatly determines the sensitivity of tumor cells to quercetin. As described in the literature, the GI50 was about 12-24µM in the p53 wild type tumor cells [23, 24], but was more than 190µM in the p53 mutant cell [25]. The phenomenon was also demonstrated in our study, although the numerical value was different, the trend was same.

As a star molecule, the expression of miR-34a was influenced by several factors, such as loss of 1p36 heterozygosity, the CpG island methylation of miR-34a genes and functional p53 [26, 27]. In the apoptosis pathway, miR-34a was always believed to transduct the apoptosis signal from the p53 to other apoptosis related proteins, such as cell cycle arrest related genes Cyclin E, CDK4 and CDK6, and cell survival related genes Bcl-2 [28]. In our study, The above genes were detected by RT-PCR, and it was found that the mRNA of CDK4, CDK6 and Bcl-2 were upregulated after transfecting with miR-34a inhibitor in quercetin treated cells (data not show). It was also found that the acetylation of p53 was up-regulated when HepG2 cells were treated with quercetin, but the acetylation of p53 and downstream genes of p53 such as p21 and Caspase 3 cleavage were down-regulated after transfecting with miR-34a inhibitor. The above results indicated that the miR-34a is a component of a very important feed back loop which could affect the p53 stability in cells exposed to quercetin.

SIRT1 is a class III nuclear deacetylase that can activate or repress genetic programs by modifying histones and transcription factors. The first finding of this work is that the enzyme is overexpressed and overfunctional in CLL cells, taking normal B lymphocytes as a comparison [29]. SIRT1 is reported to inactivate p53 by deacetylating a critical lysine residue, blocking the SIRT1 may lead to the activation of the p53 pathway. miR-34a was verified that it could bind the site within the 3' UTR of SIRT1 to silence the mRNA [30]. It was confirmed by our results that miR-34a expression, SIRT1 inhibition and p53 activity were enhanced significantly when HepG2 cells were exposed to quercetin. miR-34a just likes a tiemolecule between the p53 and SIRT1, miR-34a with p53 and SIRT1 forms a positive feedback loop structure (p53/miR-34a/SIRT1), in which quercetin induces p53 and then miR-34a, which lead to the repression of SIRT1, finally, the acetylation of p53 is increased, which will enhance the stability of p53 and promote the p53 related apoptosis, therefore increase the sensitivity of HepG2 cell to quercetin.

In conclusion, based on the results of the present study, we can deduce that the anti-tumor mechanism of quercetin in liver cancer was mainly through the p53 related pathway. In this pathway, the miR-34a is an element of a feedback loop including p53 and SIRT1, which will greatly enhance the p53 related apoptosis signal and promote the sensitivity of cells to quercetin.

Abbreviations

HCC (hepatocellular carcinoma); DMEM (Eagle's minimal essential medium); DMSO (Dimethyl sulfoxide); GAPDH (glyceraldehyde-3-phosphate dehydrogenase).

Disclosure Statement

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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