The anti-cancer effect of Quercetin in renal cancer through regulating survivin expression and caspase 3 activity

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Abstract

Background: Renal cancer is less sensitive to traditional chemotherapy and radiation therapy. Quercetin exhibits a broad-spectrum anti-cancer role and less side effects across different tumour types, but its effect in renal caner remains unknown. This study aimed to investigate the correlation between renal cancer and Quercetin.

Methods: Renal cancer ACHN cells were cultured and treated with different doses of Quercetin. Cell viability was tested by MTT assay. Cell apoptosis was measured by flow cytometry. Survivin expression was determined by Real time PCR and Western blot.

Results: Quercetin significantly suppressed ACHN cell proliferation and induced early cell apoptosis in a dose-dependent manner ($P<0.05$). Quercetin significantly enhanced caspase 3 activity ($P<0.05$), but downregulated survivin mRNA and protein expression ($P<0.05$).

Conclusion: This study has found that Quercetin may have anti-cancer effect as it induced renal cancer cell apoptosis possibly through inhibiting survivin expression and caspase 3 activation.

Key words: Quercetin; Survivin; Caspase 3; apoptosis; renal cancer

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Introduction

Renal cancer places second among malignant tumors of the urogenital system and accounts for 2% of all adult malignancies. The incidence of renal cancer is increasing in China, and tend to happen with young men [1,2]. Most primary renal cancers can be definitively diagnosed only after advanced stage due to the lack of typical symptoms for early diagnosis and ineffective render surgical treatments [3]. Patients with advanced renal cancer would normally be processed with traditional chemotherapy and radiation therapy, to which renal cancer is less sensitive though [4]. Therefore, there is still an urgent need for identifying effective treatments of renal cancer.

Quercetin is a natural hydroxyl flavonoid that can be extracted from a variety of Chinese herbal medicines, vegetables, and fruits [5]. Quercetin has various pharmacological activities, such as scavenging free radicals, reducing blood fat, anti-inflammation effects, anti-tumor effects, and anti-platelet aggregation

[6, 7]. Quercetin exerts its anti–cancer roles through suppressing tumor cell growth, regulating the cell cycle, and inducing cell apoptosis [8]. The molecular mechanisms involved in the anti–cancer effect of Quercetin have been reported in several types of tumors. For example, the cytotoxic effect of Quercetin on HL–60 human promyelocytic leukemia cells has been found associated with the rapid and transient induction of caspase 3/CPP32 activity [9]. Quercetin has been found that it can block H_2O_2–induced phosphorylation of ERK and p53 protein in rat glioma C6 cells [10]. Moreover, Quercetin inhibited the invasion of breast carcinoma cells through suppressing PKC delta/ERK/AP–1–dependent matrix metalloproteinase–9 activation [11]. In addition, the Quercetin nanoliposome–induced C6 glioma cell death has been found associated with the JAK2/STAT3 and mitochondrial pathways [12]. A recent study has found that Quercetin can increase the sensitivity of breast cancer cells to Doxorubicin (DOX) through increasing PTEN expression and downregulating and subsequent Akt phosphorylation [13]. However, the anti–cancer effect of Que on renal cancer cells remains to be clarified.

A study performed by Zhu and Liehr reported that Quercetin increased the severity of estradiol–induced tumorigenesis in hamster kidneys [15,16]. The re–evaluation of a 2–year carcinogenicity bioassay of Quercetin confirmed an increase in renal tumors in male Fischer 344 rats that received mid– and high–doses of Quercetin [17]. Liu et al revealed that a combination of Quercetin and hyperoside induced cytotoxicity in 786–O renal cancer cells through inducing caspase–3 and PARP cleavage [20]. Meng et al observed that Quercetin suppressed cell proliferation and migration, and induced cell cycle arrest and apoptosis in Caki–2 renal cell carcinoma cells [21]. However, the function and mechanism of Quercetin on renal cancer apoptosis still needs to be elucidated.

In this study, the apoptotic effect of Quercetin has been tested in human renal adenocarcinoma cells and the effects of Quercetin on the expression of survivin and activity of caspase–3 have been measured.

Materials and methods

Main reagents

RPMI–1640 medium, FBS, and penicillin–streptomycin were purchased from Hyclone (Logan, UT, USA). MTT was purchased from Gibco (Grand Island, NY, USA). PVDF membrane was purchased from Pall Life Science (Port Washington, NY, USA). ECL reagents were purchased from Amersham Biosciences. Rabbit anti–human survivin primary antibody and HRP–conjugated IgG secondary antibody were purchased from Cell Signaling Technology (Danvers, MA, USA). Caspase 3 activity kit was purchased from Pall Life Sciences. Annexin V–FITC apoptosis detection kit was purchased from BD BioScience (Sparks, MD, USA).

Cell culture

Human renal cell adenocarcinoma cell line was purchased from ATCC (American Type Culture Collection). ACHN cells were cultured in RPMI–1640 medium that containing 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 ℃, 5% CO_2.

MTT assay

ACHN cells in logarithmic phase were seeded in a 96–well plate at 3.0×10^3 cells/well for overnight. The cells were treated with 0 μM, 20 μM, and 40 μM Quercetin respectively for 48 hours. 20 μL of MTT (5 g/L) was added to each well. After incubation for 4 hrs at 37 ℃, the supernatant was removed and 150 μL of DMSO was added and the plate was gently shaken for 10 minutes at room temperature (RT). The plate was read at 570 nm wavelength. The proliferation rate was calculated. The experiment was repeated for at least three times.

Flow cytometry

The cells were seed in a 50 ml culture flask at 5×10^5 cells/mL and the cells in logarithmic phase were treated with 0 μM, 20 μM, and 40 μM Que for 48 hours. Cells were then collected and washed with 1 × PBS. After centrifugation at 1000 rpm for 5 minutes, the cells were fixed in pre–cooled 75% ethanol at 4 ℃ overnight. After removed the ethanol and washed with 1 × PBS, the cells were resuspended in 800 μL 1 × PBS with 1% BSA. After adding of 100 μg/mL PI solution (3.8% Sodium Citrate, pH7.0) and 100 μL RNAse A (10 mg/ml) at 37 ℃ for 30 minutes in the dark environment, the cells were tested by flow cytometry. The data was analyzed by FCSExpress 3.0 software.

Caspase 3 activity detection

Caspase 3 activity was measured according to the manufacturer' s protocols. The cells were treated as above, digested by enzyme, and centrifuged at 600 g for 5 minutes at 4 ℃. The cells were then incubated with RIPA buffer on ice for 15 minutes and centrifuged at 20,000 g at 4 ℃ for 5 minutes to remove debris. After adding 2 mM Ac–DEVD–pNA, the cell lysate was tested at 405 nm to calculate caspase 3 activity.

Real time PCR

Total RNA was extracted from ACHN cells using Trizol reagents and reverse transcribed to cDNA according to the user manual. The primers used in the experiment are listed in Table 1. Real time PCR was run for 55 ℃, 1 minute, followed by 35 cycles of 92 ℃, 30 s, 58 ℃, 45 s, and 72 ℃, 35 s respectively. GAPDH was amplified as internal control. Relative gene expression was analyzed by the $2^{-\triangle Ct}$ method.

Table 1. Primer sequence

Gene	Forward, 5'–3'	Reverse, 5'–3'
GADPH	ACCAGGTATCTGCTGGTTG	TAACCATGATGTCAGCGTGGT
Survivin	GACTCTGTCCCAGTCCCAT	GCCTGGATTACATTAGCTATT

Western blot

The treated ACHN cells were incubated with RIPA buffer on ice for 15 ~ 30 minutes. The cells were then ultrasonicated at 4 x 5s and centrifuged at 10,000 × g for 15 minutes at 4 ℃. The supernatant was stored at −20 ℃. The protein was separated on 10% SDS–PAGE gel and transferred to PVDF membranes. After blocking with 1 × PBST buffer containing 5% skim milk for 2 hours, the membranes were incubated with survivin primary antibody (1:1000 dilution) overnight at 4 ℃. After washing with 1 × PBST buffer, the membranes were further incubated with secondary antibody (1:2000) at RT for 30 minutes. The membranes were then incubated with chemiluminescent agent for 1 minute and exposed to X–ray films. The densities of protein bands were scanned using Protein image processing system and analyzed by Quantity one software. All experiments were repeated for 4 times (n = 4).

Statistical analysis

Data were analyzed using SPSS16.0 software and presented as mean ± standard error ($\bar{x} \pm S$). One–way ANOVA was used for comparison analysis. $P < 0.05$ was considered as statistically significant.

Results

Quercetin inhibited ACHN cell proliferation

MTT assay showed that Quercetin significantly inhibited ACHN tumor cell proliferation compared to untreated control cells ($P < 0.05$). 40 μM Quercetin showed a more obvious suppressive effect than 20 μM Quercetin ($P < 0.05$). This suggested that Quercetin dose–dependently inhibited renal cancer cell proliferation (Fig. 1).

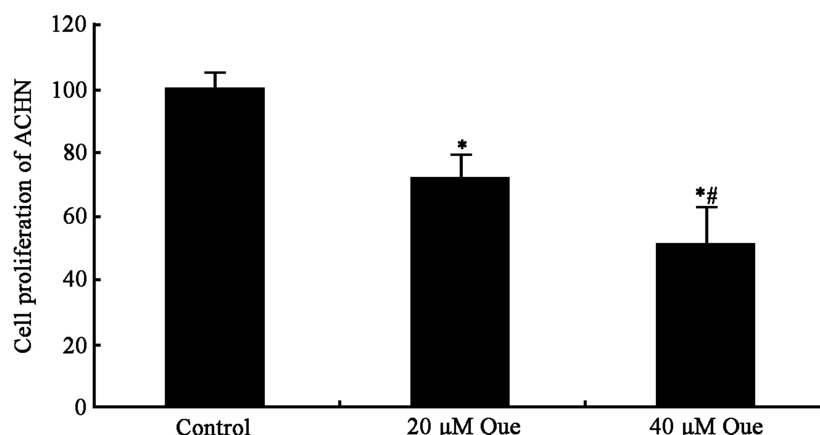

Figure 1. Que inhibits cell proliferation in ACHN cells. $^{*}P < 0.05$ *vs.* control; $^{\#}P < 0.05$ *vs.* cells treated with 20 μM Que. N = 3.

Quercetin induced ACHN cell apoptosis

Flow cytometry assay showed that Quercetin induced significant apoptosis in ACHN cells compared to untreated cells ($P<0.05$). 40 μM Quercetin exhibited more obvious effect than 20 μM Quercetin ($P<0.05$), which suggested that Quercetin can induce renal cancer cell apoptosis in a dose-dependent manner (Fig. 2A, 2B).

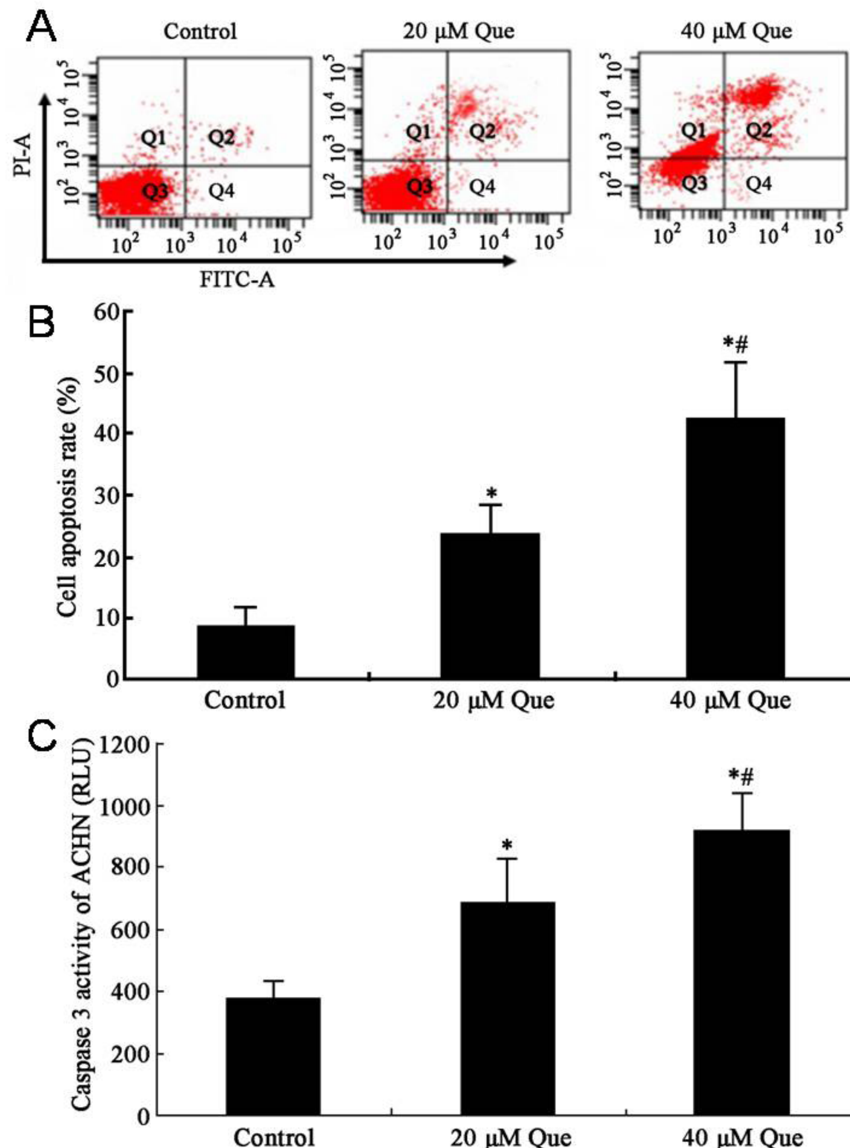

Figure 2. Que induced cell apoptosis inACHN cells. A) Representative of flow cytometry assay. B) Percentage of apoptotic cells. C) Que increased caspase 3 activity in ACHN cells. $^{*}P<0.05$ *vs.* control; $^{\#}P<0.05$ *vs.* cells treated with 20 μM Que. N = 4.

Quercetin increased Caspase 3 activity in ACHN cells

After treatment with different concentrations of Quercetin for 48 hours, caspase 3 activity was detected. Quercetin significantly increased caspase 3 activity compared to untreated control cells ($P<0.05$). 40 μM Quercetin showed more significant effect than 20 μM Quercetin ($P<0.05$), which suggested a dose-dependent effect of Quercetin on caspase 3 activity (Fig. 2C).

Quercetin inhibited survivin mRNA and protein expression in ACHN cells

Real time PCR revealed that Quercetin treatment significantly suppressed survivin mRNA and protein expression ($P<0.05$). 40 μM Quercetin showed more remarkable effect on survivin mRNA and protein expression than 20 μM Quercetin (Fig. 3).

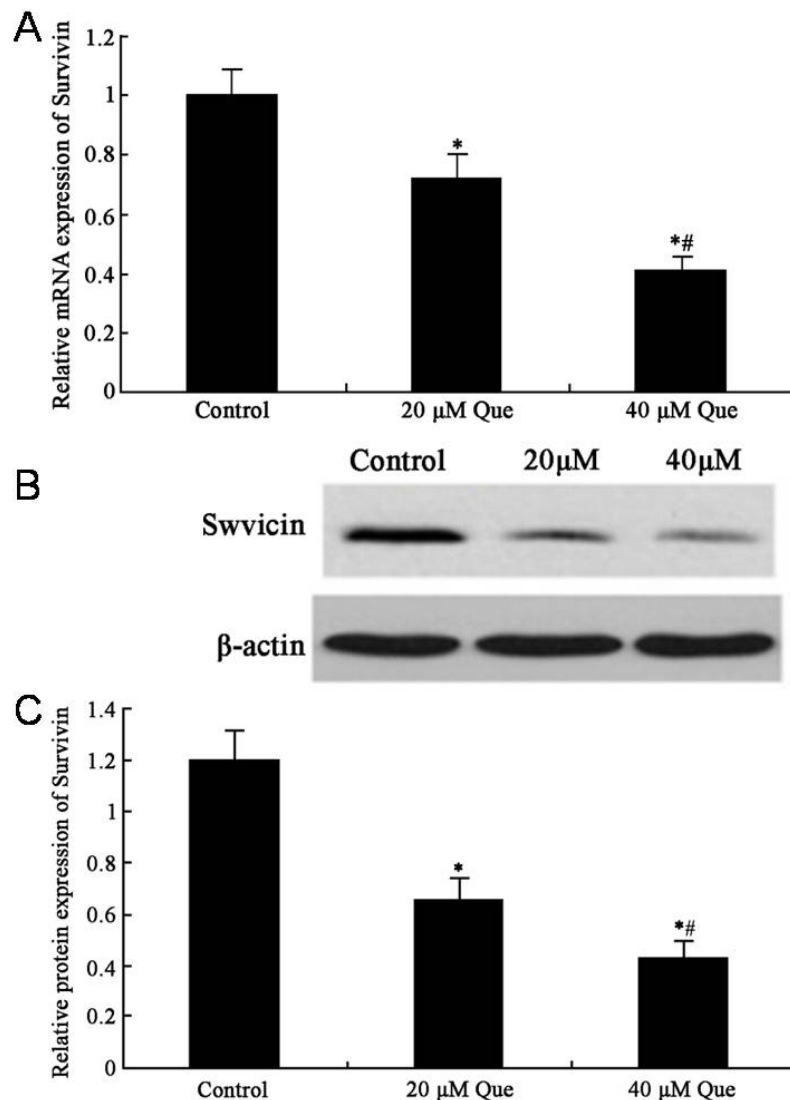

Figure 3. Que inhibited Survivin mRNA and protein expression in ACHN cells. A) Real-time PCR of survivin mRNA expression. $^{*}P<0.05$ *vs.* control; $^{\#}P<0.05$ *vs.* cells treated with 20 μM Que. N=4. B) Representative Western blot of survivin protein expression in ACHN cells. C) Semi-quantitative analysis of survivin protein expression in Western blot. $^{*}P<0.05$ *vs.* control; $^{\#}P<0.05$ *vs.* cells treated with 20 μM Que. N = 4.

Discussion

The anti-cancer effects of Quercetin have been widely reported in a variety of cancer cells, the effect of Quercetin in renal cancer cells remain uncertain though. The early studies observed a carcinogenic activity of Quercetin in animals' kidneys [14-18]. There is few recent studies have reported the anti-cancer effect of Quercetin in renal cancer cells [19-21]. This study demonstrated that Quercetin significantly inhibited cell proliferation and induced apoptosis in human renal cell adenocarcinoma cells. Quercetin exerted an apoptotic effect through inhibiting survivin mRNA and protein expression and activating caspase 3.

Quercetin is a naturally occurring flavonoid and can be extracted from food and plants. Quercetin was found to play anti-tumor effects through regulating tumor cell proliferation and apoptosis [22]. Moreover, Quercetin can specifically kill tumor cells with low toxicity to normal cells. Thus, Quercetin can reduce the side effects of traditional anti-tumor drugs. Currently, Quercetin has been putting through clinical trial as an anti-tumor agent [23]. Since renal cancer is more resistant to current anti-tumor drugs, finding new drugs to treat renal cancer with high efficiency and less side effects is important. However, there are few studies about the role of Quercetin in renal cancer. This study confirmed Quercetin's anti-cancer effect in renal cancer cells through inhibiting proliferation and inducing cancer cell apoptosis.

Caspase 3 is a one of the major regulatory factors of apoptosis activity. Previous studies have revealed that Quercetin can induce tumor cell apoptosis through increasing caspase 3 activity in liver cancer, nasopharyngeal carcinoma, and lung cancer [24, 25]. Survivin is an anti-apoptosis protein that can inhibit cell apoptosis and promote cell division. Survivin is highly expressed in many tumor tissues and inhibits caspase 3 activity in tumor cells through suppressing the release of mitochondrial cytochrome C. Also, survivin can restrain the hydrolytic effect of caspase 3 on spindles maintain cell integrity during mitosis, and reducing the inhibition of apoptosis and drug resistance [26]. This study has showed that Quercetin inhibited survivin mRNA and protein expression, and enhanced caspase 3 activity, which can be associated with Quercetin-induced apoptosis in renal cancer cells.

In conclusion, Quercetin induced renal cancer cell apoptosis possibly through inhibiting survivin expression and caspase 3 activation.

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