Gene 664 (2018) 50–57

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Metformin combined with quercetin synergistically repressed prostate cancer cells via inhibition of VEGF/PI3K/Akt signaling pathway

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ARTICLE INFO

Keywords:
Metformin
Quercetin
Prostate cancer
VEGF/Akt/PI3K
Apoptosis

ABSTRACT

The aim of present study was to examine whether metformin in association with quercetin has any synergistically anti-tumor effects on prostate cancer. Our findings showed that metformin in combination with quercetin synergistically inhibited the growth, migration and invasion of both PC-3 and LNCaP cells. Co-treatment of these two agents induced more apoptosis than single agent treatment. The co-treatment-induced apoptosis was caspase-dependent and accompanied by the down-regulation of Bcl-2 family members. Our data also indicated that co-treatment of metformin and quercetin strongly inhibited the VEGF/Akt/PI3K pathway. Moreover, these two agents acted synergistically to repress the growth of human prostate cancer cell xenograft in vivo in nude mice. In conclusion, our findings indicate that the combination therapy of metformin and quercetin exerted synergistic antitumor effects in prostate cancers via inhibition of VEGF/Akt/PI3K pathway. Thus, combination treatment of metformin and quercetin would be a promising therapeutic strategy for prostate cancer patients.

Abbreviation list

VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
PI3K	Phosphatidylinositol-4,5-bisphosphate 3-kinase
CRPCs	castration-resistant prostate cancers
z-VAD	carbobenzoxy-valyl-alanyl-aspartyl-[*O*-methyl]
CCK-8	Cell Counting Kit-8
DMSO	Dimethyl sulfoxide
ECL	enhanced chemiluminescence
PVDF	polyvinylidene difluoride
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
RIPA buffer	Radioimmunoprecipitation assay buffer
CI	combination index

1. Introduction

Prostate cancer is the second most common cancer in men worldwide, accounting for nearly 15% of all male cancers (Center et al., 2012). Over the past decades, many therapeutic strategies against prostate cancer have been developed such as androgen ablation therapy, radical prostatectomy and high dose radiotherapy 2. However, these treatments often failed in late stage and castration-resistant prostate cancers (CRPCs) (Hirst et al., 2012; Luo et al., 2015). Therefore, chemotherapy is still a vital option for advanced-stage patients. Currently, drugs such as docetaxel have been the first-line therapeutic agent against CRPC; however, the outcome is still unsatisfactory (Ryan and Tindall, 2011). Traditional chemotherapy is of limited use due to its low efficacy and side effects in the management of prostate cancer (Clarke, 2006). Thus, it is vital to develop novel chemotherapeutic agents to treat prostate cancer.

Metformin (1,1-dimethylbiguanide hydrochloride), a first-line therapeutic agent for type II diabetes mellitus, has recently been recognized as a potential anti-cancer agent due to its wide pharmacological effect and relatively low toxicity (Hirsch et al., 2009). Interestingly, amounting studies indicated that diabetic patients treated with metformin showed a decline incidence of carcinomas (Evans et al., 2005; Heckman-Stoddard et al., 2017). Experiments have shown that metformin inhibits cell proliferation, cell cycle progression and induce apoptosis of prostate cancer (Chen et al., 2016). Mouse xenograft data also suggests that metformin could prevent prostate tumorigenesis in vivo as well (Saha et al., 2015). Moreover, metformin has been shown to improve survival in patients with prostate cancer (Xiao et al., 2017). The antitumor effects of metformin suggest that it can be used as a novel agent in combined chemotherapy for the clinical treatment for

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https://doi.org/10.1016/j.gene.2018.04.045
Received 5 February 2018; Received in revised form 15 April 2018; Accepted 16 April 2018
Available online 18 April 2018
0378-1119/

prostate cancer.

Men at high risk for prostate cancer frequently supplement their diet with minerals, combinations of vitamins, fruit/seed extracts. Quercetin, a ubiquitous bioactive flavonoid, is one of the components of fruits and seeds that can inhibit the proliferation of various cancer cells (Sun et al., 2013; Yi et al., 2014). Quercetin is able to induce apoptosis and increase the activity of antioxidant enzymes in prostate cancer (Senthilkumar et al., 2010; Sharmila et al., 2014). In vitro and in vivo studies have found that quercetin can potentiate the efficacy of concomitant agents via enhancing their bioavailability as well as sensitizing tumor cells to chemotherapy agents (Gibellini et al., 2011; Fantini et al., 2015).

Plenty studies indicate that the combination of different chemotherapeutic agents may generate synergistic anti-tumor effects, resulting in less dose of each agent used in the combination and thereby reduced side effects compared with the single agent used. In previous studies, metformin has been examined in combination with agents such as valproic acid, simvastatin and vitamin D3 in prostate cancer cells and showed synergistic tumor inhibitory effects (Babcook et al., 2014; Li et al., 2015; Tran et al., 2017). However, the combinational effects of metformin and quercetin in prostate cancer have not yet been studied yet. Therefore, in the present study we utilize the PC-3 and LNCaP cells, and PC-3 xenograft mouse model to examine the combinational effects of metformin and quercetin on prostate cancer in vitro and in vivo, and found that these two agents act synergistically anti-tumor activity. Our findings may offer a promising new therapeutic approach in the treatment of prostate cancer.

2. Materials and methods

2.1. Reagents

Metformin, Quercetin, z-VAD, CCK-8, and DMSO were purchased from Sigma (St. Louis, MO, USA). Nucleosome ELISA kit was obtained from Oncogene Research Products (Cambridge, MA, USA). Caspase-3 activity kit (Beyotime, Shanghai, China). All other routine chemicals were purchased from Sigma.

2.2. Cell culture

Human prostate cancer cell lines PC-3 and LNCaP were obtained from ATCC (American Type Culture Collection). Both cells were cultured in RPMI1640 medium (Gibco, Gran Island, NY, USA) containing 10% FBS (Fetal Bovine Serum) (Gibco), 1% penicillin and streptomycin (Sigma, St. Louis, MO, USA) at 37 °C with 5% CO2.

2.3. Cell viability assay and combination index calculation

To assess cell viability, cells were seeded in 96-well plates at a density of 1 × 104 cells/well. Then cells were incubated with various doses of metformin, quercetin or their combination for 24 h. After treatment, 10 μl of CCK-8 solution (Sigma) was added to each well followed by incubation for 2 h at 37 °C. The cell viability was assessed by measurement of absorbance at 450 nm using a Microplate reader (BioTek, Winooski, VT, USA). The experiment was repeated at least three times. Combined Index (CI) was calculated using the CompuSyn, CI < 1 was considered as synergism, CI = 1 was considered as additive effect and CI > 1 was considered as antagonism.

2.4. Cell migration and invasion assay

Human prostate cancer cells PC-3, LNCaP were cultured to 95% confluence in 6-well plate. The adhesive cells were scratched using a 200 μl pipette tip to form wound gaps, cells were then washed three times with PBS to remove the debris. Fresh serum free medium containing metformin, quercetin, or their combination were added to 6-well plates, and the cells were allowed to repair the scratches for 24 h. Photos were taken at 0 and 24 h at the same place where was scratched. The cell migration distances were measured in three different areas to indicate the migration ability of treated cell. For invasion assay, 24-well BD Matrigel invasion chambers (8 μm pore, 6.5 mm polycarbonate, BD Biosciences, Bedford, MA, USA) were used according to the manufacturer's guide. Cells were re-suspended in 500 μl serum-free medium containing metformin, quercetin, or their combination and plated on the upper chamber, and 600 μl full medium including 10% FBS was added to the lower chamber. The transwell filters were put in a humidified incubator at 37 °C for 24 h. Afterwards, noninvading cells were removed from the top well with a cotton swab, the cells attached to the lower surface of membrane were fixed with 4% paraformaldehyde at room temperature for 30 min and stained with 0.5% crystal violet. Then the number of stained cells on the lower surface was counted using the microscope (magnification, 100 ×). A total of 5 fields were counted for each transwell filter.

2.5. Apoptosis assay

Cellular apoptosis was measured by Nucleosome ELISA Kit (Oncogene Research Products, Cambridge, MA, USA) and flow cytometry. For apoptosis nucleosome ELISA assay, cells were harvested and washed with PBS after treatment. Cells were lysed at room temperature for 15 min, and the lysates were centrifuged at 200 ×*g* for 5 min. Then 20 μl of the supernatant was transferred into 96-well plate and 80 μl of the provided reagents containing anti-histone conjugated with biotin and anti-DNA conjugated with peroxidase was added to each well. After incubation for 2 h at room temperature, plates were washed with wash buffer for three times. HRP catalyzes the conversion of colorless tetramethylbenzidine to blue and addition of a stop solution changes the color to yellow, the intensity of which is proportional to the number of nucleosomes in the sample. Quantitation was performed using ELISA via the construction of a standard curve. Absorbance was measured at dual wavelengths of 450/595 nm using the spectrophotometric plate. The values obtained are expressed as a ratio relative to corresponding untreated cells. For apoptosis detection by flow cytometry, the cells were stained with PI and annexin V-FITC (Invitrogen, Carlsbad, CA) and then determined by BD FACSVia™ flow cytometry system (BD bioscience, Franklin Lakes, NJ).

2.6. Caspase-3 activity assay

Caspase-3 activity was analyzed using the Caspase 3 Colorimetric Assay Kit (Beyotime Biotechnology, Shanghai, China) according to the manufacturer's guide. Briefly, PC-3 and LNCaP cells were cultured in 6-well plate and allowed to attach overnight. After drug treatment, cells were washed with PBS and lysed. The cell lysates were centrifuged at 13,000*g* for 15 min. The blank solution, containing 90 μl reaction buffer and 10 μl Ac-DEVD-pNA, and the sample solution, containing 75 μl reaction buffer, 15 μl sample, and 10 μl Ac-DEVD-pNA, were incubated in a 96-well microplate overnight at 37 °C. Absorbance was measured at 405 nm in a microplate reader.

2.7. Western blot analysis

Cells were lysed with RIPA buffer (Beyotime, Shanghai, China). Equal amounts of lysates (20 μg) were resolved by 12% SDS-PAGE and transferred to PVDF membrane. Primary antibodies were incubated with membranes at 4 °C overnight. The membranes were then washed three times with PBS, incubated for 1 h with the appropriate horseradish peroxidase-conjugated secondary antibodies, washed three times with PBS. Then signals were visualized by ECL reagent (Pierce, Rockford, IL). The following primary antibodies were used: anti-Caspase-3, anti-VEGF, anti-VEGFR, anti-phospho-Akt, anti-Akt (all from Cell Signaling Technology, Danvers, MA, USA). Anti-Bcl-2, anti-Bcl-xl,

anti-Mcl-1, anti-phospho-PI3K, anti-PI3K, (all from Abcam, San Diego, CA, USA). Anti-GAPDH (Sigma). Goat anti-rabbit IgG-HRP or anti-mouse IgG-HRP used as the second antibodies (Cell Signaling Technology). Intensity of individual band was quantified using ImageJ densitometry software (NIH, USA), and GAPDH was used as the loading control.

2.8. Xenograft mouse model

The animal study was approved by Ethics Committee on Animal Research of Affiliated Hospital of Ningbo Medical University. Male nude mice (BALB/c, 4–5 weeks old) were purchased from the Shanghai Laboratory Animal Center, Shanghai Institute for Life Sciences, Chinese Academy of Sciences (Shanghai, China). Exponentially growing PC-3 cells (1×10^6) were injected subcutaneously into the flank of mouse to obtain prostate cancer xenografts. The mice were divided into four groups (6–10 mice/group). When the size of tumors reached to 6–9 mm diameter, the tumor-bearing mice were randomly divided into four groups. Mice in the control group received an equal volume of normal saline. Mice in the experimental groups were intraperitoneally injected with metformin, quercetin, or their combination. The sizes of tumor were periodically measured by calipers, and the volume was obtained by following formula: tumor volume (mm^3) = maximal length (mm) × (perpendicular width) $(mm)^2/2$. At the end of study, the mice were killed, and the tumors were removed for further measurement of weight.

2.9. Statistical analysis

Statistical analysis was performed using the SPSS software (Chicago, IL, USA). Data are expressed as mean ± SD. Differences among groups were tested by one-way ANOVA. A value of $P < 0.05$ was considered as significantly different.

3. Results

3.1. Co-treatment of metformin and quercetin exerts synergistic cytotoxicity against prostate cancer cells

In order to determine the potential anti-tumor activity of metformin and quercetin in combination, we firstly co-treated prostate cancer cell lines PC-3 and LNCaP with various doses of metformin (Met) (10–40 μM) and quercetin (Que) (10–40 μM) for 48 h. Cell viabilities were assayed by CCK-8 assay. As indicated in Fig. 1A, metformin or quercetin treatment alone resulted in a dose-dependent growth inhibition of both PC-3 and LNCaP cells. However, co-treatment of metformin with quercetin significantly enhanced the growth inhibition compared to single agent treatment. The CI (combination index) values of all doses combination were less than one, suggesting that the growth inhibitory effect of metformin and quercetin in combination was synergistic rather than additive or antagonistic (Fig. 1B). Since metformin at 40 μM and quercetin at 20 μM used alone caused slight cytotoxicity, however, combination of these two doses showed significantly synergistic effects. Therefore, these two doses of metformin and quercetin were used for latter experiments.

3.2. Combining metformin with quercetin shows strong synergistic inhibition of migration and invasion of prostate cancer cells

Cell migration is a key characteristic of tumor cells. To investigate the effects of the metformin and quercetin combination on human prostate cancer cell migration and invasion, we used wound-healing and transwell assays to measure the effects of the metformin and quercetin combination on prostate cancer cells PC-3 and LNacP. As shown in Fig. 2A, Cells treated with metformin or quercetin slightly inhibit the migration when compared with the untreated controls but we observed almost complete inhibition of cell migration after treatment of both cells with combination of metformin and quercetin. Then we evaluated changes in cell invasion after the single and combination treatments with metformin and quercetin. Matrigel transwell assay demonstrated that combination of metformin and quercetin showed almost complete inhibition of tumor cell invasion when compared with either treatment alone.

3.3. Metformin and quercetin synergistically induce apoptosis in prostate cancer cells

Then we evaluated whether metformin and quercetin treatment induced apoptosis in prostate cancer cells. After treated with metformin, quercetin or their combination for 48 h, cells were then subjected to apoptosis ELISA assay as described earlier (Larisch et al., 2000) and flow cytometry analysis. In both prostate cancer cells, we observed that the combination of metformin and quercetin induced more apoptosis than treatment of metformin or quercetin alone (Fig. 3A, B). Then the apoptosis related proteins like caspase-3, Mcl-1, Bcl-2 and Bcl-xl proteins were examined by the western blots. The results show that co-treatment of metformin and quercetin lead to more cleavage of caspase-3 (Fig. 3C). Meanwhile, the anti-apoptotic Bcl-2 proteins Mcl-1, Bcl-2 and Bcl-xl were significantly repressed by the combination of metformin and quercetin (Fig. 3C). Moreover, the activation of caspase-3 was assayed by the caspase-3 activity assay. In line with the western blot results, the combination of metformin and quercetin led to more activation of caspase-3 than the treatment of metformin or quercetin alone (Fig. 3D). In addition, the apoptosis induced by co-treatment was effectively inhibited by the pan-caspase inhibitor (z-VAD) (10 μM) in both prostate cancer cell lines (Fig. 3E). Taken together, these results demonstrated that metformin and quercetin in combination synergistically induce apoptosis in prostate cancer in a caspase-dependent manner.

3.4. Co-treatment of metformin and quercetin synergistically inhibited the activity of VEGF/PI3K/Akt signaling pathway in the prostate cancer cells

To explore the molecular mechanisms, we performed western blotting to measure the expression levels of p-Akt, Akt, p-PI3K, PI3K VEGFR2 and VEGF in PC-3 and LNCaP cells which were treated by metformin, quercetin alone and in combination. As shown in Fig. 4, the expressions of p-Akt, p-PI3K, VEGFR2 and VEGF protein were markedly repressed in the combination treatment group compared with the single treatment group, while the total Akt, PI3K remained the same. Those data indicated that the VEGF/PI3K/Akt signaling pathway was inhibited by the combination of metformin and quercetin.

3.5. Combining metformin and quercetin has synergistic effect against the growth of prostate cancer cell xenograft in nude mice

To investigate the combinational effect of metformin and quercetin on tumor growth in vivo, we established a xenograft model in BALB/c nude male mice injected with PC-3 cells and then treated with metformin (500 mg/kg body weight) and quercetin (600 mg/kg body weight), alone or in combination, for 40 days. As shown in Fig. 5A and B, both the tumor volume and weight in the combination treatment group was significantly less than in the single-agent treatment groups, suggesting that the combination of metformin and quercetin significantly suppressed the growth of PC-3 cells in BALB/c mice. After 40 days of treatments, the final body weights in tumor-bearing mice remained almost unchanged by the administration of metformin, quercetin or their combination compared with the control group (Fig. 5C). We also measured caspase-3, Mcl-1, Bcl-2 and Bcl-xl protein expression in xenograft tumor tissues. Compared with the single-agent treatment groups, the expression of pro-caspase-3, Mcl-1, Bcl-2 and Bcl-xl was significantly decreased, while the expression of cleaved caspase-

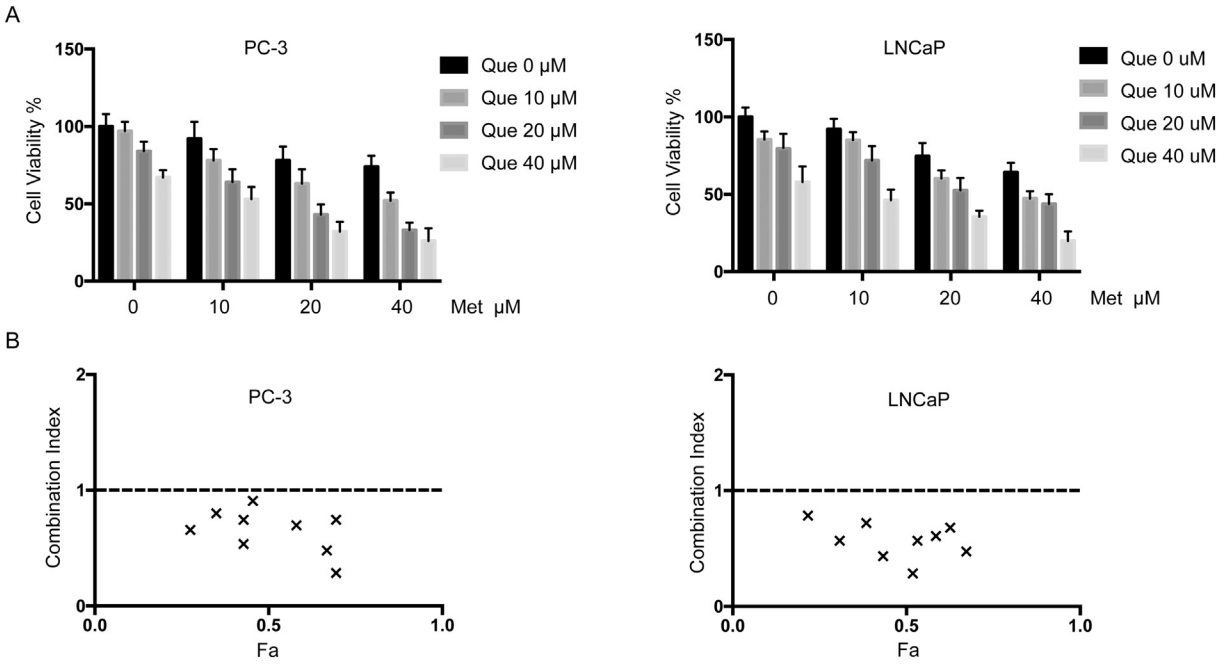

Fig. 1. Co-treatment of metformin and quercetin exerts synergistic cytotoxicity against prostate cancer cells.
A. PC-3 and LNCaP cells were treated with indicated doses of metformin (Met) and quercetin (Que) alone or in combination for 48 h. Cell viability was detected by CCK-8 assay. (B) Combination Index for PC-3 and LNCaP cells were analyzed by CompuSyn software. CI $<$ 1, CI = 1 and CI $>$ 1 stand for synergistic, additive and antagonistic effects, respectively. "Fa" refers to inhibitory rate. All data are shown as the mean $\pm$ SD of three independent experiments.

3 remarkably increased in the combination treatment group, which is consistent with the in vitro results. These findings suggest that the combination of metformin and quercetin significantly inhibits tumor growth in mice than the single agent treatment.

4. Discussion

Amounting evidence indicated that metformin, an agent that reduces blood glucose levels for type II diabetes and exhibits anti-tumor action in various cancer cell lines (Zakikhani et al., 2006; Gotlieb et al., 2008). In addition, plenty of evidence has emerged that quercetin inhibits the tumorigenesis of many different cancer cells (Brito et al., 2015). Many studies indicated that metformin or quercetin combined with other anti-tumor agents has an additive or synergistic anti-tumor effect on various cancer cell lines. However, there is little information about the combinational effects of metformin and quercetin on prostate cancer cells. In the current study, we assessed the synergistic inhibitory effect of metformin combined with quercetin on the proliferation of PC-3 and LNCaP prostate cancer cells as well as their action mechanisms.

It is well recognized that combination therapies consisting of anti-tumor agents with different mechanism of action result in synergistic effect that is more effective than monotherapy (Mandrekar, 2014; Webster, 2016). Since both metformin and quercetin possess the ability to repress the tumorigenesis of prostate cancer via different mechanisms (Baruah et al., 2016; Whitburn et al., 2017). Therefore, we hypothesized that combination of metformin and quercetin could have synergistic effects against human prostate cancer cells. Indeed, we found that the combination of metformin and quercetin exhibited markedly higher inhibition than either metformin or quercetin alone on growth of human prostate cancer cell lines PC-3 and LNCaP in vitro, as well as growth of PC-3 xenograft tumor in vivo. In our experiments, metformin (10–40 μM) and quercetin (10–40 μM) treatment alone resulted in a dose-dependent inhibition of cell viability of PC-3 and LNCaP cells. Meanwhile, co-treatment of metformin and quercetin at the same range of doses caused significantly more inhibition of both cell lines than the agents treated alone. Besides, all CI values of these agents in combination were smaller than one, indicating that these two agents in combination markedly exerted synergistic growth inhibitory effects on PC-3 and LNCaP cells. In addition, our findings showed that both metformin and quercetin could inhibit the migration and invasion ability of PC-3 and LNCaP cells, while the combinative treatment exerted more significant inhibitory effect.

We investigated the underlying mechanisms by which the metformin/quercetin combination inhibits cancer cell growth. Apoptosis, one major form of programmed cell death, can be divided into two types: caspase-dependent and caspase-independent (Kitanaka and Kuchino, 1999). In most cases, apoptosis is undergoing in a caspase-dependent manner via the extrinsic or intrinsic pathways. During apoptosis, cells are characterized by shrinkage, chromatin condensation, blebbing of plasma membrane, and nuclear condensation without cell lysis (Burke, 2017). At the molecular level, intrinsic apoptosis is subjected to the regulation of Bcl-2 family members. In the present study, we found that metformin at 40 μM and quercetin at 20 μM treatment alone slightly induce apoptosis ($<$ 10%) in both prostate cancer cells, however, combination treatment synergistically enhanced apoptosis to $>$ 20% in both cell lines. In addition, we observed that the cleavage and activities of caspase-3 were also significantly increased in both cell lines co-treated with these two agents comparing to that of either agent alone. Meanwhile, we also observed that the co-treatment of metformin and quercetin greatly decreased the expression of anti-apoptotic Bcl-2 proteins Mcl-1, Bcl-2 and Bcl-xl. Interestingly, addition of pan-caspase inhibitor z-VAD greatly diminished the apoptosis-inducing effects of metformin and quercetin. Our findings indicated that the synergistic pro-apoptotic activities of co-administration of metformin and quercetin might via inducing caspase-dependent intrinsic pathway. Our findings are in line with previous studies when either compound used alone (Granado-Serrano et al., 2006; Isakovic et al., 2007).

VEGF is a vital target for the inhibition of tumor vascularization and tumor development (Kim et al., 1993). There are three VEGF receptors: VEGFR1, VEGFR2 and VEGFR3. Among them VEGF-VEGFR2 is the dominant signal pathway that regulate tumor angiogenesis. Inhibition of this pathway could repress metastases, proliferation and induce

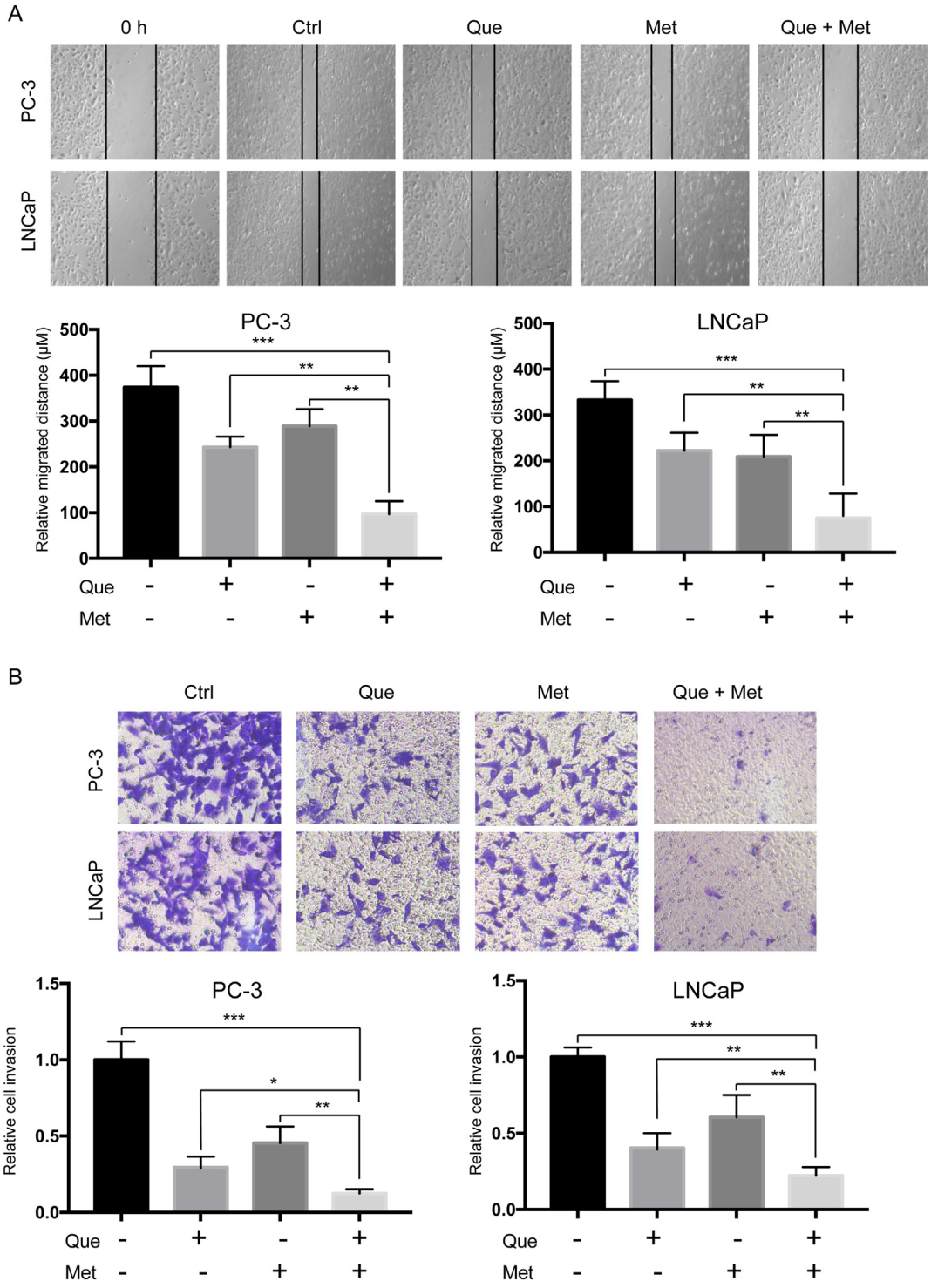

Fig. 2. Metformin and quercetin suppressed migration and invasion ability of PC-3 and LNacP cells.
A. Represent images of wound healing after 24 h treatment with metformin (Met), quercetin (Que) alone and their combination. Histograms show the average migrated distance. B. Represent images of transwell assay after 24 h treatment with metformin (Met), quercetin (Que) alone and their combination. Histograms show the relative amount of invasion cells. All data are shown as the mean ± SD of three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

apoptosis in cancer cells (Verheul and Pinedo, 2000). Therefore, targeting VEGF/VEGFR2 is a potential anti-angiogenic strategy for cancer therapy.

Among the various downstream targets of VEGFR2, PI3K/Akt signaling pathway is an important carcinogenic pathway that involved in the tumorigenesis of various cancers. In the present study, co-treatment of metformin and quercetin significantly reduced expression of VEGF, VEGFR2, p-PI3K and p-Akt proteins, and total PI3K and Akt proteins remained almost unchanged. Therefore, metformin and quercetin synergistically induce apoptosis was likely via inhibition the VEGF/PI3K/Akt signaling pathway. In previous studies, metformin has been found to reduce VEGF level in type 2 diabetic patients (Ersoy et al., 2008). Quercetin was also able to inhibit VEGFR2 and its downstream targets (Pratheeshkumar et al., 2012). Our study further confirmed these

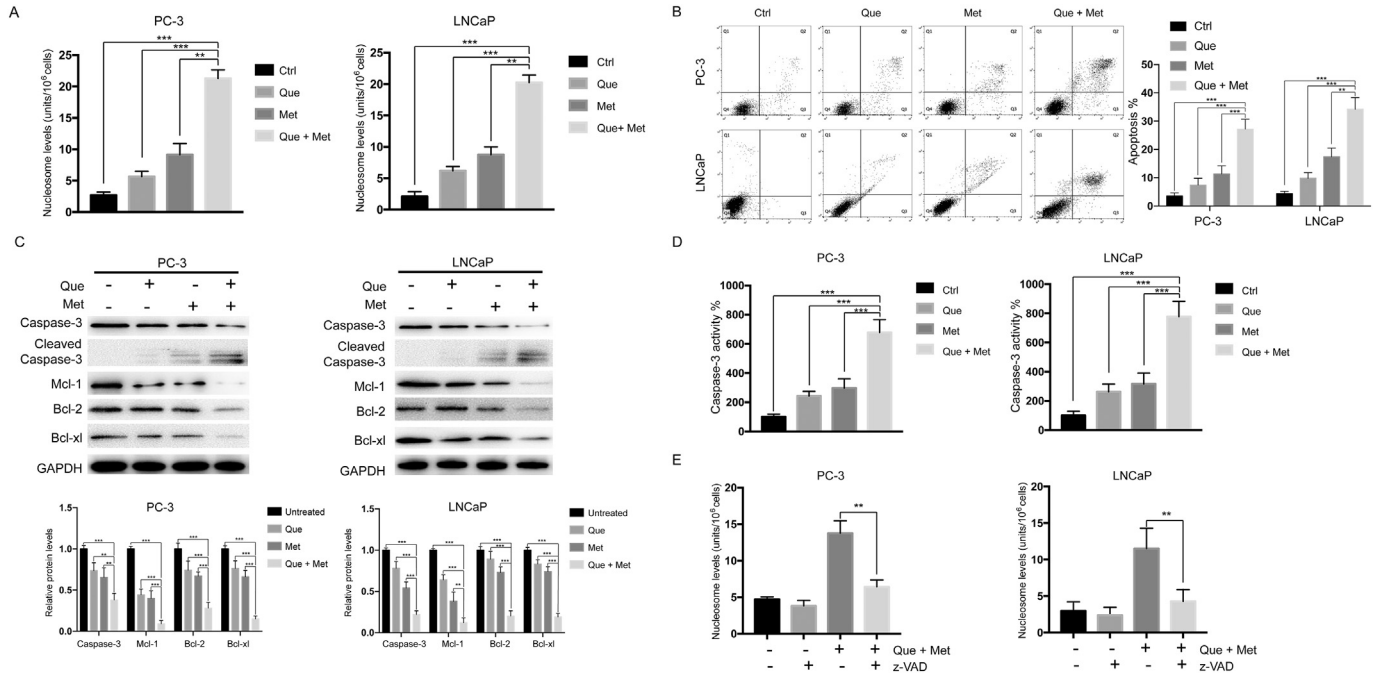

Fig. 3. Co-treatment of metformin and quercetin induce apoptosis in prostate cancer cells.
A. PC-3 and LNCaP cells were treated with metformin (Met) and quercetin (Que) alone or in combination for 48 h, then cells were subjected to apoptosis ELISA assay. B. PC-3 and LNCaP cells were treated with metformin (Met) and quercetin (Que) alone or in combination for 48 h, then cells were subjected to flow cytometry analysis. Histograms show the quantification of flow cytometry results. B. After treatment, cells lysates were subjected to western blot assay with indicated antibodies. Histograms show the densitometric analysis of western blot results. C. After treatment, cells were subjected to caspase-3 activity assay. D. PC-3 and LNCaP cells pretreated with z-VAD (10 μM) for 1 h and then treated with metformin (Met) and quercetin (Que) in combination for 48 h for apoptosis ELISA assay. All data are shown as the mean ± SD of three independent experiments. $^{**}P < 0.01$; $^{***}P < 0.001$.

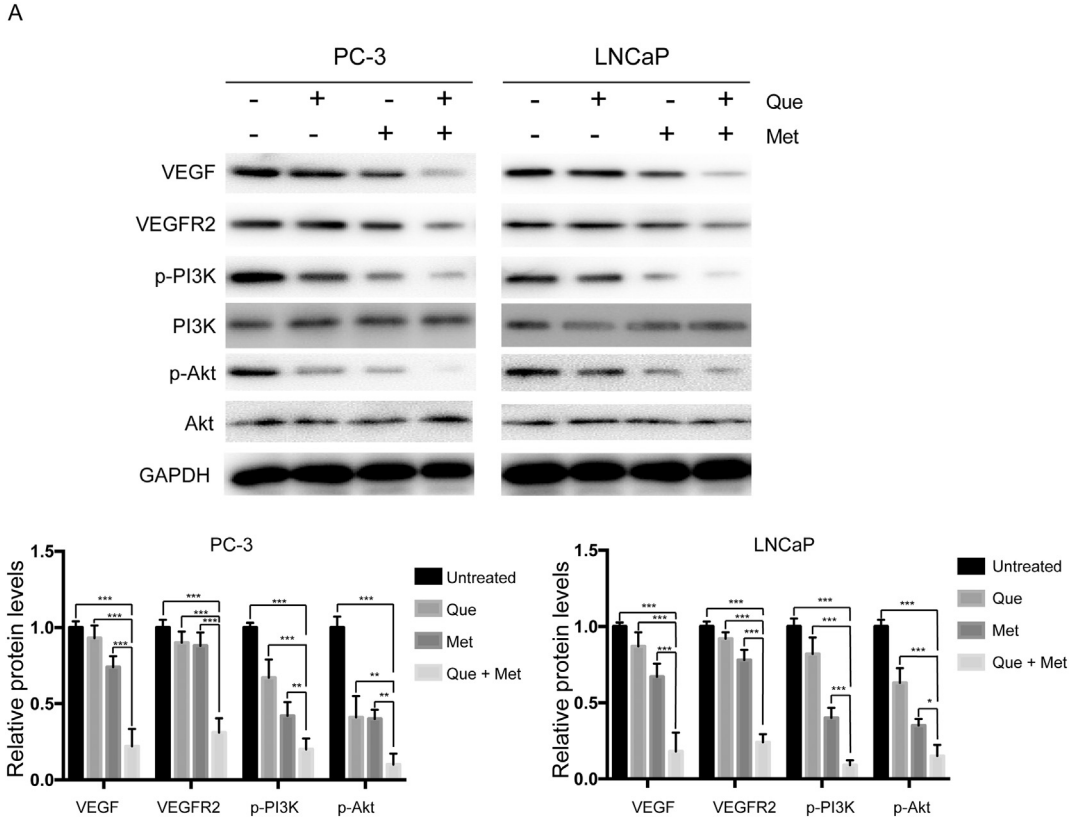

Fig. 4. Co-treatment of metformin and quercetin inhibited the VEGF/PI3K/Akt signaling pathway.
A. PC-3 and LNCaP cells were treated with metformin (Met) and quercetin (Que) alone or in combination for 48 h, then cellular lysates were subjected to the western blot analysis with indicated antibodies. Histograms show the densitometric analysis of western blot results.

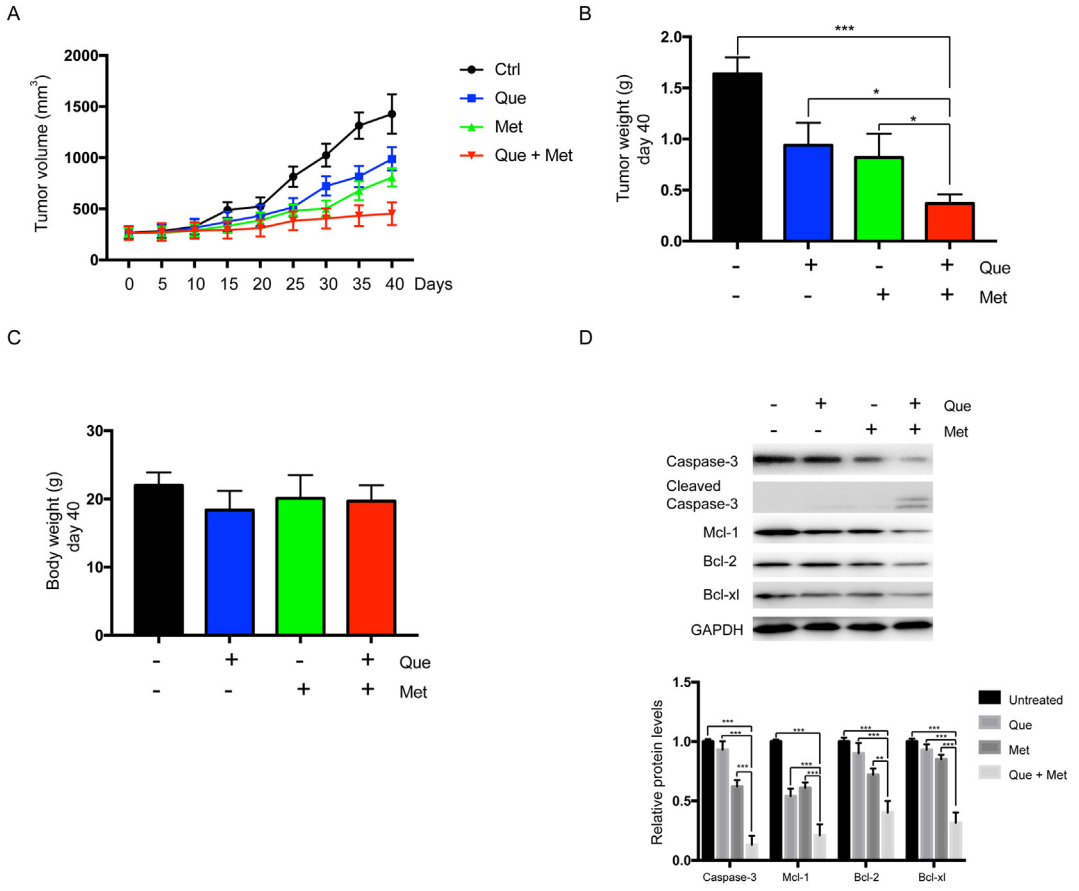

Fig. 5. Inhibition of tumor growth in mouse model by metformin and quercetin, alone or in combination.
A. Tumor volume from homografts in different treatment groups was recorded every 5 days. B. Tumor weight was detected at the time of killing for different treated groups. C. The mice body weight of different groups was measured at the time of killing. D. Western blot analysis of indicated proteins in different groups. Histograms show the densitometric analysis of western blot results. Data are represented as the mean ± SD. $^{**}P < 0.01$; $^{***}P < 0.001$.

findings.

Moreover, in vivo study found that both metformin and quercetin could induce tumor inhibition, especially when combination treatment was applied. Moreover, co-treatment of metformin and quercetin significantly lead to the cleavage of caspase-3 and downregulation of anti-apoptotic Bcl-2 proteins Bcl-2, Mcl-1 and Bcl-xl, which was consistent with the findings in vitro.

In summary, our present study reveals that the combination of metformin and quercetin could synergistically inhibit proliferation, migration, invasion and induce apoptosis in prostate cancer cells. The synergistic effects of metformin and quercetin may be associated with the inactivation of VEGF/PI3K/Akt signaling pathway. Moreover, co-administration of metformin and quercetin synergistically reduce tumorigenicity in vivo. Therefore, the combination of metformin and quercetin may be an efficient strategy to achieve antitumor synergism in the clinical treatment of prostate cancer, which needs further investigation in a clinical setting.

Acknowledgements

This study is supported by the Ningbo Natural Science Foundation (2017A610191).

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