PF-04691502 triggers cell cycle arrest, apoptosis and inhibits the angiogenesis in hepatocellular carcinoma cells

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HIGHLIGHTS

- PF-04691502 induces cell cycle arrest through regulating cyclinD1 and p27.
- PF-04691502 triggers apoptosis via a mitochondrial pathway.
- Growth factors-induced tube formation and migration of HUVECs are inhibited by PF-04691502 treatment.
- PF-04691502 inhibits the expression of VEGF and HIF-1α.

ARTICLE INFO

Article history:
Received 25 February 2013
Received in revised form 12 April 2013
Accepted 22 April 2013
Available online 29 April 2013

Keywords:
PF-04691502
PI3K/Akt
Cell cycle
Apoptosis
Angiogenesis

ABSTRACT

Hepatocellular carcinoma (HCC) is a major cause of morbidity and mortality in the world. The aim of the present study is to determine the antitumor effect of PF-04691502, a potent inhibitor of PI3K and mTOR kinases, on the apoptosis and angiogenesis of the hepatoma cancer cells. Our results indicate that treatment of cancer cells with PF-04691502 reduces cell viability and inhibits cell growth in a dose-dependent manner. PF-04691502 triggers apoptosis via a mitochondrial pathway, accompanied by activation of caspase-3, caspase-9, and poly(ADP-ribose) polymerase (PARP). Pre-treatment of hepatoma cells with the caspase-3 inhibitor (z-DEVD-fmk) blocks the PF-04691502-induced death of these cells. In addition, growth factors-induced tube formation and the migration of HUVECs are markedly inhibited by PF-04691502 treatment. The mechanisms of anti-angiogenesis of PF-04691502 are associated with inhibiting the expression of VEGF and HIF-1α. Based on the overall results, we suggest that PF-04691502 reduces hepatocellular carcinoma cell viability, induces cell apoptosis, and inhibits cell growth and tumor angiogenesis, implicating its potential therapeutic value in the treatment of HCC.

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1. Introduction

Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related deaths worldwide, with the average annual incidence on the rise both in China and abroad (Malucco and Covey, 2012). Discoveries of genomic and molecular markers in hepatocellular carcinoma have improved understanding the complexity of the signal transduction pathways in liver cancer cells. The phosphatidylinositol-3 kinases (PI3K)/Akt signaling pathway is central to cell growth and survival, entry into the cell cycle, and regulate cell death. Activation of this signaling pathway plays an important role in a variety of human cancers, including HCC (Fu et al., 2011; Zhou et al., 2012). PI3Ks are a group of lipid kinases consisting of a catalytic and a regulatory subunit, which are activated by signaling inputs transmitted to the inner cell after growth factor ligand binding to RTK such as EGFR or IGF (Psyrri et al., 2012). Once activated, PI3K catalyzes the production of the lipid secondary messenger phosphatidylinositol-3,4,5-triphosphate (PIP3), which in turn activates a wide range of downstream targets, including the Akt (also known as protein kinase B) (Zhou et al., 2011; Sadeghi and Gerber, 2012). Akt is a serine/threonine protein kinase which is activated by a number of growth factors in a PI3K-dependent manner. Activated Akt can promote protein synthesis through increasing the phosphorylation of the mammalian target of rapamycin (mTOR) kinase (Bibollet-Bahena and Almazan, 2009). There are two distinct mTOR complexes, mTORC1 and mTORC2, and the latter is involved in a feedback activation of the cascade through the phosphorylation of Akt on the Ser473 residue (Sheppard et al., 2012; Hers et al., 2011).
PI3K/Akt signaling pathway plays a major role in regulation of vasculature and angiogenesis (Shijoina and Walsh, 2006; Jiang and Liu, 2008). Angiogenesis is the formation of new blood vessels from preexisting ones, which is indispensable for growth, development, and wound healing. However, angiogenesis is also a fundamental step in tumor growth and metastasis. Tumor angiogenesis occurs by sprouting the new vessels from preexisting blood vessels or by inserting interstitial tissue columns into the lumen of pre-existing vessels, which is associated with an imbalance between pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and endogenous anti-angiogenic factors such as angiotatin (Weis and Cheresh, 2011). VEGF is the first member of angiogenic factors to be cloned and remains as the best-characterized angiogenic growth factor. One of the most potent stimuli for increased VEGF production by tumor cells is hypoxia, a common feature of the tumor microenvironment (Xu et al., 2010). In rapidly growing tumor tissues, critical mediator of the hypoxic response is the hypoxia-inducible factor 1 (HIF-1). This heterodimeric basic-helix-loop-helix transcription factor has been shown to have several transcriptional targets including the VEGF (Ahlualwala and Tarnawski, 2012; Zhang et al., 2010). It has been shown that PI3K/Akt signaling pathway regulates VEGF and HIF-1α expression, and inhibitors targeting the PI3K/Akt decrease the angiogenesis and VEGF secretion (Lee et al., 2013; Park et al., 2011).

The prevalence of PI3K/Akt signaling abnormalities in human cancer cells has suggested the potential use of PI3K/Akt pathway modulators as novel targeted therapeutic agents. Thus, inhibitors of this signaling pathway are being actively developed over the last few years. Wortmannin and LY294002 are two first-generation PI3K inhibitors which have been used as research tools to elucidate the value of PI3K as a therapeutic target, but whose lack of specificity to specific PI3K isoforms and considerable toxicity in animals limit their clinical usefulness (Kurtz and Ray-Coquard, 2012; Marone et al., 2008). In recent years, new compounds targeting PI3K, mTOR, or both have been increasingly developed and some of them are currently tested in phase I/II trials (Karar et al., 2012; Wang et al., 2012).

In this study, we investigate the antitumor activity of PF-04691502, a potent ATP competitive PI3K/mTOR dual inhibitor, in vitro models of hepatocellular carcinoma. We demonstrate that PF-04691502 inhibits the proliferation and induces the apoptosis of human hepatoma cell lines through activation of caspase. Moreover, PF-04691502 shows inhibitory properties against angiogenesis by inhibiting the expression of VEGF and HIF-1α. Our results indicate that the PI3K/mTOR dual inhibitor PF-04691502 is a potent inhibitor of hepatoma carcinoma and might represent a promising drug in the therapy of hepatocellular carcinoma.

2. Materials and methods

2.1. Reagent

PF-04691502 was obtained from Selleck Chemicals (Houston, USA), and dissolved with DMSO. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Genview (Houston, USA). Propidium iodine (PI) was purchased from Sigma–Aldrich (St. Louis, USA). VEGF was obtained from Pepro Tech (NJ, USA). Annexin V-FITC apoptosis detection kit was purchased from KeyGEN Biotech (Nanjing, China). Caspase-3 inhibitor (z-DEVD-fmk) was purchased from Santa Cruz Technology (CA, USA). ECL Western blotting reagents was purchased from Thermo Scientific Pierce (Rockford, USA).

Antibodies specific to β-actin was obtained from Sigma–Aldrich, Rabbit anti-Akt, p-Akt, caspase-9, caspase-3, and PARP polyclonal antibodies were purchased from Cell Signaling Technology (Shanghai, China). Mouse anti-cyclinD1, PI3K, and rabbit anti-VEGF were purchased from Santa Cruz Technology. Rabbit anti-HIF-1α was obtained from Bioswold Technology (St. Louis, USA). Mouse anti-p27 was purchased from BD Biosciences (California, USA) and p-STAT3 was purchased from Signalway Antibody (Shanghai, China).

2.2. Cells and cell culture

HepG2 cell line was obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Bel7402 cell and HUVEC were obtained from the KeyGEN Biotech. HepG2 cells and Bel7402 cells were cultured in RPMI 1640 medium containing 10% fetal calf serum (GIBCO, USA), 100U/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere (37 °C; 5% CO2). HUVECs were grown in M199 containing 20% fetal bovine serum (Biowest, Rue de la Caille, France), 20 ng/ml fibroblast growth factor.

2.3. Cell viability and cell colony formation assay

Cell viability was measured by MTT assay. Briefly, cells was added to 96-well plates and incubated with PF-04691502 for 24 h. An amount of 20 μL MTT (5 mg/mL) was added and incubated for 4 h. After removing supernatant, 150 μL dimethyl sulfoxide (DMSO) was added to resolve formazan crystals, and the value of optical density (OD) was detected at 490 nm. Cell colony formation assay was performed in 6-well cell culture plates. HepG2 cells were seeded at 1500 cells per well and treated with either dimethyl sulfoxide or PF-04691502 for 2 weeks. Cells were fixed in 1% glutaraldehyde, and stained with 0.5% crystal violet (Sigma) in methanol. Colonies with >30 cells were counted under an inverted microscope at low magnification.

2.4. Cell cycle analysis

Cellular DNA content was determined by flow cytometry. After incubation with PF-04691502 for 24 h, cells were harvested, washed twice with ice-cold PBS, fixed with 70% ethanol for 18 h at 4 °C. Cells were then washed twice with ice-cold PBS and resuspended in PBS. RNA was digested with RNase A (10 μg/mL) and DNA was stained with PI (10 μg/mL). Stained cells were analyzed by a FACScan flow cytometry and CellQuest analysis software (Becton Dickinson, San Jose, CA).

2.5. Annexin V-FITC/PI staining

To assess the extent of apoptosis induction after treatment with PF-04691502, a flow cytometric analysis of Annexin V-FITC/PI-stained samples was performed according to the manufacturer’s instructions. Samples were analyzed with the FACScan flow cytometry and CellQuest analysis software (Becton Dickinson).

2.6. Endothelial tube formation assay in vitro

Angiogenesis was determined using MatrigelTM matrix (BD-354234, Bedford, MA), a solubilized basement membrane preparation including various growth factors. The tube formation assay has been reported elsewhere (Fei et al., 2012). Tube-forming activity was estimated by counting the number of complete capillaries connecting individual points of the polygonal structures in a light microscope 8 h after transferring the cells to Matrigel.

2.7. Migration assay

The HUVEC migration assay was performed using the transwell apparatus (8.0 μm pore size, BD). HUVECs (5 × 10^5 cells/mL) were incubated with or without indicated concentrations of PF-04691502, then was performed as previously described (Wang et al., 2012).

2.8. Enzyme-linked immunosorbent assay (ELISA)

For the measurement of VEGF, HepG2 cells were seeded in 12-well plates and grown to 75–80% confluence, then cells were switched to fresh serum-free medium in the presence or absence of PF-04691502 and incubated for another 12 h. Cell-free culture supernatants were harvested and assayed for secreted VEGF using commercially available ELISA kits (R&D Systems, Minneapolis, MN, USA).

2.9. Western blotting assay

After cells were lysed with ice-cold lysis buffer (150 mmol/L NaCl, 20 mmol/L Tris–HCl, pH 7.4, 0.1% SDS, 1% NP-40, 0.5% Na-DOC, 0.2 mmol/L PMSF, and protease inhibitor cocktails) for 30 min on ice, lysates were centrifuged at 13,000 × g with 20 min and the supernatants were used as total cell lysates. Protein concentration was determined by Bradford protein assay (Bio-Rad, USA). A quantity of 30 μg total protein per lane was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Bedford, USA). Membranes were blocked with 5% milk powder in 0.05% Tween-20, incubated with the specific antibodies. Detection of the target proteins on the membranes was performed using the ECL Western Blotting Detection Reagents.

2.10. Statistical analysis

All data were expressed as mean ± SD. Statistical analysis was performed by student’s t-test. P < 0.05 was indicated to be statistical significant.
3. Results

3.1. PF-04691502 inhibits Akt phosphorylation and PTEN expression in human hepatoma cells

Since PF-04691502 is claimed to be a PI3K/mTOR inhibitor, we perform western blotting analysis to show the inhibitory effect of it on the phosphorylation of Akt, a key mediator of PI3K/Akt/mTOR pathway in hepatoma cancer cells. As shown in Fig. 1, incubation with PF-04691502 significantly reduced the amount of phosphorylated Akt (p-Akt547), whereas no change in total Akt levels was observed showing that PF-04691502 has no effect on total Akt protein expression. The tumor suppressor phosphatase and tensin homolog deleted on chromosome 10 (PTEN) negatively regulates the PI3K pathway by dephosphorylating PIP3 to PIP2. Therefore, the expression of PTEN was also detected in PF-04691502-treated hepatoma cells. PF-04691502 was shown to upregulate the PTEN expression both in HepG2 cells and in Bel7402 cells.

3.2. PF-04691502 inhibits hepatoma cells proliferation and induces cell cycle arrest in G1 phase

To investigate the effects of PF-04691502 on the growth of hepatocellular carcinoma cell, MTT assay was used to evaluate the cell viability. The obtained results showed that PF-04691502 significantly decreased the viability of HepG2 cells in a dose-dependent manner (IC50 = 14.43 μM). Long-term treatment (14 days) with PF-04691502 significantly suppressed the colony-forming abilities of HepG2 cells in vitro (Fig. 2B). To examine whether PF-04691502-induced growth inhibition was associated with cell cycle regulation, the cell cycle distribution was analyzed by flow cytometry. After HepG2 cells were incubated with PF-04691502 for 24 h, cells were harvested and further prepared for cell cycle analysis. Fig. 2C showed that HepG2 cells accumulated in the G1 phase of the cell cycle, whereas the percentage of cells in S phase reduced significantly. Flow cytometry analysis was also repeated in Bel7402 cells. As expected, 24 h of PF-04691502 treatment resulted in accumulation of Bel7402 cells in G1 phase.

In order to determine whether PF-04691502 was able to inhibit cyclins production, western blotting experiment was performed in the cell extracts prepared from PF-04691502-treated hepatoma cells. The results showed that PF-04691502 incubation reduced the levels of cyclinD1 and increased the amount of the cyclin-dependent kinase inhibitor p27 both in the HepG2 cells and in the Bel7402 cells.

3.3. PF-04691502 induces hepatoma cells apoptosis through caspase activation

To determine the apoptotic effect of PF-04691502 on the HepG2 cells, Annexin V-FITC and PI staining assay was used to detect the cell apoptosis. After cells were incubated with increasing concentrations of PF-04691502 for 24 h, cells were harvested and further processed for flow cytometry analysis. As shown in Fig. 3A, significant induction of apoptosis occurred in hepatoma cells. After treatment with 20 μM PF-04691502, 14.91% of the HepG2 cells were in early apoptotic stage (low-right phase), 7.62% of cells were in late apoptotic/necrotic stage (up-right phase). In contrast, 3.63% of Bel7402 cells were in early apoptotic stage and 27.93% cells were already in the late phase of apoptosis/necrosis. Thus, Bel7402 cells may be more susceptible to apoptosis induced by PF-04691502.

Next, we test whether PF-04691502-treated hepatoma cells express typical apoptotic markers. Western blotting analysis results revealed that cleavages of apical pro-caspase-3, pro-caspase-9, and PARP into the characteristic activate fragments in hepatoma cells were evident after treatment with PF-04691502 (Fig. 3B).

Caspase-3 activity can be blocked by the z-DEVD-fmk (Arora et al., 2012). To investigate if apoptosis upon PF-04691502 treatment was induced via activation of caspase, we incubated z-DEVD-fmk to the cultures and measured cell viability with the MTT assay. As shown in Fig. 3C, PF-04691502-reduced viability and caused cell death were partially but significantly blocked in the presence of z-DEVD-fmk. These results suggest that PF-04691502-induced apoptosis in hepatoma cells is associated with the caspase-dependent apoptotic pathway.

3.4. PF-04691502 inhibits tube formation and migration of HUVECs

It is now widely recognized that angiogenesis is necessary to sustain tumor growth, invasion and metastasis. Then, the tube formation assay of endothelial cells was used to determine the anti-angiogenic action of PF-04691502. HUVECs incubated on Matrigel substratum for 8 h resulted in the formation of tube-like structure in the control group, whereas HUVECs exposed to PF-04691502 formed incomplete tube networks (Fig. 4A).

Next, cell cycle distribution was analyzed by flow cytometry to examine whether PF-04691502 was associated with cell cycle regulation of HUVECs. As shown in Fig. 4B, treatment of HUVECs with PF-04691502 resulted in an accumulation of cells in the G1 phase and the percentage of cells in S phase was reduced dramatically compared with the control cells. Western blotting analysis results indicated that PF-04691502 also inhibited the expression of cyclinD1 and up-regulated the levels of p27 in HUVECs.

As endothelial cell migration is another important step of angiogenesis, transwell assay was performed to determine the effects of PF-04691502 on HUVEC migration. Fig. 4D showed that PF-04691502 significantly blocked VEGF-induced migration of HUVECs.
Fig. 2. PF-04691502 inhibits hepatoma cell growth. (A) PF-04691502 reduces the viability of HepG2 cells. Cells were incubated with PF-04691502 at the indicated concentrations for 24 h and then processed for MTT assay. (B) PF-04691502 inhibits the colony formation ability of HepG2 cells. The histogram represents the mean colony number of 3 independent experiments (mean ± SD). *P < 0.01 compared with the control. (C) Treatment of hepatoma cells with PF-04691502 results in cell cycle arrest at G1 phase. HepG2 cells and Bel7402 cells were exposed to different concentrations of PF-04691502 for 24 h, then cell cycle was determined by PI staining and flow cytometry. (D) PF-04691502 down-regulates cyclinD1 level and up-regulates p27 expression in hepatoma cells. Cells were incubated with different concentrations of PF-04691502 for 24 h and harvested, and then total protein lysates were subjected to western blotting analysis with the use of antibodies against cyclinD1, p27, or β-actin.

Fig. 3. Induction of caspase-mediated apoptosis by PF-04691502 in hepatoma cells. (A) PF-04691502 induces apoptosis in human hepatocellular carcinoma cells. Cells were incubated with the various concentrations of PF-04691502 for 24 h; induction of apoptosis was determined by Annexin V-FITC/PI staining analysis. (B) Cleavage of caspase-3, caspase-9, and PARP in hepatoma cells treated with PF-04691502. Cells were treated with indicated concentrations of PF-04691502 for 24 h and proteins were extracted as described in Section 2. Caspase-3, caspase-9, and PARP cleavage were assessed by western blotting analysis; β-actin was used as loading control. (C) Effects of the caspase-3 inhibitors (z-DEVD-fmk) on PF-04691502-induced apoptosis. Hepatoma cells were pre-treated with or without the z-DEVD-fmk and then treated with 20 μM of PF-04691502. The cellular viability was assessed by MTT assay. Data are mean ± SD and representative of three independent experiments. *P < 0.05 compared with the control group. †P < 0.05 compared with cells treated with PF-04691502 alone without the z-DEVD-fmk.
3.5. *PF-04691502 inhibits expression of VEGF and HIF-1α in HepG2 cells*

Hypoxia-inducible factor and VEGF are important for tumor angiogenesis. Therefore, we evaluated the effects of PF-04691502 on the expression patterns of HIF-1α and VEGF in HepG2 cells. As shown in Fig. 5A, PF-04691502 suppressed the expression of HIF-1α and VEGF in a dose-dependent manner. Previous reports have identified activation of STAT3 directly correlates with VEGF (Boreddy et al., 2012; Auzenne et al., 2012), we then want to see whether PF-04691502 inhibits the phosphorylation of STAT3 in HepG2 cells. We found that PF-04691502 drastically decreased the phosphorylation of STAT3 at Tyr-705 (Fig. 5A).

Solid tumors secrete various proangiogenic factors, such as VEGF, to activate the nearest endothelial cells in the host tissue for neoangiogenesis. Therefore, we intend to elucidate whether the PF-04691502 could inhibit VEGF secretion from HepG2 cells. By using ELISA assay, we found that PF-04691502 treatment for 12 h inhibited secreted levels of VEGF in HepG2 cells (Fig. 5B).

4. Discussion

The PI3K/Akt signaling transduction pathway, a critical driver of tumorigenesis, is tightly controlled and negatively regulated by several phosphatases. PTEN, also known as MMAC1/TEP1, has been implicated in regulating cell survival signaling through the PI3K/Akt pathway. PTEN blocks the action of PI3K by
dephosphorylating the signal lipid phosphatidylinositol 3,4,5-triphosphate (PIP3), and genetic inactivation of PTEN leads to constitutive activation of the PI3K/Akt/mTOR axis (Martelli et al., 2011). Inhibition of PI3K/Akt signaling pathway can inhibit cell proliferation and in some circumstances, induce cell death. Consequently, significant efforts have been made to generate inhibitors of the PI3K/Akt pathway to treat cancers. A number of PI3K/Akt pathway inhibitors have now been disclosed and are being evaluated in preclinical studies (Courtney et al., 2010; Jakubowiak et al., 2012).

Here we describe the antitumor activity of PF-04691502, a recently developed inhibitor of PI3K and mTOR kinases, that is discovered through high-throughput screening and structure-based drug design (Yuan et al., 2011). We have confirmed that PF-04691502 inhibits the phosphorylation of Akt and upregulates the expression of PTEN. We hypothesize that, like many inhibitors of PI3K/Akt pathway, the inhibition of PI3Kinase/mTOR by PF-04691502 leads to decreased cell proliferation. As expected, PF-04691502 strongly decreased proliferation of HepG2 cell as shown by the MTT and colony formation assay. Treatment of HepG2 cells and Bel7402 cells with PF-04691502 resulted in a robust growth arrest in the G1 phase. Western blotting experiments showed that G1 cell cycle arrest observed with PF-04691502 treatment in hepatoma cells seems to be well correlated with the inhibition of cyclinD1 and induction of the p27 cyclin-dependent kinase inhibitor.

Apoptosis is a process of programmed cell death that serves as a major mechanism for the precise regulation of cell numbers and as a defense mechanism to remove unwanted cells. In mammals, there are two main pathways, extrinsic pathway (cytoplasmic) and intrinsic pathway (mitochondrial), which lead to caspase activation during apoptosis (Estaquier et al., 2012). In the mitochondrial signaling pathway, a key initiator of the apoptotic caspase activity is the formation of apoptosome between the apoptotic protease-activating factor-1 (Apaf-1) and caspase-9 with cytochrome c following its release from mitochondria. Activated caspase-9 in turn cleaves executioner caspase-3. The activated caspase-3 then cleaves PARP, a 116 kDa nuclear protein related to the process of programmed cell death (Maximilian et al., 2012). Our studies clearly showed that after 24 h of treatment with 20 μM PF-04691502, the level of active caspase-9 was increased, along with a rapid increased in the active caspase-3 level (Fig. 3B). These data, together with partial protection of the caspase-3 inhibitor z-DEVD-fmk from PF-04691502-induced cell death, suggest that mitochondrial pathway is involved in PF-04691502-induced hepatoma cells apoptosis.

HCC is a particularly vascular tumor and angiogenesis plays an important role in the pathogenesis of the disease. Tumor-induced angiogenesis is important in supporting tumor growth and progression by providing the necessary blood supply and by allowing metastatic cells to enter circulation. A growing body of evidence indicates blockade of cancer angiogenesis can decrease cancer progression by inhibiting tumor growth, and development angiogenesis inhibitor is a desirable anticancer target. Rapid proliferation of cancer cells in the absence of adequate amounts of oxygen and nutrient availability results in hypoxia developing in the majority of solid tumors, with increasing the expression of angiogenic factors such as HIF-1α and pro-angiogenic factor VEGF (Mucal et al., 2012). HIF activation allows modulation of cell metabolism and gene expression to adapt to the ever-changing landscape of the tumor microenvironment. PI3K/Akt signaling plays a major role in tumor angiogenesis by regulating HIF-1α and VEGF expression. In the present study, we have also shown for the first time that PF-04691502 exerts anti-angiogenic activity by inhibiting the expression of HIF-α and VEGF in HepG2 cells. Results of ELISA assay showed that PF-04691502 inhibited secretion of VEGF from HepG2 cells.

Taken together, PF-04691502 inhibits hepatoma cell viability and induces of cell G1 phase arrest in a dose-dependent manner. Inhibition of PI3K/Akt signaling by PF-04691502 resulted in induction of apoptosis as well as inhibition of angiogenesis. Our results indicate that PF-04691502 may prove to be a valuable tool for treatment hepatocellular carcinoma.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (No. 81272683) and Shandong Provincial Natural Science Foundation, China (No. ZR2011HQ034).

References


