ApoG2 as the most potent gossypol derivatives inhibits cell growth and induces apoptosis on gastric cancer cells

J. Xin a,1, Y.H. Zhan a,1, L.M. Xia c, H.W. Zhu c,d, Y.Z. Nie c, J.M. Liang a,*, J. Tian a,b,**

a School of Life Sciences and Technology, Xi'an University, Xi'an, 710071 Shaanxi, China
b Institute of Automation, Chinese Academy of Sciences, 100190 Beijing, China
cDepartment of Gastroenterology and State Key Laboratory of Cancer Biology, Xijing Hospital, Fourth Military Medical University, Xi'an, 710032 Shaanxi, China
dDepartment of Gastroenterology, Guangzhou General Hospital of Guangzhou Military Command, Guangzhou, 510010 Guangdong, China

ARTICLE INFO
Article history:
Received 28 August 2012
Accepted 22 October 2012

Keywords:
ApoG2
Small-molecule inhibitor
Gastric cancer

ABSTRACT

Gastric cancer is one of the most common types of malignancies and proteins from the Bcl-2 family are highly expressed in human gastric cancer. Apogossypolone (ApoG2), the most potent gossypol derivative, has been defined as a novel small-molecule inhibitor of anti-apoptotic Bcl-2 family proteins. However, whether or not it can inhibit the growth and proliferation of gastric cancer cell lines has not been demonstrated to date. Here, we assessed the effects of anti-growth of ApoG2 on gastric cancer cell lines in vitro and explored the possible molecular mechanisms of ApoG2. Using the MTT assay and flow cytometry, we found that ApoG2 has the significant anti-growth effect on MKR28, MKR45 and AG5 cell lines in a time- and dose-dependent manner. Compared to (-)-gossypol, MTT assay and flow cytometry results showed that anti-growth effect of ApoG2 is inferior, but the colony formation ability of ApoG2 is superior. Furthermore, western blot results revealed that ApoG2 inhibits the growth and proliferation of gastric cancer cells by down-regulating of Bcl-2 protein expression, up-regulating of Bax and activating of Caspase-3. Taken together, albeit the ApoG2 inferior to (-)-gossypol in many ways on gastric cancer in vitro, our results suggest that ApoG2 could effectively inhibit the growth and proliferation of gastric cancer cell lines through the mitochondrial pathway of apoptosis.

© 2012 Elsevier Masson SAS. All rights reserved.

1. Introduction

Gastric cancer is a common malignancy worldwide, and is the second leading cause of cancer-related death for men in developing countries [1,2]. Chemotherapy is a frequent treatment method for gastric cancer; however, the poor prognosis and unsuccessful therapy usually happens [3,4]. One of the main reasons is that the resistance of drug to chemotherapy [5]. The intrinsically cause is that these cancers are resistant to the apoptosis-inducing effect of clinical drugs [6,7]. Thus, discovering new chemotherapeutic drugs or effective treatment strategies is important for the management of gastric cancer.

Over-expressed in gastric cancer cells, the Bcl-2 family proteins play an important role in gastric cancer progression [8]. The Bcl-2 family proteins are divided into pro-apoptotic members and anti-apoptotic members. But possessing at least one of four Bcl-2 homology domains (BH1–BH4) is their notable similar feature. The anti-apoptotic Bcl-2 protein has a hydrophobic groove. It forms a binding pocket that can bind to pro-apoptotic members BH3 domains, thereby interferes with pro-apoptotic members' inducing-apoptotic function [9–11]. Targeting the binding pocket of anti-apoptotic members using non-peptidic small-molecule inhibitors (SMIs) is a new and appealing strategy for tumor therapy [12–16].

Gossypol is a natural product isolated from cotton seeds and roots, including levo-gossypol ((−)-gossypol) and dextro-gossypol ((+)-gossypol). Gossypol as the effective male contraceptive drug is widely used in clinical practice [17]. Recently, several studies have been reported that gossypol, especially (−)-gossypol, has anti-proliferative activities in vitro against several cancer cells and anti-tumor effects in vivo on nude mice [18–24]. Now it has been tested in Phase II human clinical trials for cancer [25]. (−)-Gossypol as a natural BH3 mimetic is one of the successful tumor therapy drugs of non-peptidic SMIs. However, the drawback of (−)-gossypol is the two aldehyde groups in its chemical structure which are associated with toxicity and the potential non-specific reactivity [26]. Therefore, many researchers have studied on optimization of chemical structure and improvement its anti-cancer effect. Apogossypolone (ApoG2), a novel gossypol derivatives, which was synthesized by removing the two aldehyde groups, has been found to have superior anti-cancer activity with less toxicity in nasopharyngeal carcinoma cells [27,28], prostate cancer cells [29].
human leukemic monocyte lymphoma cells, diffuse large-cell lymphoma cells, follicular lymphoma cells [30–34], pancreatic cancer cells [35], human hepatocellular carcinoma cells [36] and so on. However, whether or not it can inhibit the growth and proliferation of gastric cancer cell lines has not been demonstrated to date.

Therefore, in this study, we investigated whether ApoG2 can inhibit the growth and proliferation in gastric cancer cell lines or not. We demonstrated the anti-cancer effectiveness between ApoG2 and (-)-gossypol on gastric cancer in vitro. The results show that ApoG2 has significant anti-cancer activities in a time- and dose-dependent manner although the anti-growth effect and induced apoptosis ability of (-)-gossypol is more significant than ApoG2 in short time, which is perhaps attributing to (-)-gossypol’s higher cell toxicity. ApoG2 may have a fascinating inhibition effect on gastric cancer for a long time. Further, the possible mechanism for inducing apoptosis of ApoG2 in gastric cancer was also demonstrated in present study. It has been reported that the ratio of Bcl-2/Bax may determine whether cancer cells are progressing towards apoptosis or not [37]. Therefore, in this report, we observed the changes of the protein expression level of Bcl-2 and Bax. The result we found that apoptosis was triggered after the cells were exposed to ApoG2 through up-regulating pro-apoptotic protein Bax and down-regulating anti-apoptotic protein Bcl-2 and interfering with the anti-apoptotic protein binds to pro-apoptotic protein. The activation of cleavages of Caspase-3 occurred when the cells were treated with ApoG2. These results suggest that ApoG2 is a successful non-peptidic pan small molecular inhibitor of anti-apoptotic Bcl-2 proteins in gastric cancer therapy.

2. Materials and methods

2.1. Cell lines and reagents

The human highly differentiated gastric carcinoma cell MKN28, poorly differentiated gastric carcinoma cell MKN45 and AGS was donated by State Key Laboratory of Cancer Biology, the Digestion Department of Xijing Hospital, the Fourth Military Medical University. All the cells were cultured in RPMI1640 medium (HyClone) supplemented with 10% fetal bovine serum (HyClone) and 1% penicillin/streptomycin in a humidified incubator at 37 °C with 5% CO2. ApoG2 and (-)-gossypol was synthesized by Yonghua Zhan in our laboratory [38,39]. The chemical structures were shown on Fig. 1. ApoG2 and (-)-gossypol was dissolved in pure dimethyl sulfoxide (DMSO) at the stock concentration of 20 mM and stored at -20 °C. Working solutions were prepared by dilution of the stock solution in fresh culture medium on the day of use. - 3(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich. The Hoechst 33258 Staining Kit was purchased from Beyotime Company. The primary antibodies (Bcl-2, Bax, Caspase-3, β-actin) used for western blotting were purchased from Santa Cruz.

2.2. MTT assay

The cytotoxic effects of ApoG2 and (-)-gossypol on gastric cancer cell lines in vitro were measured by MTT assay. Briefly, MKN28, MKN45 and AGS cells (2.5 × 10^4 cells/mL) were seeded to sterile 96-well flat-bottomed plates, and incubated overnight. Then diluted ApoG2 or (-)-gossypol was added to each well with the final concentration of 1, 5, 10, 20, 30, 40, 50 μM, respectively. To evaluate the cell viability, in a 96-well flat-bottomed plate, four wells containing only tumor cells suspended in the mixture solution of 200 μL complete medium and 0.2 μL DMSO were used as control, and four wells containing only 200 μL complete medium were used as blank control. The plates were then incubated at a humidified incubator at 37 °C with 5% CO2 for 24 h, 48 h and 72 h, respectively. After the incubation, 20 μL of 5 mg/mL MTT solution was added to each well, then the plates were incubated for 4 h. Formazan crystals formed at the bottom of the well. Culture supernatant in the plates was removed carefully. One hundred and fifty microliters DMSO was added to all wells. Shaking 15 min at least, the formazan crystals were dissolved completely. The plates were then read on MICROPLATE READER with 490 nm test wavelength. The inhibition rate of cell to drug with different concentration and different time was calculated as: ((OD of drug-treated-OD of blank control)/(OD of control-OD of blank control)) × 100%. The 50% inhibition concentration of drug was defined as the concentration, which causes 50% growth inhibition rate. The experiments were tested in triplicates.

2.3. Detection of cell apoptosis by Hoechst 33258 assay and flow cytometry analysis

The nuclei changes caused by ApoG2 induced apoptosis were visualized by Hoechst 33258 nuclear staining Kits according to the manufacturer’s instructions. Briefly, MKN28, MKN45 and AGS cells were harvested at logarithmic growth phase and grown on bio-clean cover slips in 6-well plates at the density of 2 × 10^5 and allowed to attach overnight. Then, the cells were treated with 10, 20, 30, 40, 50 μM ApoG2 or 0.1% DMSO (control) for 48 h at 37 °C or with 10 μM ApoG2 for different time span (24 h and 72 h). To determine the difference of inducing apoptosis between ApoG2 and (-)-gossypol, (-)-gossypol-induced apoptosis was also detected at 48 h. After treating, the cells were fixed in mounting medium for 10 min at room temperature, rinsed with PBS for two times, and stained with Hoechst 33258 for 5 min. Cover slips were then washed and mounted on slides with glycerol, and imaged with Olympus fluorescence microscope. To quantify the percentage of apoptosis, we counted the number of cells with apoptotic characteristics among 200 cells at high power field. According to the Hoechst 33258 assay results, apoptosis induced by ApoG2 or
(-)-gossypol was also evaluated by flow cytometry at 48 h and 72 h with the desired doses. Flow cytometry is a powerful technology for the analysis of apoptosis, which quantifies phosphatidylserine exposure on the surface of apoptotic cells using Annexin-V FITC and Propidium Iodide stain. Various concentrations of ApoG2 or (-)-gossypol (10, 20, 30, 40, 50 μM) and 0.1% DMSO were added to the cells for 48 h incubation and the cells were treated with ApoG2 at 10 μM for 72 h, additionally. Then the cells were harvested, centrifuged at 800 rpm for 5 min, washed with PBS, centrifuged again, resuspended in PBS and stained with Annexin-V FITC for 1 h and Propidium Iodide for 30 min, analyzed by a FACScan system in the end.

2.4. Colony formation assay

To evaluate anti-proliferation ability of ApoG2, the colony formation assay was done. 1 × 10^3 cells of MKN28, MKN45 and AGS were seeded into sterile 24-well flat-bottomed plates and incubated for 16 h. The cells were then treated continuously with ApoG2, (-)-gossypol or 0.1% DMSO from 100 nM to 50 μM for 14 days. After the treatment, the cells were washed with PBS for two times, fixed with 4% paraformaldehyde fixative for 10 min, stained with crystal violet for 10 min, washed again and photographed. Adobe Photoshop was used to quantify the result [40].

2.5. Western blot analysis

To determine the changes of protein levels, the cellular proteins of MKN28, MKN45 and AGS were extracted after treatment with 10 μM ApoG2 and 0.1% DMSO for 24 h, 48 h and 72 h. The proteins were separated by SDS-PAGE gels and subsequently semi-dry transferred to a nitrocellulose membrane. TBS includes 5% non-fat dry milk was used to block the non-specific binding of protein for 1 h at the room temperature. The first antibodies were incubated with membranes overnight at 4 °C. The appropriate peroxidase-conjugated secondary antibodies corresponding to the first antibodies were incubated with membranes for 1 h at room temperature. The reactive bands were detected using enhanced chemiluminescence (Pierce).

2.6. Statistical analyses

Data in this present study are presented as the mean ± SD of three replicate experiments. All statistical analyses were done by SPSS 16.0 software (Chicago, IL). Statistical difference between the means was analyzed with Student’s t test. Significance was set at the 5% level.

3. Results

3.1. ApoG2 and (-)-gossypol inhibit cell survival of gastric carcinoma cell lines

In this study, to investigate the inhibition effect on gastric cancer cell lines of ApoG2 and to compare ApoG2 and (-)-gossypol’s cytotoxicity, MTT assay was performed. ApoG2 inhibited the MKN28, MKN45, AGS cell lines in a time- and dose-dependent manner (Fig. 2A). Among the three gastric cancer cell lines MKN28, MKN45 and AGS, ApoG2 had apparent anti-growth activities in MKN45 and AGS. At 10 μM, the cell survival rate of MKN45 and AGS was about 43% and 35% at 72 h. In contrast, the cell survival rate of MKN28 was about 51% by treatment 10 μM ApoG2 for 72 h. We also compared the anti-tumor effectiveness between ApoG2 and (-)-gossypol on gastric cancer cell lines at 72 h. The cell survival rate of the three cell lines after treated with ApoG2 and (-)-gossypol was given (Fig. 2B). The 50% inhibition rate of ApoG2 for the MKN28, MKN45 and AGS cell lines about 72 h was 11.37, 7.71, 5.6 respectively. The 50%
Fig. 3. Apoptosis induced by ApoG2 and (-)-gossypol in MKN28, MKN45 and AGS cell lines. MKN28, MKN45 and AGS were treated with 10 μM ApoG2 for 24 h to 72 h and determined by Hoechst 33258 nuclear staining. A. Or quantified by FACS analysis. B. Apoptosis of MKN28, MKN45 and AGS cells after treated with a range of concentration (10–50 μM) of (-)-gossypol or ApoG2 for 48 h was observed by Hoechst stain. C. And flow cytometry. D. Apoptotic cell in MKN28, MKN45 and AGS cells induced by (-)-gossypol or ApoG2 for 48 h was analyzed statistically. E. The histogram represents the percentage of cells with apoptotic characteristics among 200 cells at high power field, data represent the average of three experiments and the bar is SD. *P < 0.05, represent statistically difference in the number of apoptotic bodies compared with control group or represent statistically difference in the number of apoptotic bodies between ApoG2 and (-)-gossypol. **P < 0.01, represent statistically significant difference in the number of apoptotic bodies compared with control group or represent statistically significant difference in the number of apoptotic bodies between ApoG2 and (-)-gossypol. All the Hoechst stain images were acquired at 400 × magnification. The scale bar represents 50 μm.
inhibition rate of (-)-gossypol for MKN28, MKN45 and AGS is 8.77, 7.24, 5.15 respectively. There was not significant difference between ApoG2 and (-)-gossypol about the 50% inhibition concentration. But for the cells exposed to (-)-gossypol of 30 μM above, the decreasing trend of cell viability was extremely evident. This may be caused by higher toxicity of (-)-gossypol.

3.2. ApoG2 induces apoptosis in gastric carcinoma

To evaluate whether ApoG2 induces apoptosis to inhibition of gastric tumor cells growth, Hoechst 33258 stain and flow cytometry were performed. The nuclear morphological change of the cells exposed to ApoG2 or (-)-gossypol was observed with Hoechst 33258 staining using fluorescence microscopy. The cells exhibited obvious apoptotic characteristics, such as cell shrinkage and nuclear fragmentation at 10 μM on 48 h (Fig. 3A). The flow cytometry results also illustrated that ApoG2 led to apoptosis at 10 μM on 72 h is two- to six-fold more potent than 48 h in MKN28, MKN45 and AGS cell line (Fig. 3B). The results demonstrated that ApoG2 led to apoptosis in a time-dependent manner. We also evaluated apoptosis induced by ApoG2 with the variation of dose and compared the apoptosis activities of ApoG2 and (-)-gossypol.

ApoG2 bodies increased as the dose rose (Fig. 3C). But in contrast to (-)-gossypol, ApoG2 led to apoptosis with less extent. Exposure to 50 μM of ApoG2 induced apoptosis was 56% of MKN28, 63.7% of MKN45 and 46% of AGS. The possible reason of fewer apoptotic cells of AGS than MKN28 and MKN45 is ApoG2 led to more necrotic cells than MKN28 and MKN45 on 48 h (Fig. 3D).

Treatment with 50 μM of (-)-gossypol led to apoptosis in more than 80.73% of MKN28, 83.73% of MKN45 and 93.5% of AGS. We analyzed the difference between ApoG2 and (-)-gossypol, at 48 h, as shown on Fig. 3D, apoptotic cells induced by ApoG2 were mainly the early apoptotic cells and apoptotic cells induced by (-)-gossypol were mainly the late apoptotic cells. Numbers of apoptotic bodies were shown on Fig. 3E statistically. Both ApoG2 and (-)-gossypol showed significant difference compared with DMSO-treated control statistically.

3.3. Effect of ApoG2 on anti-proliferation activity

To test the anti-proliferation ability of ApoG2, the colony formation assay was performed. MKN28, MKN45, AGS were treated with increasing concentration of ApoG2 (100 nM–50 μM) as the treatment group and treated with DMSO as the

![Image](image-url)

**Fig. 4.** Inhibition of proliferation activities by ApoG2 or (-)-gossypol on gastric cancer cell lines. MKN28, MKN45 and AGS cells were treated with 0 to 1000 nM ApoG2 or (-)-gossypol continuously for 14 days. Fixed with 4% paraformaldehyde, stained with crystal violet and photographed. Data was quantified by Adobe Photoshop. The experimental were tested in triplicates. *, P < 0.05, represent statistically difference in colony formation rate between ApoG2 and (-)-gossypol.
induced gastric MKN45 and ApoG2 that completely.

activation after apoptotic control. After 14 days, we statistically accounted the percentage of colony formation. From Fig. 4, we found the ApoG2 and (-)-gossypol strongly inhibited the proliferation of these gastric tumor cell lines. The percentage of colony formation of ApoG2 is higher than (-)-gossypol at 1 μM. It revealed that ApoG2 has stronger anti-proliferation effect with long time. After 14 days, treatment with ApoG2 of 1 μM, MKN28, MKN45 and AGSs' proliferation ability was nearly inhibited completely.

3.4. Change of the protein expression level of Bcl-2 family members induced by ApoG2

To investigate the molecular mechanism for triggering apoptosis, western blotting analysis was performed to observe the changes of the protein expression level of Bcl-2 family members after treated with 10 μM ApoG2 at 24 h, 48 h and 72 h. Fig. 5 showed the change of anti-apoptotic protein Bcl-2 and pro-apoptotic protein Bax expression level. The results showed that ApoG2 inhibits the growth and the proliferation of gastric cancer cells by down regulating of Bcl-2 protein expression, up regulating of Bax expression.

3.5. ApoG2 promotes Caspase-3 activation

To verify ApoG2 could promote apoptosis signaling in gastric cancer cells, we investigated the activation of Caspase-3. The activation of Caspase-3 usually occurs during drug-induced apoptosis. Western blotting revealed that ApoG2 efficiently cleaved Caspase-3 with the extension of treated time (Fig. 5).

4. Discussion and conclusion

Resistance of drug usually led to the failure of chemotherapy against malignant cancers. For reversing resistance of drug to chemotherapy, the strategies that blocking the anti-apoptotic proteins activities have become more attractive. Used non-peptidic small-molecule inhibitors may be an innovation and effectiveness therapy strategy in gastric cancer prevention.

Earlier studies have shown that gossypol has chemopreventive effect on gastric adenocarcinoma cell line through reducing of nucleic acid contents and modulating the oxidant-antioxidant status and inducing apoptosis. Existing problems of (-)-gossypol are instability, high toxicity and insolubility. Thus, it is necessary to modify the chemical structure of gossypol. ApoG2, is a derivative from gossypol, has been found to have superior anti-cancer activity with less toxicity. Our report is the first one investigating the anticancer effect of ApoG2 on gastric cancer. The results in this paper are as follows:

- MTT results showed that IC50 concentration of ApoG2 as same as (-)-gossypol in gastric cancer line MKN45 and AGS and slightly inferior in MKN28 cell line;
- the induced apoptosis activity of ApoG2 in short time is lesser than (-)-gossypol;
- colony formation assay showed that anti-proliferation activity with 1000 nM is higher than (-)-gossypol.

The over-expressed Bcl-2 protein is known to play an important role in the inhibition of apoptosis and the promotion of cell survival during oncogenesis and development of gastric cancer. ApoG2 as one of the Bcl-2 family non-peptidic small-molecule inhibitors revealed that it binds to Bcl-2 family proteins via BH3 binding pocket to induce cell death. ApoG2 induced apoptosis molecular mechanism was also demonstrated concisely in this study. Only one of the possible molecular mechanisms of ApoG2 for triggering apoptosis was investigated. It is well known that the death receptor pathway, the mitochondrial pathway, and endoplasmic reticulum stress-induced apoptosis are three common ways to induce apoptosis. We examined ApoG2 induced apoptosis by the mitochondrial way. The Bcl-2 family proteins are the key regulators of the mitochondrial pathway. Bcl-2 belonging to anti-apoptotic member, a 26kDa protein, is located mainly in the mitochondrial membrane and is a key regulator of apoptosis. It can prevent cells from death induced by radiation, chemotherapy or growth factor deprivation and so on. Bax belonging to pro-apoptotic member is a homolog of Bcl-2 that promotes cell death through apoptosis. The ratio of Bcl-2/Bax may determine whether cancer cells are progressing towards apoptosis or not [41]. Thus, we selected Bcl-2 protein and Bax protein to examine the protein expression levels changing after treated with ApoG2. Our research shows that ApoG2 could alter the expression of Bcl-2 family proteins, down-regulating the level of Bcl-2 and up-regulating the level of Bax in the time-dependent manner and lead to activation of downstream apoptosis protein, such as Caspase-3. Therefore, in gastric cancer, ApoG2 could activate mitochondrial signal pathway to promote cell death. In addition, the exact function mechanism of ApoG2 is unclear to date, it is necessary to
investigate other novel function mechanism for inducing cell death at a later date and it is now actively underway in our laboratory.

In addition, according to the difference of anti-cancer effect of ApoG2 and (-)-gossypol on gastric cancer cell lines, it is reasonable to speculate that anti-cancer effect of ApoG2 may depend on the expression of Bcl-2 protein. This may explain the observation of the different sensitivities of gastric cancer cells to ApoG2. Through western blotting analysis, we found that Bcl-2 expression was negatively correlated to the degree of tumor differentiation. In other words, MKN28 is a highly differentiated gastric carcinoma cell line with high expression of Bcl-2, while MKN45 and AGS are poorly differentiated gastric carcinoma cell lines with low expression of Bcl-2. MT assay results revealed that ApoG2 is more sensitive to low level of Bcl-2 in gastric cancer. The possible reason is the Bcl-2 protein function was inhibited by ApoG2 to a great extent in gastric cancer cells which expressed low level of Bcl-2 protein, thus the dominant pro-apoptotic protein could participate in the apoptotic response.

To further study the reasons of concentration difference of MT assay and colony formation assay, it is reasonable to speculate that the environment is an important factor influencing the drug dose. In the present of drug for a long time is easy lead to adverse conditions to cell growth. It is probably due to lesser dose of ApoG2 can inhibit the proliferation effect on gastric cancer cell lines. We changed the MKN28, MKN45 and AGS culture conditions before MT assay. Briefly, MKN28, MKN45 and AGS (2.5 × 10^4 cells/mL) were seeded to sterile 96-well flat-bottomed plates in RPMI1640 medium supplemented with 2% fetal bovine serum and 1% penicillin/streptomycin, and incubated overnight in a humidified incubator at 37 °C with 5% CO₂. Then repeated the process described above in MT assay. The results showed that ApoG2 could significantly inhibit the cell survival of gastric carcinoma cell lines at even 100 nM (the data was not shown). These results were consistent with colony formation results. The main goal of our research is to find out whether non-peptidic small-molecule inhibitors can be used to gastric cancer therapy or not. As a successful compound of non-peptidic small-molecule inhibitors, the toxicity and anti-cancer effectiveness of ApoG2 in vivo is being done in our laboratory.

In conclusion, ApoG2 has significant anti-growth and anti-proliferation effect and induced apoptosis activities on gastric cancer in vitro. It can bind to Bcl-2 family proteins and prevent its association with BH3 domain of pro-apoptotic proteins, thus unleashing the pro-apoptotic protein could participate in the apoptotic response. Our findings suggested that ApoG2 could effectively inhibit the growth and proliferation of gastric cancer cells through the mitochondrial signal pathway of apoptosis.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgements

This work was supported by the Program of the National Basic Research and Development Program of China (973) under Grant No. 2011CB707702, the National Natural Science Foundation of China under Grant Nos. 81090272, 81090273, 81101100, the Natural Science Basic Research Plan in Shaanxi Province of China under Grant No. 2012JQ0415, the National Key Technology Support Program under Grant No. 2012BAI23806, and the Fundamental Research Funds for the Central Universities.

References


