Original Article
Paclitaxel exerts anticancer bioactivity on SGC7901 human gastric adenocarcinoma cell line through inhibition of Akt/mTOR signaling pathway

Dong Sun1*, Ke-Shu Shan1*, Jin-Shen Wang1, Yue-Zhi Chen1, Liang Shang1, Yarmus Khan2, Bang-Zhen Ma2, Le-Ping Li1

Departments of 1Gastrointestinal Surgery, 2Hepatobiliary Surgery, Shandong Provincial Hospital Affiliated to Shandong University, Jinan 250021, People’s Republic of China. *Equal contributors.

Received September 6, 2015; Accepted December 9, 2015; Epub February 1, 2016; Published February 15, 2016

Abstract: Gastric cancer is one of the most prevalent malignancies and the leading cause of cancer mortality. Paclitaxel, a traditional medicine, is originally extracted from pacific yew tree. It has been reported that paclitaxel can treat multiple human malignancies including gastric cancer. However, the underlying mechanism has not been fully understood. In our study, SGC-7901 human gastric adenocarcinoma cell line was cultured and treated with paclitaxel. Cell Counting Kit-8 (CCK-8) and colony formation assay were performed to examine cell viability. Cell migration and metastasis ability were measured by cell invasion assay. Moreover, cell cycle was determined by the Flow Cytometry, Hoechst33342/PI (propidium iodide) double staining and Flow Cytometry were both employed to test cell apoptosis. To further explore the underlying mechanisms of paclitaxel in gastric cancer, western blotting was performed to detect the protein expression of phospho-Akt (p-Akt) and phospho-mTOR (p-mTOR). Our results showed that paclitaxel obviously reduced SGC-7901 cell viability and cellular colony formation ratio compared with the control ($P < 0.01$). Meanwhile, paclitaxel effectively inhibited cell migration and metastasis, the number of invaded cells reduced significantly ($P < 0.01$). Flow Cytometry and Hoechst/PI double staining results also showed paclitaxel induced SGC-7901 cell apoptosis ($P < 0.01$) and let cell cycle arrest at G2/M phase. Furthermore, paclitaxel strongly decreased the expression of p-Akt and p-mTOR proteins. Collectively, our study indicated that paclitaxel exerts an obvious anticancer bioactivity on SGC-7901 human gastric adenocarcinoma cell line, which is possibly, mediated through inhibition of Akt/mTOR signaling pathway.

Keywords: Paclitaxel, gastric cancer, SGC-7901, Akt/mTOR signaling pathway

Introduction
Gastric cancer, also known as stomach cancer, is the fourth most common malignancies and the second cause of cancer related death worldwide [1, 2]. Among many kinds of malignancies, gastric cancer is the leading cause of cancer mortality in China [3]. The majority of patients diagnosed with gastric cancer at advanced stage have distance organ metastasis and only 15% of them can survive beyond six months due to its resistance to radiotherapy and chemotherapy. Even diagnosed in early stage of gastric cancer, only 60% patients can survive more than 5 years [4, 5]. Although the most effective therapy method is resection operation, chemotherapy is indispensable for gastric cancer treatment, especially for advanced stage.

Paclitaxel, a traditional medicine, was first extracted from Pacific yew tree. In 1971, researchers obtained the pure paclitaxel and verified its anticancer activity [6, 7]. Since then, paclitaxel has been proven to treat multiple human malignancies and has already been used as an chemotherapy in breast cancer, ovarian cancer, non-small cell lung cancer (NSCLC), pancreatic cancer and other malignancies [8-11]. Especially, paclitaxel is an effective chemotherapeutic drug for gastric cancer [12]. In Japan, patients received weekly paclitaxel treatment has already regarded as the perfect second-line chemotherapy in unresectable gastric cancer cases [13]. And, patients adapted periodical chemotherapy with paclitaxel after resection surgery were observed with low recurrence rate, metastasis rate and long survival time [14].
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However, the underlying mechanism has not been fully understood.

Although many studies have reported the role of paclitaxel on gastric cancer, most of them focused on the effects of paclitaxel on cell growth, apoptosis, cytotoxicity and anti-angiogenesis, etc [15]. Investigators have already found that PI3K (phosphoinositide-3-kinase) pathway plays a central role in cell proliferation, apoptosis, energy metabolism, autophagy and other cell functions. Moreover, PI3K pathway was reported aberrantly activated or over-expressed in gastric cancer [16]. Akt and mTOR belong to the classical PI3K/Akt signal pathway. Recently, Gou WF and his colleagues demonstrated that over-expression of ING5 in gastric cancer cells can induce cell autophagy, differentiation and inhibit proliferation and apoptosis, which is relative to p-Akt [17]. A recent study also suggested that inhibition of PI3K/HIF-1α pathway can enhance the therapeutic effect of paclitaxel in human hypoxic gastric cancer cells [18]. Genetic variants in the PI3K pathway may predict platinum-based chemotherapy response in lung cancer cells [19]. Thus, we hypothesize that paclitaxel can exert anticancer bio-activity through regulating Akt/mTOR signaling pathway, however, little is known about it in SGC-7901 human gastric adenocarcinoma cells.

To test our hypothesis, in this study, SGC-7901 human gastric adenocarcinoma cells were cultured and treated with paclitaxel. The cell viability, invasion, apoptosis and cell cycle were examined in cultured cells. Furthermore, western blotting was employed to determine the expression of Akt/mTOR signal pathway. Our study will be beneficial for better understanding of paclitaxel’s anticancer bioactivity and provide possibly targets for treating gastric cancer.

**Materials and methods**

**Cell culture**

The SGC-7901 cell was incubated in RPMI1640 medium (Hyclone, Logan, UT) supplemented with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT) and 1% penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37°C in 5% CO₂ incubator. The paclitaxel powder (Yuanye Company, Shanghai, China) was dissolved with dimethylsulphoxide (DMSO) at a concentration of 20 mg/ml, and then diluted to the desired concentration by RPMI1640 medium before using. The highest concentration of DMSO in culture medium was less than 0.1%, to make sure it did not affect cellular viability [20].

**Cell proliferation analysis**

Cell Counting Kit-8 (CCK-8) (Dojindo, Japan) was employed to detect cell viability according to a previous report [21]. Cells with the concentration of 3×10⁴ were seeded into the 96-well plate and incubated with normal medium or medium contains 0.25 μg/ml and 0.5 μg/ml paclitaxel. Cell viability was examined at 24 h, 48 h, 72 h and 96 h in vitro culture, respectively. 10 μl CCK-8 was added to each well and incubated for additional 1 h at 37°C. The cell absorbance was measured by microplate reader (Molecular Devices, America) at 450 nm wavelength.

**Colony formation analysis**

SGC-7901 cells (about 200 cells) treated with or without paclitaxel were suspended in 2 ml RPMI1640 medium contains 10% FBS and then cultured at cell incubator for two weeks. After washed with Phosphate Buffered Saline (PBS), the cells were fixed by 4% formaldehyde for 15 min and stained with hematoxylin for 10 min at room temperature. The cell colony number was obtained in five random sights of different groups under an inverted phase contrast microscope (Olympus, Japan). The colony formation rate was calculated by following formula: colony formation rate = (the average number of colony)/200×100%.

**In vitro cell invasion assay**

The cell migration and metastasis ability were determined by the non-coated transwell membrane (8.0 μm pore size, Corning, America) and the membrane pro-coated with Matrigel diluted by serum-free 1640 medium according to a previous report [22]. Briefly, the cultivated cells were suspended in serum-free medium and planted on the upper chamber. Medium contains 10% FBS was added to the lower chamber as a chemo-attract. After incubated for 24 h in vitro, cells can’t invade the lower chamber as a chemo-attract. After incubated for 24 h in vitro, cells can’t invade the lower chamber as a chemo-attract. After incubated for 24 h in vitro, cells can’t invade the lower chamber as a chemo-attract. After incubated for 24 h in vitro, cells can’t invade the lower chamber as a chemo-attract. After incubated for 24 h in vitro, cells can’t invade the lower chamber as a chemo-attract. After incubated for 24 h in vitro, cells can’t invade the lower chamber as a chemo-attract. After incubated for 24 h in vitro, cells can’t invade the lower chamber as a chemo-attract.
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Hoechst33342/PI (propidium iodide) double staining

Hoechst33342/PI double staining was applied to test cell apoptosis treated with paclitaxel according to manufacture protocols. After cultured in 24-well plate, cells were washed twice with PBS, and incubated with 10 μg/ml Hoechst33342 and PI for 15 min at cell incubator. Then, the cells were rinsed and observed under an inverted phase-contrast microscope (Nikon, Japan). Five random fields were selected to count the mean number of dead and surviving cells. The death rate was calculated by the following formula: Death rate = (mean number of PI positive stain cells)/Hoechst positive stain cell in the same field.

Cell apoptosis analysis

Flow Cytometry was employed to further detect cell apoptosis. SGC-7901 cells treated with or without paclitaxel were collected after digested with 0.25% trypsin. Cells were re-suspended by pre-cold PBS for three times and then stained with Annexin-V-FITC and PE-7AAD for 15 min at room temperature in the dark according to the manufacture protocol of Becton Company. Cell apoptosis was analyzed by the Flow Cytometry (Becton Dickinson, Franklin Lakes, NJ, America).

Cell cycle analysis

Cells of different groups were digested with 0.25% trypsin and washed three times with cold PBS, then the cells were fixed with pre-cold 70% ethanol for 24 h at 4°C. The next day, 500 μl PI was added to each tube and then incubated at 37°C in dark for additional 30 min according to the manufacturer’s guidelines (Leagent Company, Beijing, China). The cell cycle was analyzed by the Flow Cytometry according to the fluorescence intensity.
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Western blot

Cell proteins were extracted using RIPA lysis buffer contained 1% PMSF (Excell, Shanghai, China) according to the manufacture instructions, protein concentration was quantified used BCA kit (Excell, Shanghai, China). Equal amount of proteins (40 μg per lane) were separated on 6% SDS-PAGE gels. Then the isolation protein bands were transferred to the PVDF membrane and blocked with 5% non-fat milk for 1 h, after washed by 1×TBST for three times, all membranes were incubated overnight at 4°C with the following primary antibodies respectively: β-actin (Excell, Shanghai, China), p-Akt and Akt (CST, America), p-mTOR and mTOR (Abcam, England). In the following day, the corresponding secondary antibodies were added to specific bind the primary antibodies for 1 h at room temperature. Finally, the Amersham Imager 600 imaging system (GE Company, America) was employed to visualize and analyze the protein band.

Statistical analysis

The data were analyzed used the SPSS 17.0 software. All data were reported as the mean ± standard deviation. The differences between control and experiment groups were analyzed with one-way ANOVA test, differences between groups were analyzed with t-test. P<0.05 were considered as statistically significantly.

Figure 2. Effects of paclitaxel exerted on cell colony formation. Data shown are means ± SD, n=3, data were analyzed by the one-way ANOVA test and un-paired t-test; *P<0.05, **P<0.01.
Results

**Paclitaxel inhibits cell proliferation and viability**

SGC-7901 is an adenocarcinoma cell line derived from human gastric cancer. After cultured 24 h in normal medium, SGC-7901 cell can be found grow fast with less dead cells (Figure 1A). However, when added 0.25 μg/ml paclitaxel in medium for 24 h in vitro culture, cells became round shape while membrane swelled (Figure 1B). When cells were incubated in 0.5 μg/ml paclitaxel, dead cells could be easily found in the medium (Figure 1C). CCK-8 was performed to further test cellular viability and the absorbance data showed that the optical density of cells decreased significantly after treated with paclitaxel, among them, the 0.5 μg/ml paclitaxel exerted more obvious inhibitory effect \( (P<0.01) \) (Figure 1D). It indicates that paclitaxel can inhibit SGC-7901 growth and viability in a dose-dependent manner.

**Paclitaxel decrease the colony formation of SGC-7901**

Cell colony formation assay was determined to assess cellular viability exerted by the paclitaxel. The colony number of the control group, 0.25 and 0.5 μg/ml paclitaxel groups were 109.0 ± 12.7, 56.7 ± 7.1 and 34.0 ± 8.9, respectively (Figure 2A-C). Further analysis indicated paclitaxel could significantly inhibit the colony formation of SGC-7901 cell \( (P<0.01) \) and 0.5 μg/ml paclitaxel showed more obvious inhibitory effects than the 0.25 μg/ml group \( (P<0.05) \) (Figure 2D), means that paclitaxel could inhibit the colony formation of SGC-7901 cell in a dose-dependent manner.

**Paclitaxel inhibit the migration and metastasis ability of SGC-7901 cell**

To investigate the influence of paclitaxel exerted on cellular invasion ability, we detected cellular migration and metastasis ability using Transwell assay. For migration assay, the number of cells invaded the membrane was 195.0 ± 20.0, 119.2 ± 17.6, 82.2 ± 24.5 respectively for control, 0.25 μg/ml and 0.5 μg/ml paclitaxel group (Figure 3A-C); similarly, in the metasta-

**Paclitaxel induce SGC-7901 cell apoptosis**

Both Hoechst33342/PI double staining and the flow cytometry were employed to test cell apoptosis. The results of double staining showed after treated with paclitaxel, the dead cells (red fluorescence) was obvious increased compared to the normal SGC-7901 cell (Figure 4E), consistent with this, the flow cytometry results showed the apoptosis rate of normal SGC-7901 cell group was 7.1 ± 1.1\% (Figure 4A). However, when treated with 0.25 μg/ml and 0.5 μg/ml paclitaxel, the rate raised to 12.0 ± 1.6\% and 15.6 ± 1.2\%, almost twice than the control group with a \( P<0.01 \) (Figure 4B, 4C). Similarly to our previous results, paclitaxel also showed dose-effects on cell apoptosis.

**Paclitaxel arrest SGC-7901 cell cycle**

As shown in Figure 5, the flow cytometry results showed after treated with paclitaxel, the G2/M phase ratio of SGC-7901 was raised. The ratio was 10.81 ± 1.23\% in control group (Figure 5A), and raised to 14.57 ± 1.78\% and 15.54 ± 2.17\% when treated with 0.25 μg/ml and 0.5 μg/ml paclitaxel (Figure 5B, 5C). The results showed paclitaxel can arrest cell cycle at G2/M phase \( (P<0.05) \) (Figure 5D), but different from our previous results, paclitaxel did not show obvious dose-effect on cell cycle arrest in this experiment \( (P>0.05) \).

**Paclitaxel affect the Akt/mTOR signaling pathway**

As shown in Figure 6, paclitaxel can regulate the Akt/mTOR signaling pathway. After treated
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with paclitaxel, the expression level of p-Akt and p-mTOR were both down-regulated, their expression level showed a declined trend with the increase concentration of paclitaxel, the expression level was much lower in high concentration group. While the p-Akt and p-mTOR were down-regulated, however, the expression of Akt and mTOR maintain at a constant line (Figure 6A, 6B), confirmed that it was their phosphorylated form that involved in the paclitaxel anti-cancer mechanism.

**Discussion**

In this study, we employed the gastric adenocarcinoma cell line SGC-7901 to investigate its reaction to paclitaxel. Through the experiment, we found that paclitaxel can attenuate cell via-

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**Figure 4.** Effects of paclitaxel exerted on SGC-7901 apoptosis. A-C: The flow cytometry results of cell apoptosis induced by paclitaxel. E: The results of Hoechst33342/PI double staining. All data were presented as mean ± SD, n=3, data were analyzed by the one-way ANOVA test and un-paired t-test; *P<0.05, **P<0.01.
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Especially, we tested the possible molecular mechanisms, and found that paclitaxel can significantly inhibit the expression of Akt/mTOR signaling pathway. This experiment revealed the possible mechanism of paclitaxel in the treatment of gastric cancer.

Paclitaxel is a very effective and widely used natural chemotherapeutic drug in clinical. It was first permitted to cure advanced uterine cancer by the American FDA (Food and Drug Administration) in 1992, then it was used as a chemotherapeutic in clinical. Since large portion of gastric cancer are adenocarcinoma, through consulting literatures, we found little was done to explore the effects of paclitaxel exerts on the SGC-7901 cell, so we choose SGC-7901 cell to do the test. Through the experiment, we verified that paclitaxel can inhibit cell viability, attenuate cell invasion ability and induce cell apoptosis in a dose dependent manner. However we did not observe the same effect in cell cycle arrest, results showed paclitaxel can arrest cell cycle at G2/M phase without obvious dose effect ($P>0.05$), it was different from previous papers, the reason need to be further explored.

A growing body of report has shown that paclitaxel can exert its anti-tumor function through various signaling pathways. Previous researches have confirmed paclitaxel can induce cell apoptosis by regulating the BCL-2, BCL-x, p-53, and bax bypass pathways [23].

Figure 5. Effects of paclitaxel exerted on the cell cycle of SGC-7901 cell. Data shown are mean ± SD, n=3, data were analyzed used the one-way ANOVA test and un-paired t-test; *$P<0.05$, **$P<0.01$. 
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The influence of paclitaxel exerted on the Akt/mTOR signal pathway. A: The expression level of Akt and p-Akt in SGC-7901 cells treated with or without paclitaxel; B: The expression level of mTOR and p-mTOR in normal and paclitaxel groups. All data were presented as mean ± SD, n=3, data were analyzed by the one-way ANOVA test and un-paired t-test; #P>0.05, *P<0.05, **P<0.01.

Gastric cancer, as the fourth most common and second cause of cancer mortality worldwide, has caused great danger to people’s health. Due to its resistance to chemotherapy and radiotherapy, surgery is still the most effective therapeutic option in present, unfortunately, many patients were diagnosed at an advanced stage, some of them even lost the surgery opportunity, for them, there is still no good method. But, it is a feasible treatment strategy if we can specially induce cell death by target drugs. Researchers have reported the NVP-BEZ235 (an inhibitor of PI3K/mTOR signaling pathway) can down-regulate the expression of p-mTOR to enhance the anti-tumor effect of paclitaxel in gastric cancer [29], similarly, in NSCLC, the inhibitor of CK2 (casein kinase2), CX-4945, can also induced lung cancer cell apoptosis by lowering the expression of p-mTOR [30]. These evidences suggested that it is a new field that need to be further explored to treat gastric cancer.
Our experiments confirmed that paclitaxel can indeed exert anti-tumor effects on SGC-7901 human gastric cancer cell line and the possible mechanism maybe through suppressing the Akt/mTOR signaling pathway. Gastric cancer is a common malignancy in China, its treatment is of great importance for patients, paclitaxel has already been used as an chemotherapeutics in many malignancies including gastric cancer, detailed explore its mechanism in gastric cancer is vital for its further application in gastric cancer and can provide more effective guidance for the clinical treatment of gastric cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Leping Li, Department of Gastrointestinal Surgery, Shandong Provincial Hospital Affiliated to Shandong University, Jinan 250021, People’s Republic of China. Tel: +86 0531-68776388; E-mail: lileping060654@163.com

References

[16] Ang KL, Shi DL, Keong WW and Epstein RJ. Up-regulated Akt signaling adjacent to gastric can-


