RESEARCH ARTICLE

Taxol Produced from Endophytic Fungi Induces Apoptosis in Human Breast, Cervical and Ovarian Cancer Cells

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Abstract

Currently, taxol is mainly extracted from the bark of yews; however, this method can not meet its increasing demand on the market because yews grow very slowly and are a rare and endangered species belonging to first-level conservation plants. Recently, increasing efforts have been made to develop alternative means of taxol production; microbe fermentation would be a very promising method to increase the production scale of taxol. To determine the activities of the taxol extracted from endophytic fungus *N. sylviforme* HDFS4-26 in inhibiting the growth and causing the apoptosis of cancer cells, on comparison with the taxol extracted from the bark of yew, we used cellular morphology, cell counting kit (CCK-8) assay, staining (HO33258/PI and Giemsa), DNA agarose gel electrophoresis and flow cytometry (FCM) analyses to determine the apoptosis status of breast cancer MCF-7 cells, cervical cancer HeLa cells and ovarian cancer HO8910 cells. Our results showed that the fungal taxol inhibited the growth of MCF-7, HeLa and HO8910 cells in a dose-and time-dependent manner. IC50 values of fungal taxol for HeLa, MCF-7 and HO8910 cells were 0.1-1.0 μg/ml, 0.001-0.01 μg/ml and 0.01-0.1 μg/ml, respectively. The fungal taxol induced these tumor cells to undergo apoptosis with typical apoptotic characteristics, including morphological changes for chromatin condensation, chromatin crescent formation, nucleus fragmentation, apoptotic body formation and G2/M cell cycle arrest. The fungal taxol at the 0.01-1.0 μg/ml had significant effects of inducing apoptosis between 24-48 h, which was the same as that of taxol extracted from yews. This study offers important information and a new resource for the production of an important anticancer drug by endofungus fermentation.

Keywords: Apoptosis - fungal taxol - MCF-7 - HeLa - HO8910

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Introduction

The effects of an anticancer drug on tumor cells are dependent on not only its inhibition of tumor cell proliferation, but also the induction of apoptosis. Apoptosis is a form of cell death characterized by active cellular suicide during T-cell colonial deletion, embryogenesis, and DNA damage. Apoptotic cell death is often associated with distinctive characteristics, such as nuclear fragmentation, cytoplasmic blebbing, and nucleosomal fragmentation of DNA (Zhou and Zhu, 2003). Apoptotic cells are eventually swallowed by phagocytes or neighboring cells without causing any inflammatory reaction and the anticancer effects of chemotherapy drugs for many tumors are associated with the induction of apoptosis; therefore, apoptosis is an important symbol to evaluate the effect of anticancer drugs.

Taxol, a secondary compound with a complex diterpenoic structure, is found in various tissues of Taxus species (Srinivasan et al., 1996). This compound is the world's first billion-dollar anticancer drug, and widely used as an anticancer drug for breast cancer, ovarian cancer, prostate cancer, non-small cell lung cancers, adenocarcinoma and esophagus squamous cell carcinoma (Jones et al., 1996; Woo et al., 1996; Zhou and Zhu, 2003; Park et al., 2004; Henley et al., 2007; Tan and Yu, 2007). It is used to treat a number of other human tissue-proliferating diseases as well, alone or in combination with other anticancer agents. The anticancer properties of taxol are based on its ability of binding and stabilizing microtubules to block cell replication in the late G2-M phase of the cell cycle (Schiff et al., 1979; Fauzee, 2011).

Currently, taxol is mainly extracted from the bark of yew. The most important member of the clinically useful natural anticancer agent is Paclitaxel (Taxol®), which was first extracted from the bark of Taxus brevifolia (Wani et al., 1971). However, the amount of taxol is very low in yews, 0.01-0.03 % of Taxus bark dry weight and this has been a major factor contributing to its high prices. Therefore, this method can not meet the increasing

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demand of taxol on the market because yews grow very slowly and are a rare and endangered species belonging to the first-level conservation plants. Over the past few years, increasing efforts have been made to develop alternative methods of taxol production. The renewable sources for taxol, such as the needles of Taxus spp. or cell cultures and other alternative chemical biosynthetic methods are found to be too expensive for commercialization. A possibility of producing taxol in a cheaper way for industrial fermentation may come from the discovery of some endophytic fungi that belonging to different genera.

Using microbe fermentation in the production of taxol would be a very prospective method of obtaining a large amount of taxol. Several endophytic fungi that produce taxol have been isolated (Stierle et al., 1993; Li et al., 1996; Gangadevi and Muthumary, 2008; Kumaran et al., 2009; Zaiyou et al., 2013). Since 1993, we have screened samples from the inner bark (phloem-cambium) and xylem of Taxus cuspidata and isolated five new endophytic fungal species that can produce taxol. These fungi are Nodulisporium sylviforme (a new genus and new species in China) (Zhou et al., 2001), Pleurocytospora taxi (Sun et al., 2003), Alternaria taxi (Ge et al., 2004), Botrytis (Zhao et al., 2008a), and Aspergillus niger subsp. taxi (Zhao et al., 2009). The strain with high taxol output, N. sylviforme HDFS4-26, has been obtained after breeding by genome shuffling and can produce taxol over 516.37 μg/l (Zhao et al., 2008b).

The studies of taxol production from endophytic fungi quickly advanced, and many reports have demonstrated its effect on proliferation inhibition and apoptosis induction in various human carcinoma cell lines (Chakravarthi et al., 2013; Raj et al., 2014). In this study, we investigated the effects of the fungal taxol produced from the strain HDFS4-26 on the proliferation and apoptosis of human breast cancer MCF-7 cells, cervical cancer HeLa cells and ovarian cancer HO8910 cells. We observed that the fungal taxol exhibited the ability of inducing growth inhibition and cell apoptosis in these tumor cells in a dose- and time-dependent manner. The fungal taxol at the 0.01-1.0 μg/ml had significant effects of inhibiting cell growth between 24-48 h, which was the same as the effect of yews-derived taxol ($p > 0.05$). This is the first report on the antitumor effect of taxol produced from taxol-producing fungi on cancer cells, and our study offers important information and a new resource of producing this important anticancer drug by endofungus fermentation.

Materials and Methods

Strain and cell lines

N. sylviforme HDFS4-26 CCTCC M 208026 with a high taxol output of 516.37 μg/l, a taxol-producing endophytic fungus, was obtained by genome shuffling (Zhao et al., 2008). N. sylviforme HDFS4-26 (CCTCC M 208026) was deposited in the China Center for Type Culture Collection (Beijing, China).

Cervical cancer HeLa cells were obtained from State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute (HVRI), the Chinese Academy of Agricultural Sciences (CAAS). Human breast cancer cell line MCF-7 and ovarian cancer cell line HO8910 were obtained from the Fourth Affiliated Hospital of Harbin Medical University, China.

Reagents

Dulbecco's modified Eagle's medium (DMEM) was purchased from GIBCO (Grand Island, NY, USA). Cell Counting Kit (CCK-8) was purchased from Dojindo Laboratories (Kumamoto, Japan). Annexin V-FITC apoptosis detection kit was purchased from Bender Medsystems (Vienna, Austria). Taxol standard (from Taxus brevifolia, 99.9 % in purity), Trypan blue, Geimsa, Hoechst 33258, propidium iodide (PI) and RNase were obtained from Sigma (St. Louis, MO, USA).

Extraction and purification of taxol produced from the strain HDFS4-26

The fungal taxol was extracted from fermented fungal culture of stain HDFS4-26 by methanol and ethyl acetate method. The procedure of fungal taxol extraction, purification and analysis was carried out as previously described (Zhao et al., 2008b).

Assessment of cell viability

Cell viability was assessed by CCK-8 assay according to the supplier recommendations. HeLa, MCF-7 and HO8910 cells were cultured in DMEM and then diluted to 5×10^5 /ml, respectively. Cells were transferred to 96-well plates at 100 μl per well and cultured at 37°C and 5 % CO_2 for 24 h. 10-flod increased taxol from 0.001 to 100 μg/ml were added to the cells, followed by incubation at 37°C for 24 h, 48 h, 72 h, respectively. Ten microliters of WST-8 reagent were added and incubated for 3 h. Cell viability was expressed as the percentage of viable cells relative to untreated cells using the absorbance at 450 nm (OD450) read by an auto microplate reader (infinite M200, Tecan, Austria). Control cultures were treated with methanol. The maximum concentration of methanol added to the medium in this study was 0.01 %. OD450 was measured to determine the survival rate of the cells, which was calculated using the following formula: Survival rate (%) = [(As-Ab) / (Ac-Ab)] × 100%. The As is for the test wells (containing cell medium, cells, WST-8 and fungal taxol), Ab forthe blank wells (containing cell medium and WST-8), and Ac for the control wells (containing cell medium, cells and WST-8).

In vitro morphology

HeLa, MCF-7 and HO8910 cells were grown in 100 ml flasks. At 50 % confluence, they were treated with either DMEM medium, methanol or with concentrations of 0.001, 0.01, 0.1, 1.0, 10 and 100 μg/ml of fungal taxol, respectively. The cells were trypsinized and harvested after 24, 48 and 72 h. Subsequently the cells were fixed in 3.7 % formaldehyde and stained with Geimsa for 10 min. Cell morphological change was observed with an EVOS digital inverted microscope (Bothell, WA, USA). Photographs were taken with a Nikon FM 10 camera.

Detection of apoptosis

Apoptosis of cells was assessed by Hoechst 33258

and Geimsa staining. HeLa, MCF-7 and HO8910 cells were grown on coverslips and treated by different concentrations fungal taxol as described above. Cells were then fixed with 3.7 % formaldehyde, washed three times with Phosphate Buffered Saline (PBS), stained with the DNA intercalating dye Hoechst 33528 and observed under a Laser Scanning Confocal Microscope (LSM510, Zeiss, Germany). Hoechst 33258 staining images were acquired with a color CCD camera. In addition, apoptosis of cells was detected by Geimsa staining and observed with microscope.

Extraction of total DNA

After grinding 1.0 g of frozen HeLa, MCF-7 and HO8910 cells for enzyme extraction, the powder for each cell line was transferred to a sterilized Eppendorf tube, which 600 μl hot buffer containing 2 % (w/w) CTAB, 10 mmol/l Tris-HCl of pH 8.0, 20 mmol/l EDTA of pH 8.0, 1.4 mmol/l NaCl and 2 % (v/v) β-mercaptoethanol. The samples were incubated for 30 min at 65°C and then extracted with chloroform and isoamyl alcohol (24:1, v/v). The aqueous phase was precipitated with an equal volume of the pellet buffer (1 %, w/w CTAB, 50 mmol/l Tris-HCl of pH 8.0 and 10 mmol/l EDTA) for 30 min at 65°C. The total DNA was collected by centrifugation at 12, 000 r/min for 5 min and then re-suspended in a high salt TE buffer (10 mmol/l Tris-HCl of pH 8.0, 1.0 mmol/l EDTA of pH 8.0 and 1.0 mol/l NaCl). DNA was precipitated by addition of two volumes of ethanol at -20°C for 1 h. The DNA pellets were washed by centrifuging with 70 % (v/v) ethanol and then dried at 37°C. Finally, the total DNA was dissolved in 30 ml TE buffer (10 mmol/l Tris-HCl of pH 8.0, 1.0 mmol/l EDTA of pH 8.0) and small amount of RNase A (100 mg/ml) was added to digest RNA for 30 min at 37°C. Equal amount of DNA from each sample was separated on a 1.5 % (w/w) agarose gel by electrophoresis at 50-60 v for 2-4h and stained with ethidium bromide.

Cell cycle detection

Cell cycle distribution was analyzed using FCM. Briefly, cells were seeded into 6-well plates and grown for 24h and further grew in the presence of fungal taxol increased 10 fold from 0.01 to 100 μg/ml for 24, 48 and 72 h, respectively. The cells were harvested, washed twice with PBS, and fixed in 70 % ethanol on 4°C for 1h and centrifuged. The pellet was treated with RNase (20 μg/ml) at room temperature for 30 min and then incubated with PI (10 μg/ml) for 30 min. DNA content and cell cycle distribution were analyzed using FCM as decribed by the manufacturer.

Statistics analysis

All experiments were repeated three times and each measured in triplicate. Results were expressed as mean ± standard deviation (SD). Student's t-test was used to compare the mean differences between samples by the statistical software SPSS version 10.0. Throughout the work, P values less than 0.05 were considered to be statistically significant.

Results

Assessment of cell viability

To determine the effect of fungal taxol on the growth of HeLa, MCF-7 and HO8910 cells, we treated these cells with different concentrations of fungal taxol for 24, 48 and 72 h, and assessed the survival and proliferation of these cells by CCK8 assays (Figure 1). The results presented in Figure 1 revealed that fungal taxol at 0.001, 0.01, 0.1, 1.0, 10 and 100 μg/ml for 24h reduced the proliferation and survival of HO8910, MCF-7 and HeLa cells in a dose-dependent fashion, and that the IC_{50} values of fungal taxol fell between 0.1-1.0 μg/ml.

Figure 1 showed that fungal taxol at 0.001, 0.01, 0.1 and 1.0 μg/ml for 48h reduced significantly the

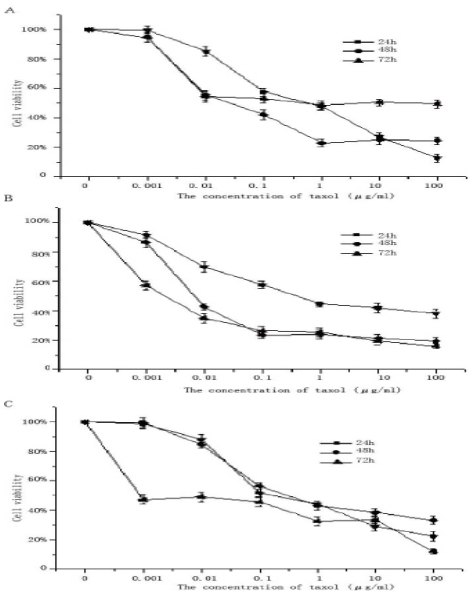

Figure 1. Figure 1 Shows the Viability of HO8910, MCF-7 and HeLa Cells after the Treatment by the Fungal Taxol. A) HO8910 cells; **B)** MCF-7 cells; **C)** HeLa cells

Table 1. Viability of HO8910, MCF-7, and HeLa Cells Treated with the Different Concentrations of Methanol for 24 h, 48 h and 72 h, Respectively

Concentration of methanol (%)/ concentration of taxol (μg/ml)	Viability of HO8910 cells (%)			Viability of MCF-7 cells (%)			Viability of HeLa cells (%)		
	24	48	72	24	48	72	24	48	72
9.6/100	40.1±2.1	38.5±1.8	50.1±1.9	18.9±0.9	14.1±0.6	10.2±0.3	29.4±0.7	52.6±2.0	40.6±2.4
0.96/10	75.2±3.3	88.8±3.9	66.5±2.2	46.2±1.9	33.6±1.3	30.2±1.1	59.7±2.3	70.7±2.7	66.3±2.5
0.096/1	100.5±4.1	95.9±3.7	75.9±3.3	59.1±2.7	77.9±3.2	41.3±1.9	83.6±3.9	86.2±3.5	94.4±3.9
0.0096/0. 1	104.2±4.5	95.2±5.2	85.3±3.7	76.5±4.1	90.1±4.8	52.4±1.8	87.6±4.1	94.9±4.3	99.8±4.8
0.00096/0.01	104.4±4.7	96.1±4.4	96.1±4.2	103.9±5.1	102.5±4.7	63.2±2.1	95.2±4.4	96.1±4.1	101.2±5.0

proliferation and survival of HO8910, MCF-7 and HeLa cells in a dose-dependent fashion ($p<0.05$), and that cell survival rate did not change significantly with doses higher than 0.001-1.0 μg/ml ($p<0.05$). IC_{50} values of fungal taxol for HO8910, MCF-7 and HeLa cells were 0.01-0.1 μg/ml, 0.001-0.01 μg/ml and 0.1-1.0 μg/ml, respectively. No significant difference was seen in the proliferation and survival of HO8910, MCF-7 and HeLa cells for the concentration ranging 0.01 to 100 μg/ml of fungal taxol for 72h after the fungal taxol treatment ($p<0.05$).

Meanwhile, we used the same concentration of methanol that used to dissolve fungal taxol as negative control. The results showed that methanol at higher than 0.096 % for 24h or 48h affected significantly the proliferation and survival of HO8910 cells ($p<0.05$), methanol at higher than 0.0096 % for 72h affected significantly the proliferation and survival of HO8910 cells ($p<0.05$) (Table 1); and that methanol at higher than 0.0096 % for 24h or 48h affected significantly the proliferation and survival of MCF-7 and HeLa cells ($p<0.05$), methanol at higher than 0.00096 % for 72h affected significantly the proliferation and survival of MCF-7 cells ($p<0.05$) (Table 1). In addition, HeLa, MCF-7 and HO8910 cells were also treated with 0.001-1.0 μg/ml of taxol extracted from yews (Sigma) for 48h and we did not observe any significant difference between the group treated by taxol extracted from yews and the group cells treated with fungal taxol ($p<0.05$). The findings indicated that fungal taxol and yews-derived taxol have the same inhibitory effect on cancer cells. Meanwhile, fungal taxol significantly reduced cell viability of HeLa, MCF-7 and HO8910 cells ($p<0.05$), and the inhibition was in a concentration- and time-dependent fashion. Therefore, we determined that the optimal inhibition concentration of fungal taxol was 0.1 μg/ml for HO8019, 0.01 μg/ml for MCF-7 and 0.1μg/ml for HeLa, the time for treatment was 48 h. Table 1 showed the same concentration of methanol as that used to dissolve the taxol for 0.1 μg/ml or 0.01 μg/ml have no significant effect on these cells alone.

In vitro morphology

We monitored the morphological changes of the HeLa, MCF-7 and HO8910 cells after 48h treatment of the fungal taxol at different concentrations. At this time point, MCF-7 and HeLa cells treated with 0.01 μg/ml of fungal taxol began to shrink and spherical in shape (Figure 2B and Figure 2C), and the cell morphology was basically the same to that of cells treated with the same concentration of taxol from yews, while some HO8910 cells began to come off from the flask sidewall and cracked (Figure 2A). Most cells treated with 10 μg/ml of fungal taxol came off from the flask sidewall, cracked and released cell contents (Figure 2). Similar to the results of cell viability, these results suggested that the fungal taxol induced cell death was concentration-dependent.

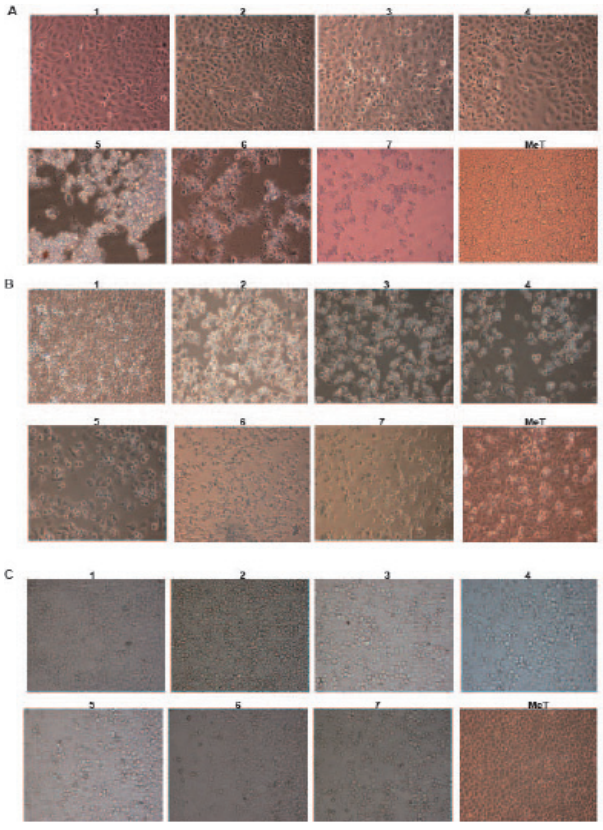

Figure 2. Figure 2 Documents the Morphology of HO8910, MCF-7 and HeLa Cells Treated with the Different Concentration of Fungal Taxol for 48 h (200×). A) HO8910 cells; **B)** MCF-7 cells; **C)** HeLa cells. 1: negative control (no taxol added); 2: 0.001 μg/ml; 3: 0.01 μg/ml; 4: 0.1 μg/ml; 5: 1μg/ml; 6: 10 μg/ml; 7:100 μg/ml. MeT: shows the morphology of HO8910 (A), MCF-7(B) and HeLa (C) cells treated with 0.0096%, 0.000096% and 0.0096% methanol respectively

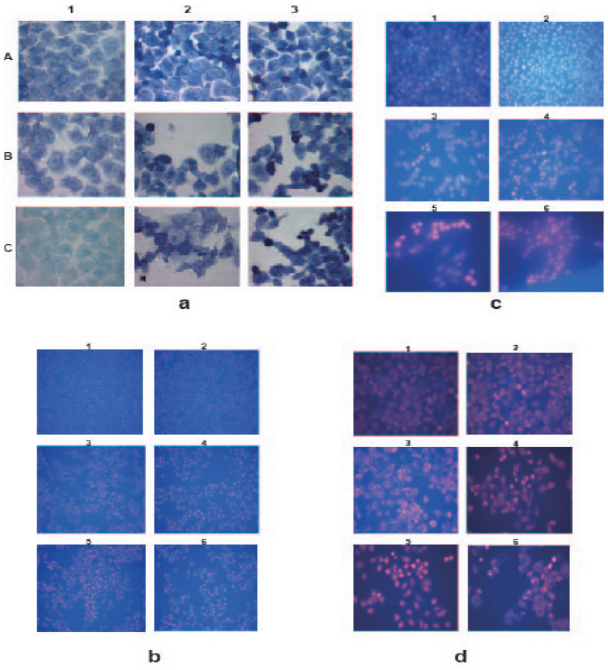

Figure 3. Figure 3a Shows the Geimsa Staining of HO8910, MCF-7 and HeLa Cells Treated with the Different Concentration of Fungal Taxol for 24 h, 48h and 72 h, Respectively. A) HO8910 cells; **B)** MCF-7 cells; **C)** HeLa cells; 1: negative control (no taxol added); 2: 0.01μg/ml; 3:0.1μg/ml. Figure 3b, c, and d documents the Hoechst 33258 staining of HO8910, MCF-7 and HeLa cells treated with the different concentrations of fungal taxol for 48 h. 1: no taxol; 2: 0.001 μg/ml; 3: 0.01 μg/ml; 4: 0.1 μg/ml; 5: 1.0 μg/ml; 6: 10 μg/ml

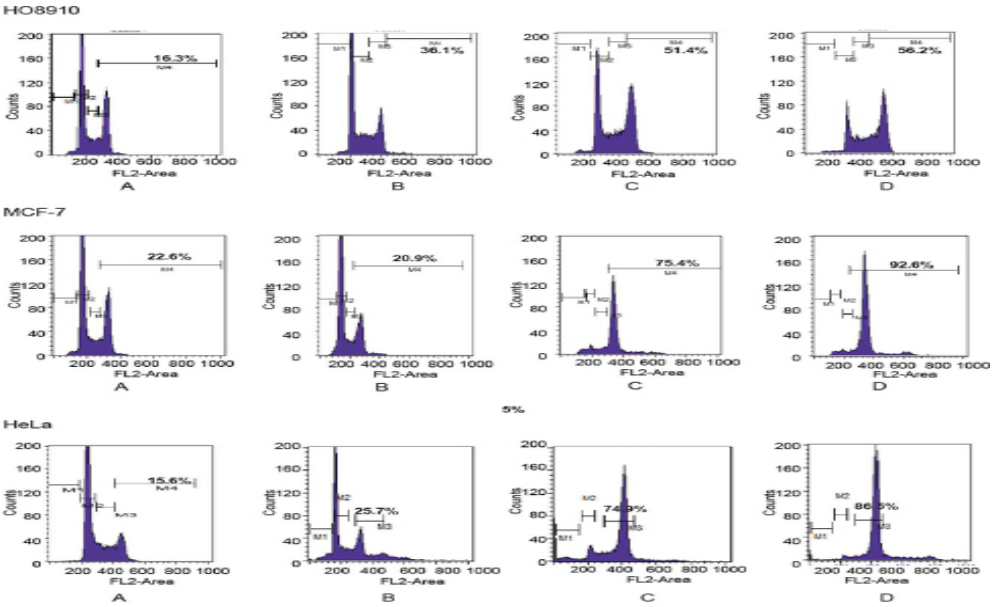

Figure 4. Figure 4 Shows the FCM Analysis of Cell Cycle of HeLa, MCF-7 and HO8910 Cells Treated with the Different Concentration of Fungal Taxol for 48 h. A: no taxol; B-D: 0.01-1.0 μg/ml

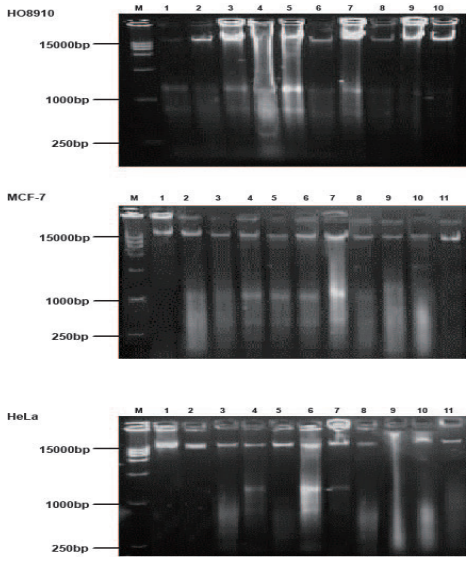

Figure 5. Figure 5 Documents the Analysis of Cell Genomic DNA of HeLa, MCF-7 and HO8910 Cells Treated with the Different Concentrations of Fungal Taxol for 24 h, 48h and 72 h, Respectively. HO8910 cells, M: DNA markers of 15000 bp; 1: 72h 0.01 μg/ml; 2: 48h 0.01 μg/ml; 3: 24h 0.01 μg/ml; 4: 72h 0. 1 μg/ml; 5: 48h 0. 1 μg/ml; 6: 24h 0.1 μg/ml; 7: 48h 1.0 μg/ml; 8: 24h 1.0 μg/ml; 9: 0.96% Meth control 10: 0 μg/ml. MCF-7 cells, M: DNA markers of 15000 bp; 1: 0 μg/ml; 2: 24h 0.01 μg/ml; 3: 24h 0.1 μg/ml; 4: 24h 1.0 μg/ml; 5: 48h 0.01 μg/ml; 6: 48h 0. 1 μg/ml; 7: 48h 1.0 μg/ml; 8: 72h 0.01 μg/ml; 9: 72h 0.1 μg/ml; 10: 72h 1.0 μg/ml; 11: 0.96% Meth control. HeLa cells, M: DNA markers of 15000 bp; 1: 0 μg/ml; 2: 0.96% Meth; 3: 24h 1.0 μg/ml; 4: 24h 0.1 μg/ml; 5: 24h 0.01 μg/ml; 6: 48h 1.0 μg/ml; 7: 48h 0.1 μg/ml; 8: 48h 0.01 μg/ml; 9: 72h 1.0 μg/ml; 10: 72h 0.1 μg/ml; 11: 72h 0.01 μg/ml

Compared to the control cells in Figure 2, no significant change was observed in cell morphology of HO8910, MCF-7 and HeLa cells after treatment with 0.0096 %, 0.000096 % and 0.0096 % of methanol at 48 h, respectively (MeT cells in Figure 2).

Detection of cell apoptosis

To determine whether the fungal taxol can induce apoptosis, we treated HeLa, MCF-7 and HO8910 cells with 0.1 μg/ml or 0.01 μg/ml of fungal taxol for 48 h, and assessed the changes of cell apoptotic morphology, nuclear condensation and nuclear fragmentation by Geimsa stain and Hoechst 33258 staining, respectively. Figure 3a showed that 48h of treatment by taxol could induce the shrinkage of the cell membrane, condensation of cytoplasm and fragmentation of the nucleus, which is the characteristic of apoptosis in eukaryotic cells.

With increasing concentration of fungal taxol, more cells began to shrink and became round in shape, separated from surrounding cells, and showed darkly stained chromatin. Figure 3b, c and d showed similar results when analyzed by Hoechst33258 staining after treatment 48h by 10-flod increased fungal taxol. The results suggested that fungal taxol induced the concentration-and time-dependent apoptosis of HeLa, MCF-7 and also HO8910 cells.

FCM analysis of cell cycle

To further determine the effect of fungal taxol on cell cycle of HeLa, MCF-7 and HO8910 cells, we used different concentrations of fungal taxol to treat these three cell lines. The treated cells were stained by PI and then analyzed by FCM. After 48h of the treatment at 0.01 to 1 μg/ml of fungal taxol, we observed an increased G2/M arrest of HeLa, MCF-7 and HO8910 cells (Figure 4). As shown in Figure 4, with increased fungal taxol from 0.01 to 1.0 μg/ml, more G2/M-enriched fraction of cells was observed and the typical apoptotic peak (M1) appeared after treatment. These results suggested that the fungal taxol induced G2/M cell cycle arrest in a dose-dependent manner. The higher the concentration of taxol, the higher the percentage of cells arresting at G2/M phase was detected, which was consistent with the results obtained by Hoechst 33258 staining.

Analysis of cell genomic DNA

To further confirm the occurrence of cell apoptosis, we extracted DNA from the treated cells and analyzed it by agarose gel electrophoresis (Figure 5). Distinct DNA ladders with multiples of 100-1000 bp showing DNA cleavage into oligonucleosomal fragments, the hallmark of programmed cell death, were clearly seen at about 24h and 48h of treatment (Lane 4, 5, 7 of HO8910 cells; Lane 7 of MCF-7 cells and Lane 6 of HeLa cells) compared with the control. The appearance of the DNA smear observed after 48h treatment (Lane 4 of HO8910 cells; Lane 7 of MCF-7 and Lane 6 of HeLa cells) reflected the transformation of cells from the apoptosis to the dying states. This result suggested that the fungal taxol could induce nucleosomal DNA degradation through apoptosis of these cancer cells.

Discussion

Taxol extracted from yews is a powerful anticancer agent known to promote microtubule assembly, inhibit microtubule depolymerization, and change microtubule dynamics, resulting in disruption of the normal reorganization of the microtubule network required for mitosis and cell proliferation (Amos and Lowe, 1999). Therefore, cells treated with taxol are unable to proceed normally through the cell cycle and are arrested in the G1 and G2/M phases (Chandar et al., 1992; Yoo et al., 1998; Mullan et al., 2001). Moreover, additional activities of taxol extracted from the bark of yews have been described including its effect on 1) cell signaling and gene expression, activation of mitogen activated protein kinases, Raf-1, protein tyrosine kinases, and c-Jun NH2-terminal kinase, 2) triggering apoptosis by caspase-dependent and -independent pathways as well as necrosis and regulating the expression of apoptosis-related proteins like Bcl-2, Bad, Bcl-xL, p21WAF-1/CIP-1, tumor necrosis factor-α (TNF-α) receptor 1 (TNFR1), and the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors DR4, DR5, and 3) inducing apoptosis in various cell lines (Park et al., 2004). Although previous studies have shown that taxol extracted from yews can induce apoptosis of many cancers, it is remains unclear whether the taxol produced from N. sylviforme can also induce apoptosis in cancer cells and whether it has similar effect to that of taxol widely used in clinical treatment.

In this study, we have investigated the effects of the fungal taxol on the proliferation and apoptosis of HeLa, MCF-7 and HO8910 cells. The cytotoxic effects of fungal taxol were tested by the MTT assay, which showed that the effect of fungal taxol on the cell viability of MCF-7 cells for 24, 48 and 72 h. Our results showed a dose-and time-dependent increase in tumor cell mortality, and its concentration-and time-effect relationships were significant (seen in Figure 1). IC_{50} values of the fungal taxol for HeLa, MCF-7 and HO8910 cells were 0.1-1.0 μg/ml, 0.001-0.01 μg/ml and 0.01-0.1 μg/ml, respectively. Thus, the fungal taxol isolated from N. sylviforme HDFS4-26, exhibited a high degree of in vitro cytotoxic activity against cancer cells.

Apoptosis is a genetically controlled mechanism essential for the maintenance of tissue homeostasis (Signore et al., 2013). In mammalian cells, two major apoptosis pathways are proposed: the first one involves signal transduction through death receptors; the second relies on a signal from the mitochondria (Dodson et al., 2013). Induced apoptosis plays a critical role in suppressing the proliferation of hepatoma cells by taxol, and many changes can be used to detect apoptosis (Davies et al., 2013; Ruden and Puri, 2013). Consistent with previous reports from taxol produced by Taxus, we for the first time demonstrated that the fungal taxol could induce apoptosis of HO8910, MCF-7 and HeLa cells (seen in Figure 2 and Figure 3). These effects lead to the cell cycle arrest of the cells in the G2/M phase and eventually to apoptotic cell death (seen in Figure 4) (Henley et al., 2007; Samadi et al., 2009). Either the assessment of cell viability, in vitro morphology analysis, Geimsa staining and Hoechst 33258 staining, cell genomic DNA analysis or FCM analysis strongly suggested that increases of concentrations or treatment time of the fungal taxol made the apoptosis more pronounced.

Although our results in this study and previous reports have determined the effects of inducing apoptosis and the accordance of taxol from endophytic fungi and yew tree, there are many unclear aspects. Therefore, more work is needed for a better understanding of the fungal taxol-induced cell apoptosis, such as the molecular mechanism of fungal taxol-induced cell apoptosis. This study offers important information and a new resource for the production of an important anticancer drug by endofungus fermentation to meet the increasing demand for taxol on the market.

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