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Proteomic analysis of anti-cancer effects by paclitaxel treatment in cervical cancer cells

Keun-Ho Lee[a,1], Eun-Kyoung Yim[b,1], Chan-Joo Kim[a], Sung-Eun Namkoong[a], Soo-Jong Um[c], Jong-Sup Park[a,b,*]

[a]*Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, The Catholic University of Korea, 505 Banpo-dong, Seocho-gu, Kangnam St. Mary's Hospital, Seoul 137-040, Korea*
[b]*Department of Medical Bioscience, Graduate Schools, The Catholic University of Korea, 137-040 Seoul, Korea*
[c]*Department of Bioscience and Biotechnology/Institute of Bioscience, Sejong University, 143-747 Seoul, Korea*

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Abstract

Objectives. Paclitaxel (Taxol®), a potent drug of natural origin isolated from the bark of the Pacific yew, is widely used in the treatment of ovarian, lung and breast cancer. At present, there is little information regarding the anti-cancer mechanism of paclitaxel against cervical carcinoma cells. We thus tried to show the anti-cancer effect of paclitaxel on cervical carcinoma cell line carrying HPV by using a proteomic analysis and to investigate the mechanism of actions.

Methods. We treated paclitaxel to cervical carcinoma cells and then carried out MTT assay to observe the anti-proliferate activity. Using proteomics analysis including two-dimensional (2-DE) gel electrophoresis and MALDI-TOF-MS, we tried to find the anti-proliferate activity-related proteins. Among them, paclitaxel treatment suppressed the expression of the mitotic checkpoint protein BUB3. Functional proteomic analysis by small interfering RNA (siRNA) targeting was tried to illuminate a role of mitotic checkpoint protein BUB3 in cell cycle progression.

Results. The cytotoxicity effects of paclitaxel were determined in HPV-16 positive CaSki, HPV-18 positive HeLa and HPV-negative C33A cervical carcinoma cell lines. Using efficient proteomics methods including 2-DE/MALDI-TOF-MS, we identified several cellular proteins that are responsive to paclitaxel treatment in HeLa cells. Paclitaxel treatment elevated mainly apoptosis-related, immune response-related and cell cycle check point-related proteins. On the other hand, paclitaxel treatment diminished growth factor/oncogene-related proteins and transcription regulation-related proteins. Paclitaxel showed anti-proliferate activity through the membrane death receptor (DR)-mediated apoptotic pathway involving activation of caspase-8 with a TRAIL-dependent fashion as well as the mitochondrial-mediated pathway involving down-regulation of bcl-2 by cytochrome *c* release. Furthermore, we found siRNA-induced BUB3 knock down on cell cycle progression blocked by cell cycle arrest after paclitaxel treatment.

Conclusions. The proteome profiling technique provided a broad-base and effective approach for the identification of protein changes induced by paclitaxel and showed anti-proliferate activity through the membrane death receptor-mediated apoptotic pathway, the mitochondrial-mediated pathway. This study shows the power of proteomic profiling with functional analysis using RNAi technology for the discovery of novel molecular targets and a better understanding of the actions of paclitaxel at the molecular level in cervical carcinoma cells.

Keywords: Paclitaxel; HPV; Cervical carcinoma; Proteomics; Small interfering RNA (siRNA); Mitotic checkpoint protein BUB3

Introduction

Taxanes, paclitaxel and docetaxel are important anti-tumor drugs endowed with a unique mechanism of action: they inhibit microtubule from disassembled [1]. The taxanes

* Corresponding author. Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, The Catholic University of Korea, 505 Banpo-dong, Seocho-gu, Kangnam St. Mary's Hospital, Seoul 137-040, Korea. Fax: +82 2 595 8774.
E-mail address: jspark@catholic.ac.kr (J.-S. Park).
[1] Contributed equally to this work as first authors.

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bind to a subunit of the tubulin heterodimers that form cellular microtubules; the binding of the taxanes accelerates the polymerization of tubulin, effectively stabilizing and inhibiting the depolymerization of the microtubules. Nocodazole and the vinca alkaloids in contrast inhibit the polymerization of the tubulin heterodimers, in turn preventing the formation of microtubules by turn [2]. The taxanes currently in clinical use are effective against a broad spectrum of human tumors, in particular, ovarian, lung and breast cancers [3,4]. They have demonstrated a significant activity against many solid tumors as single agent or as an agent combined with other chemotherapeutic agents [5,6]. Recently, a variety of cellular and molecular effects of paclitaxel have been discovered. These include induction of cytokines and tumor suppressor genes and activation of signal transduction pathways.

Proteomics is being used to characterize the molecular events occurring in disease processes [7] to identify polymorphisms in genes that may be associated with increased (or decreased) risk of specific diseases [8] and to search for biomarkers of disease [9]. In addition, these technologies offer great opportunities to explore and characterize the mechanistic action of anti-cancer drug at a molecular level [10]. The usage of proteomics is partly limited because proteins undergo numerous post-translational modifications—phosphorylations, glycosylations, as well as many other modifications, which are critical to their functions, as they can determine activity, stability, localization and turnover. To make a further analysis of the proteomics, functional studies involving Western blot, gene transfection and gene knock down are required.

It is well known that infection with HPV is the main cause of cervical cancer, and certain types of HPV are recognized as carcinogens [11,12]. In advanced or recurrent cancer of cervix, Gynecologic Oncology Group conducted phase II trial which demonstrated the moderate activity of paclitaxel with the result of 17% response [13]. In another NCI-supported study, the activity of paclitaxel with the result of 21% partial response was shown in advanced or recurrent cancer of the cervix [14]. After these trials, paclitaxel is widely applied against cervical cancer. However, at present, there is not enough information regarding the anti-cancer mechanisms of paclitaxel against cervical carcinoma cells and even if there is, most of them do not correlate with the HPV as a potent cause of cervical carcinoma.

This report describes the first evaluation using 2-DE/MALDI-TOF-MS to profile differential protein expression in cervical carcinoma cells through paclitaxel-induced cell death. The elucidation of survival and apoptotic proteins activated or down-regulated in cancer cells will be the key solution to improve cancer therapy. Using two-dimensional gel electrophoresis to profile proteins that are unique to paclitaxel treatment, we identified an interesting protein, mitotic checkpoint protein BUB3 (budding uninhibited by benzimidazole 3). Furthermore, we suggest that BUB3 function may be crucial for cell cycle arrest through siRNA knock out experiment. Overall, this study shows the power of proteomic profiling accompanying with functional study for the discovery of potential cancer-specific biomarker.

Materials and methods

Cell culture and cytotoxicity assay

HPV-16 positive CaSki, HPV-18 positive HeLa and HPV-negative C33A cervical carcinoma cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum at 37°C in 5% CO_2. The cytotoxic studies were carried out using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazonium bromide (MTT) (Sigma, St. Louis, MO) representing the percentage of growth inhibition induced by increasing drug concentrations. The cervical carcinoma cells (1×10^4/well) were incubated with paclitaxel (Taxol®, BMS, Montreal, Canada) for 24 h, 48 h, 72 h, 96 h and 120 h on the 96-well plates. After 4 h incubation with MTT substrate (20 mg/ml), the culture medium was removed and DMSO added. The cytotoxicity was evaluated with reference to the IC_{50} value. The tests were performed at least three times.

Two-dimensional gel electrophoresis and image analysis

HeLa cervical carcinoma cells were treated with 0.02 μM Taxol® and harvested by trypsinization after 24 h incubation. Cell pellets were solved in a lysis buffer containing 7 M urea, 2 M thiourea and 4% CAHPS. After sonication, 0.05 mg of total protein was loaded onto immobilized pH 3–10 (linear) IPG strip (Amersham Bioscience, Arlington Heights, IL) at 20°C using a Ettan IPG phor (Amersham Bioscience). The IPG strip were rehydrated overnight in a solution of 7 M urea, 2 M thiourea, 4% CHAPS, 45 mM DTT, 0.5% IPG buffer, 400 mM Tris and a trace of bromophenol blue prior to use. IEF was carried out using the following conditions: 100 V, 50 VH; 300 V, 150 VH; 600 V, 300 VH; 1000 V, 500 VH; 3000 V, 1500 VH; 7000 V, 42,000 VH. Focused gels were stored at −20°C prior to SDS-PAGE then incubated for 15 min in equilibration solution (50 mM Tris–Cl, 6 M urea, 30% glycerol, 2% SDS, 0.002% BPB) with 100 mg/ml DTT followed by 15 min in equilibration buffer with 25 mg/ml iodoacetamide and rinsed with SDS-PAGE buffer (25 mM Tris–Cl, 192 mM glycine, 0.1% SDS). Proteins were visualized by silver stain. The stained 2D gels were scanned with a Bio-Rad Scanner (Bio-Rad, Philadelphia, PA). The image analysis and 2D gel proteome database management were done using the PDQuest software 6.2.1 (Bio-Rad). For identification of proteins by mass spectrometry, matching was done between analytical silver-stained gels and preparative gels in order to correlate the precise position if the spots to be excised.