RESEARCH COMMUNICATION

Identification of Pathways Involved in Paclitaxel Activity in Cervical Cancer

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Abstract

Paclitaxel is one of the key chemotherapeutic drugs widely used to treat various types of cancer. Many cervical cancer patients exhibit selectivity in response to thereapy, however, which is considered to be correlated with drug-gene-pathways. The aim of this study was to identify pathways involved in paclitaxel activity in cervical cancer. Gene expression data was obtained from the NCBI Gene Expression Omnibus and the associations between paclitaxel and genes from DrugBank, MATADOR, TTD, CTD and SuperTarget databases. Differentially expressed genes in cervical cancer were identified using the significance analysis of microarrays (SAM) statistical technique. Pathway analysis was performed according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database using the software package SubpathwayMiner to predict target genes of paclitaxel in cervical cancer and regulated pathways. We found that paclitaxel, which exhibits anticancer activity in cervical cancer, may interact with these differentially expressed genes and their corresponding signaling pathways. Our study presents the first in-depth, large-scale analysis of pathways involved in paclitaxel activity in cervical cancer. Interestingly, these pathways have not been reported to be involved in other tumors. Thus our findings may contribute to the understanding of the mechanisms underlying paclitaxel resistance in cervical cancer.

Keywords: Paclitaxel - cervical cancer - signaling pathway - resistance

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Introduction

Cervical cancer is the leading cause of cancer-related death in the female population worldwide. Tumorigenesis is associated with alterations in cellular signaling pathways that control the crucial events of cell function. These pathways are essential to normal cell growth regulation and cell fate determination, and their disregulation is crucially implicated in cancer development.

Paclitaxel is one of the key chemotherapeutic drugs widely used to treat various types of cancer such as cervical cancer, ovarian cancer, lung cancer, breast cancer, and gastric cancer (Gogas et al.; Hogberg; Kim et al.; Komuta et al.; Park et al., 2009; Saito et al., 2010). Despite impressive initial clinical responses, the majority of patients eventually develop some degree of resistance to paclitaxel-based therapy. Many cervical cancer patients exhibit selectivity for paclitaxel, which is considered to be correlated with drug-gene-pathway. But the drug-gene-pathway relationship is complex, and it has not been analyzed. Therefore, in this study we performed a bioinformatics analysis with the aim to identify pathways involved in paclitaxel activity in cervical cancer.

Materials and Methods

Description of Data Sets

Based on previously reported studies (Biewenga et al., 2008; Scotto et al., 2008; Rajkumar et al., 2009), the gene expression data were obtained from National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/, accession numbers: GSE7410, GSE9750, GSE14404), and we used the "*.cel" files as raw data for this analysis. The data regarding the association between paclitaxel and genes were obtained from DrugBank, the Manually Annotated Targets and Drugs Online Resource (Matador), Therapeutic Targets Database (TTD), Comparative Toxicogenomics Database (CTD), and SuperTarget databases.

Extraction of Differentially Expressed Genes in Cervical Cancer

Differentially expressed genes in cervical cancer were extracted using the significance analysis of microarrays (SAM) statistical tool and the false discovery rate (FDR) was set as 0.05.

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Table 1. The Statistically Significant Enriched Sub-pathways Identified by Subpathway Miner for Paclitaxel and Differentially Expressed Genes in Cervical Cancer

Pathway Name	Subpathway ID(p-value)	Differentially expressed genes (geneID)
MAPK signaling pathway	path:04010_10 (2.11E-09)	4609;5601;5599; 5595;5594;4137;
	path:04010_15 (1.55E-15)	5601;5599;836;208;207;10000;5595;5594;
	path:04010_19 (7.49E-08)	836;208;207;10000;
	path:04010_23 (1.43E-11)	5601;5599;836;208;207;10000;5595;5594;
	path:04010_5 (2.59E-09)	836;208;207;10000;
ErbB signaling pathway	path:04012_5 (6.70E-07)	208;207;10000;
	path:04012_6 (4.32E-09)	208;207;10000;1026;
	path:04012_9 (2.25E-10)	5595;5594;4609;5601;5599;
p53 signaling pathway	path:04115_1 (3.32E-09)	836;54205;
	path:04115_2 (1.08E-08)	27113;54205;581;1026;5728;
mTOR signaling pathway	path:04150_1 (1.85E-06)	208;207;10000;7422;
	path:04150_3 (4.32E-09)	208;207;10000;5595;5594;
Apoptosis	path:04210_2 (5.65E-09)	208;207;10000;581;
	path:04210_4 (0)	54205;840;330;329;836;
	path:04210_5 (0)	54205;840; 330;329;836;596;598;
	path:04210_6 (0)	840; 330;329;836;596;598;
	path:04210_7 (1.46E-10)	208;207;10000;
	path:04210_8 (1.24E-13)	54205;840;330;329;836;
VEGF signaling pathway	path:04370_2 (0.000549)	208;207;10000;
	path:04370_6 (1.17E-08)	208;207;10000;
Focal adhesion	path:04510_11 (1.01E-07)	5601;5599;5595;5594;
	path:04510_16 (1.23E-09)	5601;5599;208;207;10000;
	path:04510_5 (8.82E-08)	7422;5728;208;207;10000;
	path:04510_8 (3.23E-11)	5595;5594;596;330;329;
T cell receptor signaling pathway	path:04660_5 (2.05E-05)	208;207;10000;
	path:04660_6 (0.000444)	5595;5594;
Pathways in cancer	path:05200_15 (3.23E-08)	4609;5595;5594;
	path:05200_16 (0)	596;1026;54205;598;208;207;10000;
	path:05200_17 (0)	330;329;596;581;54205;598;208;207;10000;
	path:05200_18 (3.22E-15)	7422;596;332;4609;5595;5594;598;
	path:05200_19 (1.55E-11)	332;4609;598;208;207;10000;
	path:05200_2 (8.92E-10)	1026;208;207;10000;
	path:05200_32 (3.87E-08)	7422;5925;4609;5595;5594;
	path:05200_33 (1.04E-11)	1026; 208;207;10000;
	path:05200_36 (0)	596;581;54205;208;207;10000;

Detection of Significantly Regulated Pathways involved in Paclitaxel Activity

Differentially expressed genes in cervical cancer were mapped to the pathways involved in paclitaxel activity according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa et al., 2006). KEGG is a collection of manually drawn pathway maps based on molecular interaction and reaction networks for metabolism, genetic information processing, cellular processes and human diseases (http://www.genome.jp/kegg/kegg2.html). The SubpathwayMiner subpathway identification system (Li et al., 2009) was used to analyze the detected pathways and the differentially expressed genes.

Results

Significantly Enriched Sub-pathways involved in Paclitaxel activity in Cervical Cancer

We identified differentially expressed genes using SAM (FDR=0.05) and these genes were mapped to KEGG pathways which have been associated with paclitaxel. The predicted gene targets in cervical cancer and the regulated pathways (including entire pathways and sub-pathways) were identified by SubpathwayMiner (Tables 1 and 2).

Table 2. Gene ID Numbers(http://www.ncbi.nlm.nih.gov/gene)

geneID	gene	geneID	gene	geneID	gene
5594	MAPK1	581	BAX	840	CASP7
5595	MAPK3	598	BCL2L1	4609	MYC
5601	MAPK9	596	BCL2	1026	CDKN1A
5599	MAPK8	54205	CYCS	5728	PTEN
207	AKT1	330	BIRC3	7422	VEGFA
208	AKT2	329	BIRC2	4137	MAPT
10000	AKT3	836	CASP3	27113	BBC3

Relationship between Paclitaxel and Two Signaling Pathways in Cervical Cancer

Among the identified pathways, we described the epidermal growth factor receptor (ErbB) and mitogen-activated protein kinase (MAPK) signaling pathways in more detail. There are three sub-pathways significantly associated with paclitaxel in the ErbB signaling pathway ($P < 0.01$) (Figure 1A-C). As shown in Figure 1D, the ErbB pathway is associated with both the MAPK and mammalian target of rapamycin (mTOR) pathways. Enzymes were marked by blue letters surrounded by a red line if they were identified in the submitted sets of genes as both target genes of paclitaxel and differentially expressed genes. On the other hand, the MAPK signaling

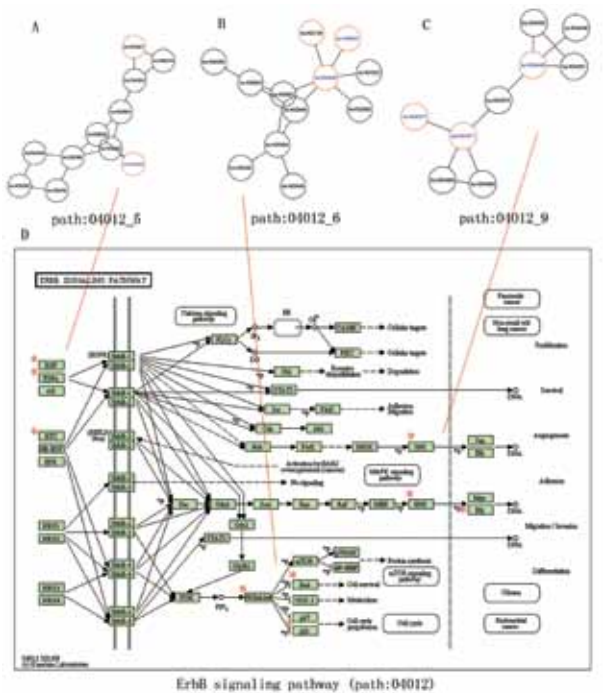

Figure 1. ErbB Signaling Pathways (Path: 04012). A-C. Visualization of sub-pathways as the undirected graph. Enzymes are colored red if the corresponding genes are identified as differentially expressed genes in cervical cancer. Enzymes are marked by blue letters if the corresponding genes are identified as both target genes of paclitaxel and differentially expressed genes in cervical cancer. D. Visualization of the ErbB signaling pathway (path: 04012) through linkage to the KEGG website. *Differentially expressed genes in cervical cancer

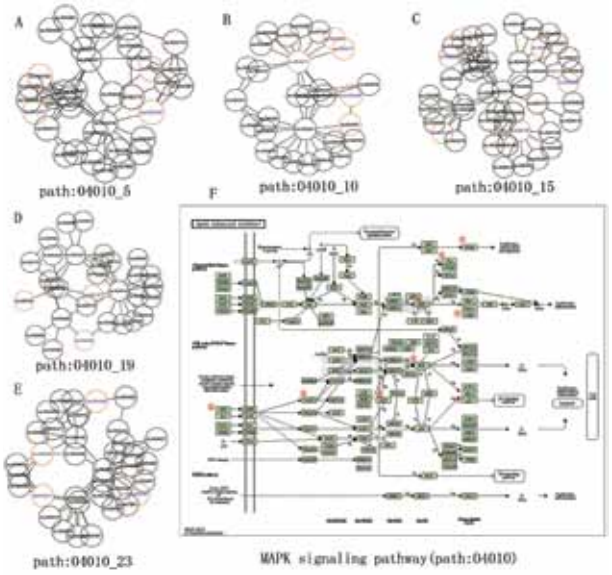

Figure 2. MAPK Signaling Pathways (Path: 04010). A-E. Visualization of sub-pathways as the undirected graph. Enzymes are colored red if the corresponding genes are identified as differentially expressed genes in cervical cancer. Enzymes are marked in blue if the corresponding genes are identified as both target genes of paclitaxel and differentially expressed genes in cervical cancer. F. Visualization of the MAPK signaling pathway (path: 04010) through the linkage to the KEGG website. * Differentially expressed genes in cervical cancer

pathway has five sub-pathways significantly associated with paclitaxel ($P < 0.01$) (Figure 2). Similarly, enzymes were emphasized by blue letters surrounded by a red line if they were the targets of paclitaxel in cervical cancer.

Discussion

Paclitaxel is an effective chemotherapeutic agent for cancer patients. Resistance to chemotherapy represents a major obstacle to improving the survival rate of cervical cancer patients. The resistance of cancer cells to paclitaxel and other chemotherapeutic agents frequently results in the subsequent recurrence and metastasis of cancer. However, the mechanisms underlying the resistance to paclitaxel in cancer cells are not fully understood.

In this study, we found the predicted gene targets of paclitaxel in cervical cancer and the regulated pathways (Table 1), i.e. apoptosis, pathways in cancer, p53 pathway, focal adhesion, MAPK pathway, mTOR pathway, ErbB pathway, vascular endothelial growth factor (VEGF) pathway, and T-cell receptor pathway. One signaling pathway is coordinately associated with another signaling pathway. For example, there are crosstalks between the p53 pathway and the IGF-1-AKT and mTOR pathways (Feng et al., 2007). Activation of p53 inhibits mTOR activity and regulates its downstream biological effects including autophagy, a tumor suppression process. P53 and mTOR signaling can crosstalk with each other and coordinately regulate cell growth, proliferation and death (Feng et al., 2005).

Among these pathways, we focus on the MAPK and ErbB signaling pathways which are associated with paclitaxel. The MAPK cascade is involved in various cellular processes including cell proliferation, differentiation and migration (Yang, Sharrocks, and Whitmarsh, 2003). It has been demonstrated that the inhibition of MAPK pathway could markedly promote the apoptosis of colon cancer cells induced by paclitaxel. Selective blockage of the MAPK pathway by small interfering RNA (siRNA) also increases the apoptotic cell death induced by paclitaxel. The alternative use of ERK1/2 and p38 MAPK pathways may be necessary for the transition from proliferation state to paclitaxel-induced apoptosis in human ovarian carcinoma cells (Seidman et al., 2001). These findings highlight the importance of the MAPK pathway in paclitaxel-induced apoptosis. As shown in Table 1, among the differentially expressed genes in cervical cancer AKT and MAPK were identified as the targets of paclitaxel in the MAPK pathway. Protein kinase B (PKB/AKT) is a serine/threonine kinase, which comprises three highly homologous members known as PKBalpha (AKT1), PKBbeta (AKT2) and PKBgamma (AKT3) in mammals. PKB/AKT is activated in cells exposed to diverse stimuli such as hormones, growth factors and extracellular matrix components (Nicholson and Anderson, 2002). When paclitaxel interferes with the activation of AKT and MAPK, the cell proliferation promoted by MAPK pathway is inhibited.

The ErbB family of receptor tyrosine kinases plays an important role in tumor formation and is a target for the development of advanced cancer drugs. ErbB2 is overexpressed in invasive breast, ovarian, stomach, bladder, salivary, and lung cancers (de Graeff et al., 2008; Abdel Salam, 2009; Pryczynicz et al., 2009; Simonetti

et al., 2009; Idirisinghe et al.). ErbB is activated by the binding of growth factors that belong to the epidermal growth factor (EGF) family (Yarden and Schlessinger, 1987). The binding of EGF-like ligands to ErbB receptor induces a conformational change in the extracellular domain of the receptor, leading to the formation of an activated receptor dimmer (Greenfield et al., 1989). The ErbB2 receptor exhibits structural features that promote its oncogenic potential. Two pathways induced by ErbB2-containing heterodimers are the MAPK and AKT, which are involved in cell proliferation. AKT activation also induces a strong anti-apoptotic response and protects cells against apoptosis. High AKT activity is responsible for the enhanced resistance of ErbB2-overexpressing cancer cells to chemotherapeutic agents. Interference with AKT activation resulted in the restoration of normal sensitivity of breast cancer cells to Taxol (Kunz et al., 2006). Drugs targeting the ErbB2 receptor not only interfere with cell proliferation but also result in an improvement of the therapeutic efficacy of chemotherapy (Piccart-Gebhart et al., 2005; Romond et al., 2005).

In conclusion, our study presents the first in-depth, large-scale analysis of sub-pathways involved in paclitaxel activity in cervical cancer. Interestingly, these pathways have not been reported to be involved in other tumors. Thus our findings may contribute to the understanding of the mechanisms underlying paclitaxel resistance in cervical cancer.

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