Introduction

Evaluation of the response to the chemotherapeutic regimen in vivo is often global, mainly based on changes in tumor mass rather than on molecular effects elicited by the drugs. Paclitaxel (Taxol®) is a microtubule binding agent routinely used in breast cancer treatment in the clinic. Recently, a variety of cellular and molecular effects of paclitaxel have been described. These include induction of cytostasis, tumor suppressor genes, and activation of signal transduction pathways. The gene profiling of tumor cells exhibiting different responsiveness to anticancer drugs has been made possible by microarray technologies. Molecular analysis of the response to pharmacological treatment has been mainly explored in vitro and with the drug tested at high concentration. But these studies do not take into account the host environment in contributing to a given response, which is clearly of greater relevance to clinical settings. Local mediations certainly influence pharmacodynamics, pharmacodynamics, molecular response, and drug efficacy.

In the present study, we sought to examine the molecular events elicited by paclitaxel in the MDA-MB231 human breast cancer cell line both in vitro and in vivo using cDNA microarray technology.

Abstract

Background: Paclitaxel (Taxol®) is a drug that is usually used in cancer chemotherapy. Paclitaxel-induced changes in cell behavior are well known in the literature. Recent studies have shown that the cellular effects of paclitaxel can be measured using various cellular assays. Some cellular assays have been used to better understand the effects of paclitaxel on tumor cells. In vivo and in vitro experiments have been conducted with Taxol® in breast cancer cells. This study was intended to understand the molecular consequences of Taxol® on MDA-MB231 tumor xenografts with and without clinical adjuvants. The in vivo and in vitro experiments were conducted to determine the molecular effects of paclitaxel on MDA-MB231 cells.

Methods: For the in vivo experiment, xenograft tumors were induced in female nude mice by subcutaneous injection of MDA-MB231 cells. The tumors were allowed to grow until they reached a size of approximately 1 cm. For the in vitro experiment, MDA-MB231 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum. The cells were treated with paclitaxel at different concentrations for various time periods. The effects of paclitaxel on the proliferation, apoptosis, and survival of MDA-MB231 cells were determined by measuring the cell viability and the number of surviving cells. The results were analyzed using appropriate statistical methods.

Results: The results of the in vivo and in vitro experiments are shown in the figure. Paclitaxel significantly inhibited the proliferation of MDA-MB231 cells in a dose-dependent manner. The in vivo experiments showed that paclitaxel induced a significant decrease in tumor volume and a significant increase in the survival time of the mice. The in vitro experiments showed that paclitaxel induced a significant decrease in cell proliferation and a significant increase in the apoptosis of MDA-MB231 cells.

Conclusions: Paclitaxel induces a significant decrease in the proliferation of MDA-MB231 cells in vivo and in vitro. Paclitaxel induces a significant decrease in the tumor volume and an increase in the survival time of the mice in vivo. Paclitaxel induces a significant decrease in the cell proliferation and an increase in the apoptosis of MDA-MB231 cells in vitro. These findings suggest that paclitaxel is a potential therapeutic agent for the treatment of breast cancer. Further studies are needed to investigate the mechanism of action of paclitaxel and to determine its clinical efficacy.

In Vitro Experimental Procedure

Tumor cell line: MDA-MB231

Test substance: Paclitaxel

Vehicle: DMSO

Cell plating:
- 6 Petri dishes: 10% suspension - 10 ml complete medium
- Incubation at 37°C/5%CO2, for 4 h

Cell treatment:
- Cells cultured on 70-70%
- TAL at 100× (1.2 ml of complete medium) or vehicle
- Incubation at 37°C/5%CO2, for 4 h

Cell collection: cyto-centrifugation
- Lysate snap frozen in liquid nitrogen and stored at -80°C

In Vivo Experimental Procedure

Model: 20 female Balb/c-nu/nu mice

Drug Treatment:
- Intraperitoneal injection of mice 24 h before cells inoculation
- SC inoculation of 10× MDA-MB231 cells

Randomization of 40 per treatment in 2 groups of 10 mice according to tumor volume

Mean tumor volume at randomization = 321 ± 214 mm³

Treatment schedule: TAL - 1 × 15 mg/kg - QOD

 Sacrifice of 2-3 mice / group (at 2 time points: 1h after the 2nd inj and 2h after the 1st inj)

Tissue collection:
- Tumor and healthy conjointive tissue
- Immerion of tissues in RNIVholders for 24 h at 4°C
- RNIVholder removal and storage at -80°C until analyses

Gene Expression Profiling Platform

DiscoveryChip
- Manufactured in compliance with GMP standards
- cDNA array (ip65x6)
- Isotopic labelling during RT
- No amplification bias
- Robust reproducibility/sensitivity (5-μg RNA)

Integrated Bioinformatics:Discovery Software (DS)
- Full processability
- Automated data processing
- cDNA quality

Data Processing/Analysis: ProfileSoftCorporate
- Chip validation
- Non-linear normalization
- Hierarchical clustering & supervised analyses

Study Design In Vivo

In Vivo MDA-MB231 cells treated for 24h with TAL at 100nm or vehicle

In Vivo MDA-MB231 mean SC tumor growth curves.

Differentially expressed genes

Comparison of Effect of Taxol in vitro & In Vivo

The cascade represents the number of differentially expressed genes between both treatments and controls conditions for the 3 experimental procedures (p-value: 4.46 × 10^-10 vs 0.1342) for 2 repetitions. The significance of the changes in the expression of these genes was confirmed by qPCR analysis.

The validated genes are the number of genes commonly modulated in 3 or 5 experimental procedures (p-value: 4.46 × 10^-10 vs 0.1342) for 2 repetitions. The significance of the changes in the expression of these genes was confirmed by qPCR analysis.

The scatter plot showing the differentially expressed genes between taxol treated (T) and not treated (NT + control) MDA-MB231 cells.

18 Genes commonly modulated after Taxol treatment in vitro & in vivo

Pathways were defined according to the Gene Ontology; the number of genes in different pathways that take part into the total number of genes evaluated on EPDOS International Chip array (N6.1)

Most important effect of Taxol on angiogenesis, proliferation and apoptosis

Effect on Proliferation:
- In vitro & in vivo: Fos Upregulated
- In vivo: CYP2D

Effect on Angiogenesis:
- In vitro & in vivo: sFlt1, CSPG
- In vivo: VEGF

Effect on Apoptosis:
- In vitro & in vivo: bax, bcl2, bcl-xl

Conclusions

- Paclitaxel induces changes in gene expression that are relevant for apoptosis, migration, cell cycle, and p53-dependent tumor suppression pathways. These changes in gene expression are comparable with published data in other studies (Biel et al., 2004). Gauthier et al., 2004.
- Taxol-induced changes are a subset of genes that are differentially affected. As an example, p53 gene expression was up-regulated in vitro but down-regulated in vivo.
- 5 genes were up-regulated and 2 genes were down-regulated after Taxol treatment both in vitro and in vivo.
- ID1 gene that encodes a protein involved in the 5q31-epithelial phenotype, was highly expressed both in vitro and in vivo.
- Cyclophilin G2, a regulatory enzyme of 5q31 phase of cancer progression, was highly expressed both in vitro and in vivo.
- The scatter plot, showing how a set of identified genes can be validated in vivo.
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