Poster 4137 Gene Expression Profile Analysis of PACLITAXEL-Induced Changes in the MDA-MB231 Human Breast Cancer Xenograft Model

A. Groulet-Martinec[1], O. Duchamp[3], A. Gonçalves[2], V. Forestier[1], N. Borie[1], S. Debono[1], V. Fert[1], P. Genne[3], A. Koki[1], N. Guilbaud[3].

[1]IPSOGEN, Marseille, France ; [2]Institut Paoli-Calmettes, Marseille, France ; [3]ONCODESIGN, Dijon, France

Introduction

Evaluation of the response to the chemotherapeutic regimens *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 cytokines, 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 analyses 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. Host metabolism certainly influences pharmacokinetics, 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

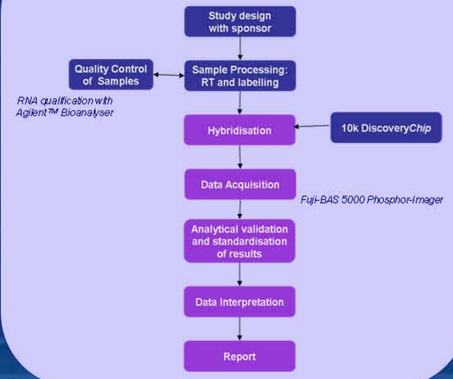
Background: Paclitaxel (Taxol®) is a microtubule binding agent routinely used in cancer treatment. Paclitaxel has been shown to be efficacious in patients with advanced and metastatic breast cancer, as well as in the adjuvant setting in early breast cancer. Numerous preclinical studies using various cancer cell lines have contributed to identify potential mechanisms of action of paclitaxel. However, little data are available on the molecular effects of paclitaxel *in-vivo*.

Methods: We conducted cDNA micro-array and antitumor activity analysis to investigate the molecular alterations and the tumor growth inhibition induced by paclitaxel exposure in MDA-MB231 mouse xenograft model. Tumor cells (2 x 107 cells/mouse) were subcutaneously implanted into Nude mice. Mice were ranked according to tumor volume and randomized to receive either 15 mg/kg paclitaxel or vehicle from day 1 to 5 following randomization. Animals were sacrified and tumor and healthy mouse subcutaneous tissues harvested 6 hr following dosing on days 2 and 5, and immediately frozen in liquid nitrogen. MDA-MB231 cells exposed for 24 hr to 100 nM paclitaxel or vehicle *in-vitro* were also obtained in parallel experiments. Total RNAs were extracted and analyzed on cDNA microarrays containing ca. 9000 genes.

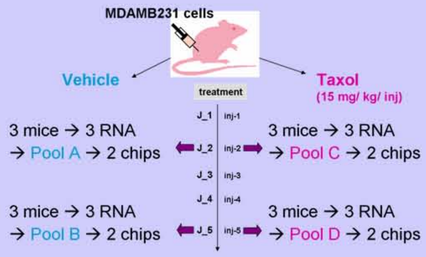
Results: Kinetic analysis of paditaxel-treated tumor tissues revealed a strong *in-vivo* antitumor activity (T/C = 6.5%) of pharmacological doses and transcriptional alterations involving various intracellular pathways or systems, including apoptosis, mitotic regulation, cell cycle control, microtubule regulation, and cytoskeletal remodeling. Several differences were identified in paclitaxel-treated tumor tissues compared to drug-exposed cultured cells, and may explain discordant phenotypes of response between *in-vitro* and *in-vivo* evaluation.

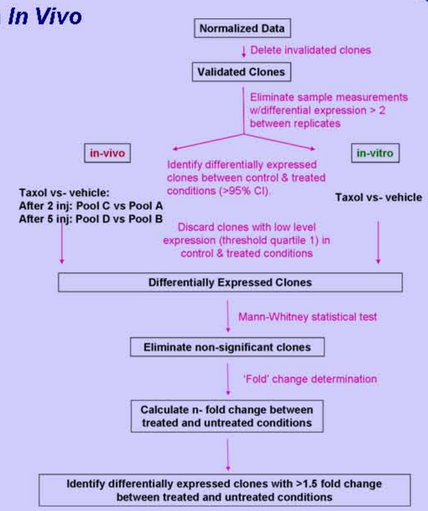
Conclusions: Gene expression profiling of drug effect *in-vivo* may improve preclinical assessment of anti-cancer compounds. In addition, such an approach may identify potential surrogate markers of drug effects that can be monitored in the clinical setting to predict clinical activity.

Gene Expression Profiling Procedure

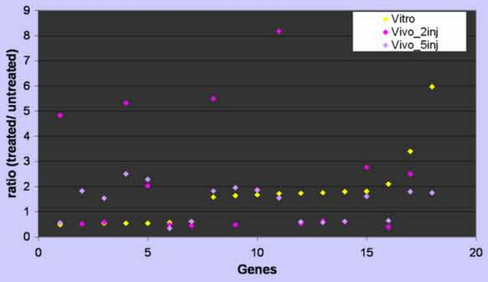


Study Design *In Vivo*





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



This graph shows the up regulation (ratio >1.5) or down regulation (ratio<1.5) of the 18 genes modulated in the 3 experimental procedures (*in vitro* & *in vivo*)

Number of genes modulated by paclitaxel treatment in different pathways

	Nb of genes	Immunity	Angiogenesis	Proliferation	Mobility metastasis	Differentiation	Apoptosis
		121	98	122	48	53	125
vitro	755	11	18	20	5	7	17
vivo_2inj	796	15	19	16	8	6	15
vivo_5inj	705	15	11	23	11	11	18

Pathways were defined according to the Gene Ontologies; the number of genes in different pathways take only into account the spotted cDNAs on IPSOGEN DiscoveryChip (grey line).

In Vitro Experimental Procedure

Tumor cell line: MDA-MB231
Test substance: Paclitaxel
Vehicle: Cremophor/ethanol/NaCl
Cells plating:
- 6 Petri dishes - 5.10^6 cells/dish - 10 ml complete medium
- incubation at 37°C 5%CO_2 for 24 h

Cells treatment:
- Cells confluence = 70-75%
- TXL at 100nM (in 10 ml of complete medium) or vehicle
- Incubation at 37°C 5%CO_2 for 24 h

Cells collection:- cytolyse with Trizol
- Lysate snap frozen in liquid nitrogen and storage at - 80°C

In Vivo Experimental Procedure

Models: 20 female Balb/c-nu/nu mice
Mice Treatment:
- Irradiation of mice 24h before cells inoculation
- SC inoculation of 10^7 MDA-MB231 cells

Randomisation at D46 post-implantation in 2 groups of 10 mice according to tumor volumes
Mean tumor volume at randomisation = 323 + 214 mm^3
Treatment schedule: TXL - IV - 15 mg/kg/inj - Q1Dx5
Sacrifice of 2-3 mice / group at 2 time points: 6h after the 2nd inj and 7h after the 5th inj
Tissues collection:
- Tumor and healthy conjonctive tissue
- Immersion of tissues in RNAlater for 24h at +4°C
- RNAlater removal and storage at –80°C until analyses.

Gene Expression Profiling Platform

DiscoveryChip:
- Manufactured in compliance with GMP standards
- cDNA array (bp>450)
- Isotopic labeling during RT
- No amplification bias
- Robust reproducibility / sensitivity (3-5 µg RNA)

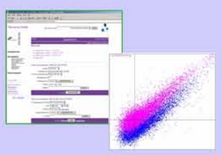
Integrated Bioinformatics: DiscoverySoftware (DS)
- Full traceability
- Automated data processing
- MIAME compliant

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

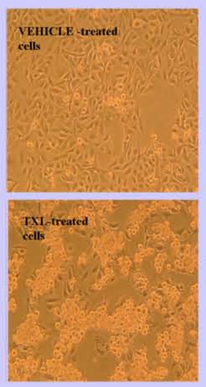
PCR Products Reorganization (Microarray Facility - DS)

Spotting of PCR Products

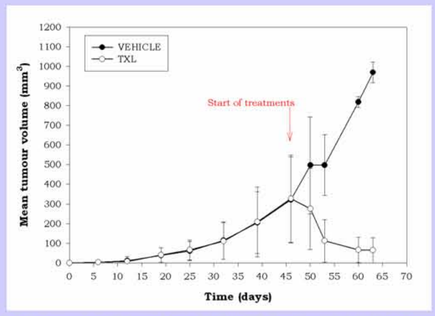
DiscoveryChip



In Vitro MDA-MB231 cells treated for 24h with TXL at 100nM or vehicle



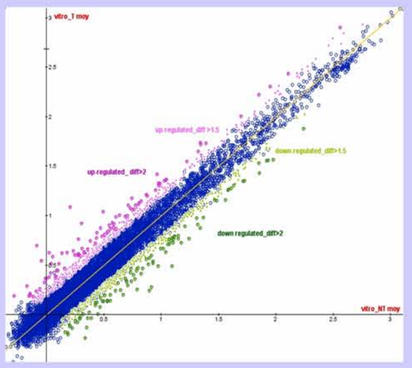
In Vivo MDA-MB231 mean SC tumor growth curves.



Tumor-bearing mice were treated with TXL or vehicle for 5 consecutive days

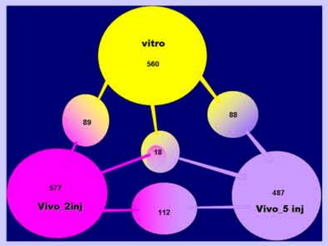
Paclitaxel was highly active in the MDA-MB231 breast cancer cells with the doses and schedules chosen for *in vitro* and *in vivo* studies

Differentially expressed genes



Scatter Plot showing the differentially expressed genes between taxol treated (T) and non treated (NT = control) MDA-MB231 cells

Comparative Effect of Taxol *in vitro* & *in vivo*



The circles represent the number of differentially expressed genes between taxol-treatment and control conditions for the 3 experimental procedures (in vitro : 560 + 89 + 88 + 18 = 755 genes; in vivo after 2 injections : 577 + 89 + 112 + 18 = 796 genes; in vivo after 5 injections :487 + 88 + 112 + 18 = 705 genes).

The smallest circles give the number of genes commonly modulated in 2 or 3 different experimental procedures (89 genes in common between in vitro and in vivo-2inj conditions; 88 genes in common between in vitro and in vivo-5inj; 112 genes in common between in vivo 2 and 5 injections; 18 are modulated by all treatments)

Most important effect of Taxol on angiogenesis, proliferation and apoptosis

Effect on Proliferation:		Down-regulated
In vitro & in vivo	fos	Up-regulated
	cyclin D2	
In vivo	ppp2ca	
	myc, tp53	
Effect on Angiogenesis:		
In vitro & in vivo	egr1	
In vivo	PDGFRb	
	VEGFb	
Effect on Apoptosis:		
In vitro and in vivo	itgb1, myc & nr2c2	
In vitro	apaf1, CASP8, MAPK8	
	CASP4, cdkn1b	
In vivo	BAX, ing1, nbkia, BAK	

Conclusions

- Taxol induces changes in genes involved in apoptosis, mitotic regulation, cell cycle control, and cytoskeletal remodeling of breast cancer cells and these changes in gene expression are consistent with published data in other systems (Bani et al., 2004; Sugimura et al., 2004).
- Taxol-induced changes in a sub-set of genes are different *in-vitro* & *in-vivo*. As an exemple, tp53 gene expression was up-regulated *in vitro* but down-regulated *in vivo*.
- 5 known genes were up-regulated and 2 genes were down-regulated after Taxol treatment both *in vitro* and *in vivo*:
  - **ID2 gene**, that encodes a protein able to abolish the S-phase entry block by binding pRb pocket proteins, was higly over expressed both *in vitro* and *in vivo*,
  - **Cyclin D2**, a positive regulator of G1 phase cell cycle progression, was largely down-regulated both *in vitro* and *in vivo*.
  - **Fos gene**, that encodes a nuclear protein involved in growth-related transcriptional control, was also up-regulated *in vitro* and *in vivo*.
- This study shows how *in vitro* - identified biomarkers can be validated *in vivo*.
- This feasibility study on GEP of xenografted tumor models as a result of taxol exposure provides insight related to drugs' action *in vivo* that will anticipate the response of the tumor in taxol – treated patients.

The identified biomarkers could be good candidates to early predict the tumor response to paclitaxel treatment and used as end point for *in vivo* studies.

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