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Effects of paclitaxel on proliferation and apoptosis in human acute myeloid leukemia HL-60 cells

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ABSTRACT

AIM: To investigate the regulatory effect of paclitaxel on proliferation and apoptosis in human acute leukemia HL-60 cells. **METHODS:** HL-60 cell growth was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay. Cell cycle kinetics and apoptosis were analyzed by flow cytometry and microscopic examination. In addition, DNA microarrays containing 14400 EST elements were used to investigate the gene expression pattern of HL-60 cells exposed to paclitaxel 1 $\mu\text{mol/L}$. **RESULTS:** Paclitaxel inhibited HL-60 cell growth significantly in a dose-dependent and time-dependent manner ($P < 0.01$). Marked cell accumulation in G₂/M phase and multinucleated cells were also observed after treatment with paclitaxel 0.1 and 1 $\mu\text{mol/L}$. Among 14400 EST elements, 277 genes were found to be markedly up- or down-expressed in the HL-60 cells treated with paclitaxel 1 $\mu\text{mol/L}$ for 0.5 h, comprising 210 known genes and 67 unknown genes. **CONCLUSION:** Paclitaxel suppresses the growth of HL-60 cells *in vitro* by causing cell-cycle arrest and apoptosis. The results of microarray suggest that paclitaxel initiates apoptosis through multiple mechanisms.

INTRODUCTION

Most chemotherapeutic drugs exert their anti-tumor effects by inducing apoptosis. A powerful new approach to facilitate the discovery of unique genes is the recently developed microarray technology^[1]. This process allows the expressions of thousands of genes to be compared and profiled simultaneously. The development of microrobotics and a computer-enhanced detection system allow up to 1×10^4 separate gene ele-

ments to be placed on a glass slide. This slide can be hybridized to two different probes, each labeled with a distinct fluorescent dye. Each probe consists of any single-stranded genetic element capable of hybridizing to the cDNA placed on the glass slide.

Paclitaxel exerts its cytotoxic effect by binding and stabilizing microtubules^[2] and induces apoptosis in a variety of cell types^[3,4]. It was demonstrated that paclitaxel released cytochrome c by direct action on the mitochondria^[5], activated small GTP-binding proteins^[6], and activated the NF- κ B/I κ B- α signaling pathway^[7]. Akt (protein kinase B, PKB), a serine/threonine protein kinase, has a pivotal role in exerting an antiapoptotic effect against various stimuli. Overexpression of Akt was reported to confer resistance to paclitaxel^[8].

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However, it was reported that paclitaxel independently induced apoptosis in human ovarian carcinoma cells of Akt^[9]. Thus, it is unclear whether paclitaxel causes apoptosis through modulation of the Akt survival pathway. The JNK/SAPK (c-Jun N-terminal kinases/stress-activated protein kinases) is frequently associated with apoptosis. It was demonstrated that activated JNK/SAPK was required for the early phase of paclitaxel-induced apoptosis^[10]. However, it was reported that paclitaxel induced prolonged activation of the JNK/SAPK signaling pathway independently of activating the programmed cell death machinery^[11]. The precise roles of these activated kinases in this context remain obscure. Although these actions were implicated as the mechanisms through which paclitaxel induced apoptosis, these studies were impaired by the treatment of high concentrations of paclitaxel and by the long exposed time (over 16 h). And the upstream expressing molecules of the apoptotic-signaling pathway activated by paclitaxel are unclear.

In an attempt to identify the genes responsive to or responsible for paclitaxel-induced apoptosis and to reveal the apoptosis signaling pathways triggered by paclitaxel, we have utilized the microarray technology to simultaneously display changes of gene expression. Characterization of these paclitaxel-responsive genes may lead to the identification of novel target (s) for the discovery of anticancer drugs.

MATERIALS AND METHODS

Agents Paclitaxel, MTT, propidium iodide (PI) and RNase were purchased from Sigma Chemical Co; TRIzol and RPMI-1640 medium were the products of Gibco. Human cDNA expression arrays, representing 14 400 human genes, were obtained from United Gene Scientific Technology Company, Ltd (Shanghai, China). All other reagents were analytical reagents.

Cell culture and cytotoxicity assay *in vitro* Human leukemia HL-60 cell line was from Shanghai Institute of Cell Biology, Chinese Academy of Sciences. Cells were cultured in RPMI-1640 medium, supplemented with 10 % heat-inactivated calf serum, streptomycin 100 mg/L, benzylpenicillin 100 kU/L, in a 5 % CO₂ humidified incubator at 37 °C.

Apoptosis and cell cycle assay HL-60 cells were planted in T-75 cm² flasks and treated for 24 h with either Me₂SO (dimethyl sulfoxide) or paclitaxel. Single-cell suspensions were collected, and pellets were fixed

in ice-cold ethanol (70 %) for 30 min. After centrifugation of the samples, propidium iodide (50 mg/L) and RNase (30 kU/L) were added to the pellets at 37 °C for 20 min. After filtration, samples were analyzed by flow cytometry (FACSalibur, Becton Dickinson, USA). For each sample, at least 1×10⁴ cells were analyzed by flow cytometry. Results were analyzed by software of CELLQUEST and ModFIT for macV1.01 (Becton Dickinson, USA).

Morphology of apoptotic cells After treatment with or without paclitaxel, 5×10⁴ cells were washed with PBS, pH 7.3, and resuspended in the same buffer. Cytospin preparations of the cell suspensions were fixed and then stained with Wright's stain. Cell morphology was determined by light microscopy. In all, five different fields were randomly selected for counting at least 500 cells. The percentage of apoptotic cells was calculated for each experiment. Cells designated as apoptosis were those that displayed the characteristic morphological features of apoptosis, including cell volume shrinkage, chromatin condensation, and the presence of membrane-bound apoptotic bodies.

Chip preparation The 14 400 EST elements were used in the cDNA chips. These genes were amplified through PCR using universal primers and purified by the standard method. The quality of PCR was monitored by agarose electrophoresis. Target genes were dissolved in 3×SSC spotting solution, and spotted on silylated slides (Telechem, Inc, USA) by Cartesian 7500 spotting robotics (Cartesian, Inc, USA). Each target gene was dotted twice. After spotting, the slides were hydrated (2 h), dried (0.5 h, room temperature), cross-linked under UV light and then treated with 0.2 % SDS, H₂O and 0.2 % NaNBH₄ for 10 min, respectively. The slides were then dried in the cold and ready for the probe preparation.

Total RNA in the treated samples with or without drug were extracted by a single step method^[12]. The suspensions of HL-60 cells were centrifugated, then washed with PBS, pH 7.3, and homogenized in D solution plus 1 % mercaptoethanol. After centrifugation, the supernatant was extracted twice with phenol:chloroform (1:1), then once with NaAc and acidic phenol:chloroform (5:1), the aqueous phase was precipitated by equal volumes of isopropanol.

The precipitate was centrifuged and dissolved with milli-Q H₂O. After purification by a LiCl precipitating method, UV analysis and electrophoresis detection showed the good quality of purified RNA. mRNA were

isolated and purified using an Oligotex mRNA MidiKit (QIAGEN, Inc, USA). The fluorescein-labeled cDNA probe was prepared through reverse transcription and purified by the method of Schena^[13]. Probes from the control samples were labeled with Cy3-dUTP; those from tested samples were labeled with Cy5-dUTP. The probes were mixed, then precipitated by ethanol and resolved in 20 μ L hybridization solution (5 \times SSC+2 % SDS).

Hybridization and washing Hybridizing probe and chip were denatured in a 95 °C bath for 5 min. The probe was added on the chip and covered with glass. The chip was hybridized in a sealed chamber at 60 °C for 15-17 h. After removing the covered glass, the slide was washed in a solution of 2 \times SSC+0.2 % SDS, 0.1 \times SSC+0.2 % SDS, and 0.1 % SSC for 10 min each, respectively, then dried at room temperature.

Fluorescence scanning and analysis The chip was scanned with a Scan Array 3000 Scanner (General Scanning Inc, USA). Cy3 and Cy5 overall intensity were normalized and corrected by a coefficient according to the location ratios of the 40 housekeeping genes. The acquired image was analyzed by ImeGene 3.0 Software using a digital computer. Intensity of the fluorescent signal and each ratio of Cy3 to Cy5 were compared. The data were obtained on an average of two repeated spots. The standards to screen out each differentially expressed gene were defined: (1) the absolute value of the natural logarithm of the signal ratio of Cy5/Cy3 was greater than 0.69 (gene expression change >2-fold). (2) One of the signal values of Cy3 and Cy5 was greater than 600. (3) The results of PCR were good.

RESULTS

Cytotoxic effect of paclitaxel on HL-60 cells HL-60 cells at 4 \times 10⁸ cells/L were incubated with different concentrations of paclitaxel for different time (24 h, 48 h, and 72 h), and the effect of paclitaxel on the cell growth was examined by MTT assay. The growth of HL-60 cells was markedly inhibited by paclitaxel 1 nmol/L-0.1 mmol/L (Fig 1). Moreover, the cytotoxicity of paclitaxel was concentration-dependent. The time-effect analysis of paclitaxel showed that its cytotoxicity against the human leukemia cells was in a time-dependent manner (Fig 1).

Effect on cell-cycle kinetics of HL-60 cells Cell-cycle kinetics of HL-60 cells treated with paclitaxel was analyzed by flow cytometry. Paclitaxel 0.1 μ mol/L and 1 μ mol/L for 24 h caused an accumulation of the

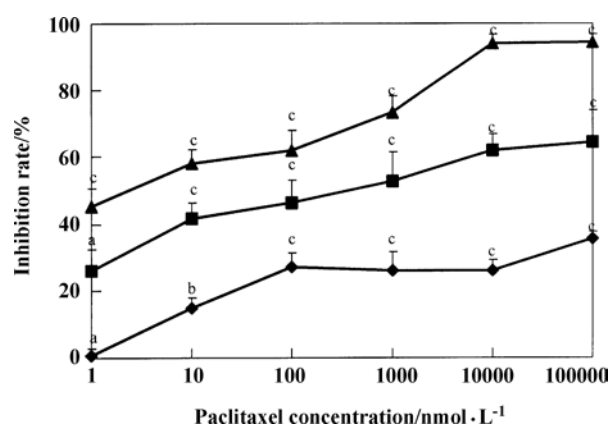


Fig 1. Effect of paclitaxel treatment on the cytotoxicity of HL-60 cells. *n*=3. Mean \pm SD. ^a*P*>0.05, ^b*P*<0.05, ^c*P*<0.01 vs control. (♦) 24 h; (■) 48 h; (▲) 72 h.

cells in G₂/M phase about 70.5 % and 78.5 %, respectively, and that of the control about 10.27 %. Moreover, the G₂/M cell cycle arrest of paclitaxel in HL-60 cells was concentration-dependent (Tab 1).

Tab 1. Effect of 24 h-paclitaxel treatment on the cell cycle of HL-60 cells. *n*=3. Mean \pm SD. ^c*P*<0.01 vs control.

Groups/ μ mol·L ⁻¹	Cell cycle/%		
	G ₀ /G ₁	S	G ₂ /M
Control	41.2 \pm 2.4	48.5 \pm 1.6	10.3 \pm 1.5
Paclitaxel 0.1	0.20 \pm 0.8 ^c	29.3 \pm 2.0 ^c	70.5 \pm 2.9 ^c
1	0.7 \pm 0.8 ^c	20.8 \pm 2.4 ^c	78.5 \pm 3.0 ^c

Induction of HL-60 cells apoptosis As analyzed by flow cytometry, paclitaxel induced the apoptosis of HL-60 cells. Apoptosis was induced with the cells exposed to paclitaxel 0.1 μ mol/L and 1 μ mol/L for 24 h, the apoptotic percentages were 40.5 % and 37.5 % respectively, compared with 2.27 % of the control (Tab 2). The results from light microscope were similar to

Tab 2. Effect of 24 h-paclitaxel treatment on inducing apoptosis of HL-60 cells. *n*=3. Mean \pm SD. ^c*P*<0.01 vs control.

Groups/ μ mol·L ⁻¹	Apoptosis/%
Control	2.3 \pm 0.5
paclitaxel 0.1	40.5 \pm 1.4 ^c
1	37.5 \pm 1.3 ^c

those mentioned above (data not shown). Morphologic observation further confirmed paclitaxel-induced apoptosis in HL-60 cells. The results from light microscope displayed shrinkage and morphologic abnormality of the cells after treatment with paclitaxel 0.1 $\mu\text{mol/L}$ for 48 h. On the contrary, the untreated cells did not show these apoptotic characteristics (photo not shown).

Identification of the genes responsive to apoptosis induced by paclitaxel: the use of microarray technology In an attempt to identify genes whose expressions changed during paclitaxel-induced apoptosis, we utilized microarray technology to obtain an overall profile of gene expression. In order to identify early apoptosis-responsive genes, poly (A⁺) mRNA was isolated from cells treated with paxlitaxel (1 $\mu\text{mol/L}$) for 0.5 h and subjected to cDNA chip hybridization. Cells treated with Me₂SO for 0.5 h were used as the control. The hybridization results from all chips were compiled and sorted on the basis of fold change compared to the control cells. Genes that displayed approximately two-fold or greater changes were scored as having significant changes^[14]. Hybridization results obtained by parallel comparison of gene expression of the two samples demonstrated: 277 genes were differentially expressed between the tested samples and the control. Of these 277 mRNA species, comprising of 210 known genes and 67 unknown genes, 121 were significantly induced and 156 were significantly repressed. According to their functions, these genes could be divided into 13 groups (Tab 3). The biochemical function of the genes in this expression profile are diverse, including: transcription factors, protein kinases and phosphatases, cell cycle regulators, proteases, apoptotic and antiapoptotic factors, protein synthesis-related genes, proliferation-related genes, as well as a large number of metabolic genes. Altered expressions of the transcription factors included down-regulation of the general transcription factor II-I (GTF2I), the activating transcription factor 5 (ATF5), the transcription elongation factor B (TCEB), and the cyclin D binding myb-like transcription factor 1 (DMTF1). These changes seemed to be consistent with a general shutdown of transcription response to paclitaxel treatment. Changes in the expressions of a group of zinc finger transcription factors were also noted. Altered expressions of the protein kinases and phosphatases included down-regulation of the protein kinase B (PKB), the protein kinase C (PKC), the protein phosphatase 1 regulatory subunit 9A (PPP1R9A), the serine/threonine protein kinase 18 (STK18), the dual

Tab 3. Classification of gene function. Gene expression difference between the tested samples and the control.

Gene function classification	Number of different gene expression	
	Induced genes	Decreased genes
Cell cycle proteins	6	7
Metabolism-related proteins	8	16
Protein synthesis-related genes	0	13
DNA binding, transcription and its factor	6	14
DNA synthesis, repair and recombinant proteins	0	6
Ion-channel and transporters	4	7
Proliferation-related genes	0	7
Cell receptors	4	1
Cell surface antigen and adhesion proteins	4	5
Cell antiapoptosis-related proteins	6	14
Cell signs and transducing proteins	6	9
Extra-pressure reaction proteins	3	0
Other genes	27	37

specificity phosphatase 3, the adenylate cyclase 6 and the AMP-activated protein kinase 2 (PP2A), up-regulation of the protein tyrosine kinase 6 (PTK6), the Rho-associated coiled-coil containing protein kinase 1 (ROCK1) and the protein phosphatase 2. Different expressions of the cell cycle regulators included down-regulation of the cyclin E2, the exportin 1 (XPO1/CRM1), the quiescin Q6, the retinoblastoma-binding protein 9 (RBBP9) and up-regulation of the E2F transcription factor 5 (E2F5). Altered expressions of the apoptotic and antiapoptotic factors included down-regulation of gelsolin, the death effector domain-containing protein (DEDD), the MAP kinase-activating death domain (MEDD), the TNF receptor-associated factor 5 (TRAF5) and the partner of RAC1. Altered expressions of the protein synthesis-related genes and metabolic genes included down-regulation of the eukaryotic translation elongation factor 2 (EEF2), the eukaryotic translation initiation factor 4A (EIF4A), the adenine nucleotide translocation 1, the isocitrate dehydrogenase 2, COX15 and the NADH-ubiquinone oxidoreductase flavoprotein 1 (NDUFV1). We also found that the DNA ligase I, the DNA polymerase Kappa (POLK), the ubiquitin-specific protease 5 (USP5) and the prosaposin (PSAP) were decreased. In addition, the heterogeneous nuclear ribonucleoprotein H1 (HNRPH1), the heterogeneous nuclear ribonucleoprotein B1 (HNRPB1), the het-

erogeneous nuclear ribonucleoprotein A/B (HNRPAB), the oxysterol-binding protein and the valosin-containing protein (p97) were depressed. These changes were consistent with the cytotoxic effects of paclitaxel.

DISCUSSION

Recent accumulating reports have demonstrated that paclitaxel-induced microtubular bundling and mitotic arrest of human leukemia cells are followed by DNA fragmentation and morphological features of apoptosis^[2,3,15]. We found that an increased apoptotic population in HL-60 cells caused by paclitaxel treatment was accompanied by a pronounced mitotic arrest. We also found that paclitaxel inhibited the cell cycle G₂/M of HL-60 cells in a dose-dependent manner (Tab 1), but the apoptosis was not increased after paclitaxel treatment (Tab 2), according to the previous report^[16]. We speculate that the mitotic arrest induced by paclitaxel was not due to paclitaxel-induced apoptosis.

In regard to the biochemical events downstream of paclitaxel binding to microtubules, which lead to apoptosis, our results provide a genome-wide analysis of the cellular responses to paclitaxel. The cytotoxic response to paclitaxel in HL-60 cells involves the induction, as well as the repression, of genes of various biochemical and regulatory pathways (Tab 3). Paclitaxel exposure leads to the down-regulation of GTF2I, ATF5, TCEB, DMTF1 and HDAC1. Decreased expression of these genes may trigger a general repression of transcription in cells in response to the deadly insult of a cellular poison. Moreover, it is also intriguing that the expressions of a group of previously unknown putative zinc finger transcription factors, were prominently targeted for alterations in response to paclitaxel. Although the functional consequences of these alterations are unclear, we speculate that the downstream target genes of these zinc finger transcription factors may be a response to the cytotoxic insult.

Another cluster of genes that showed changes after paclitaxel treatment were the protein kinases and phosphatases, including PKB, PKC α , STK18, ROCK1 and PP2A, which were down or up-regulated after paclitaxel treatment. A role of PKC α in signal transduction pathways was related to growth control^[17]. STK18 may be involved in the promotion or progression of cancers^[18]. ROCK1 may have a role in the progression of the cell cycle^[19]. PP2A modulated cell cycle progression^[20] and apoptosis^[21]. Whether these protein

kinases and phosphatases have some influences on cell cycle and apoptotic regulation in HL-60 cells treated by paclitaxel remains to be further explored.

DNA microarray also showed an interesting pattern of expression of cell cycle genes following exposure to paclitaxel. Cyclin E2, CRM1, and RBBP9 were depressed, and E2F5 was induced by paclitaxel. We suggested that these genes may determine the terminal fate of HL-60 cells in G₂/M after exposure to paclitaxel.

Cell death induces the activation of proapoptotic factors and the inactivation of antiapoptotic factors. One of the mechanisms that triggers cell death involves the release of cytochrome c from the mitochondria, which subsequently causes apoptosis by activation of caspases. Kamada *et al* ascribed the antiapoptotic activity of gelsolin to prevention of a step leading to cytochrome c release from the mitochondria into the cytosol^[22]. The N terminus of DEDD induces apoptosis and the C terminus has antiapoptotic activity^[23]. Overexpression of MADD activated the mitogen-activated protein (MAP) kinase ERK2 and JNK1^[24]. TRAF5 specifically interacts with the lymphotoxin- β receptor and activates the transcription factor NF- κ -B^[25]. Partner of RAC1 mediates synergy between the ARF and RHO family signaling pathways^[26]. Our results showed repression of gelsolin, DEDD, MADD, TRAF5 and partner of RAC1 after paclitaxel treatment, which presumably lead to the eventual death of the cells treated with paclitaxel.

A subset of genes that showed striking changes after paclitaxel treatment were the genes involved in protein synthesis, DNA biosynthesis, repair and metabolism, including EEF2, EIF4A, adenine nucleotide translocation 1, isocitrate dehydrogenase 2, COX15, DNA ligase I, POLK, and hnRNPs, which were depressed after paclitaxel treatment. These results reveal that the drug exerts an inhibiting action on protein synthesis, DNA biosynthesis, repair and metabolism in tumor cell.

We also found that two interesting genes of USP5 and PSAP were depressed after paclitaxel treatment. USP5 is a member of the ubiquitin specific protease family (UBP). It may exhibit a role in the regulated catabolism of the polymeric ubiquitin, including the polyubiquitin protein degradation signal^[27]. The precise mechanisms by which protein degradation may affect the drug effect remain to be determined. The sphingolipid ceramide is an important second signal molecule that regulates diverse signaling pathways involving apoptosis, the cell cycle, and differentiation. It has been

shown that PSAP is the ceramidase activator, and the protein-deficiency caused the ceramide accumulation^[28]. Although the functional consequences of PSAP are unclear, we speculate that inhibition of PSAP and ceramide accumulation may be another important factors on the apoptosis of HL-60 after paclitaxel treatment.

The use of DNA microarrays containing 14 400 EST elements in our analysis has provided a global view of the response of HL-60 cells to paclitaxel at the genomic levels. The ultimate identification of patterns of responsive genes for any given treatment of agents will have a profound influence in the target identification and validation in future drug discovery.

REFERENCES

- Schena M, Shalon D, Davis RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 1995; 270: 467-70.
- Diaz JF, Strobe R, Engelborghs Y, Souto AA, Andreu JM. Molecular recognition of taxol by microtubules. Kinetics and thermodynamics of binding of fluorescent taxol derivatives to an exposed site. *J Biol Chem* 2000; 275: 26265-76.
- Moos PJ, Fitzpatrick FA. Taxanes propagate apoptosis via two cell populations with distinctive cytological and molecular traits. *Cell Growth Differ* 1998; 9: 687-97.
- Shen SC, Huang TS, Jee SH, Kuo ML. Taxol-induced p34cdc2 kinase activation and apoptosis inhibited by 12-*O*-tetradecanoylphorbol-13-acetate in human breast MCF-7 carcinoma cells. *Cell Growth Differ* 1998; 9: 23-9.
- Andre N, Braguer D, Brasseur G, Goncalves A, Lemesle-Meunier D, Guise S, *et al*. Paclitaxel induces release of cytochrome c from mitochondria isolated from human neuroblastoma cells. *Cancer Res* 2000; 60: 5349-53.
- Lou PJ, Chen WP, Lin CT, Chen HC, Wu JC. Taxol reduces cytosolic E-cadherin and beta-catenin levels in nasopharyngeal carcinoma cell line TW-039: cross-talk between the microtubule- and actin-based cytoskeletons. *J Cell Biochem* 2000; 79: 542-56.
- Huang Y, Johnson KR, Norris JS, Fan W. Nuclear factor-kappaB/IkappaB signaling pathway may contribute to the mediation of paclitaxel-induced apoptosis in solid tumor cells. *Cancer Res* 2000; 60: 4426-32.
- Page C, Lin HJ, Jin Y, Castle VP, Nunez G, Huang M, *et al*. Overexpression of Akt/AKT can modulate chemotherapy-induced apoptosis. *Anticancer Res* 2000; 20: 407-16.
- Mitsuuchi Y, Johnson SW, Selvakumaran M, Williams SJ, Hamilton TC, Testa JR. The phosphatidylinositol 3-kinase/AKT signal transduction pathway plays a critical role in the expression of p21WAF1/CIP1/SDI1 induced by cisplatin and paclitaxel. *Cancer Res* 2000; 60: 5390-4.
- Wang TH, Popp DM, Wang HS, Saitoh M, Mural JG, Henley DC, *et al*. Microtubule dysfunction induced by paclitaxel initiates apoptosis through both c-Jun N-terminal kinase (JNK)-dependent and -independent pathways in ovarian cancer cells. *J Biol Chem* 1999; 274: 8208-16.
- Okano J, Rustgi AK. Paclitaxel induces prolonged activation of the Ras/MEK/ERK pathway independently of activating the programmed cell death machinery. *J Biol Chem* 2001; 276: 19555-64.
- Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987; 162: 156-9.
- Schena M, Shalon D, Heller R, Chai A, Brown PO, Davis RW. Parallel human genome analysis: microarray-based expression monitoring of 1000 genes. *Proc Natl Acad Sci USA* 1996; 93: 10614-9.
- De Saizieu A, Certa U, Warrington J, Gray C, Keck W, Mous J. Bacterial transcript imaging by hybridization of total RNA to oligonucleotide arrays. *Nat Biotechnol* 1998; 16: 45-8.
- Lieu CH, Chang YN, Lai YK. Dual cytotoxic mechanisms of submicromolar taxol on human leukemia HL-60 cells. *Biochem Pharmacol* 1997; 53: 1587-96.
- Milross CG, Mason KA, Hunter NR, Chung WK, Peters LJ, Milas L. Relationship of mitotic arrest and apoptosis to antitumor effect of paclitaxel. *J Natl Cancer Inst* 1996; 88: 1308-14.
- Johannes FJ, Prestle J, Eis S, Oberhagemann P, Pfizenmaier K. PKCm is a novel, atypical member of the protein kinase C family. *J Biol Chem* 1994; 269: 6140-8.
- Hudson JW, Chen L, Fode C, Binkert C, Dennis JW. Sak kinase gene structure and transcriptional regulation. *Gene* 2000; 241: 65-73.
- Maekawa M, Ishizaki T, Boku S, Watanabe N, Fujita A, Iwamatsu A, *et al*. Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase. *Science* 1999; 285: 895-8.
- Anderson SC, SundarRaj N. Regulation of a Rho-associated kinase expression during the corneal epithelial cell cycle. *Invest Ophthalmol Vis Sci* 2001; 42: 933-40.
- Santoro MF, Annand RR, Robertson MM, Peng YW, Brady MJ, Mankovich JA, *et al*. Regulation of protein phosphatase 2A activity by caspase-3 during apoptosis. *J Biol Chem* 1998; 273: 13119-28.
- Kamada S, Kusano H, Fujita H, Ohtsu M, Koya RC, Kuzumaki N, *et al*. A cloning method for caspase substrates that uses the yeast two-hybrid system: cloning of the antiapoptotic gene gelsolin. *Proc Natl Acad Sci USA* 1998; 95: 8532-7.
- Stegh AH, Schickling O, Ehret A, Scaffidi C, Peterhansel C, Hofmann TG, *et al*. DEDD, a novel death effector domain-containing protein, targeted to the nucleolus. *EMBO J* 1998; 17: 5974-86.
- Schievella AR, Chen JH, Graham JR, Lin LL. MADD, a novel death domain protein that interacts with the type 1 tumor necrosis factor receptor and activates mitogen-activated protein kinase. *J Biol Chem* 1997; 272: 12069-75.
- Nakano H, Oshima H, Chung W, Williams-Abbott L, Ware CF, Yagita H, *et al*. TRAF5, an activator of NF-kappaB and putative signal transducer for the lymphotoxin-beta receptor. *J Biol Chem* 1996; 271: 14661-4.
- Tarricone C, Xiao B, Justin N, Walker PA, Rittinger K,

- Gamblin SJ, *et al*. The structural basis of Arfaptin-mediated cross-talk between Rac and Arf signalling pathways. *Nature* 2001; 411: 215-9.
- 27 Wilkinson KD, Tashayev VL, O'Connor LB, Larsen CN, Kasperk E, Pickart CM. Metabolism of the polyubiquitin degradation signal: structure, mechanism, and role of isopeptidase T. *Biochemistry* 1995; 34: 14535-46.
- 28 Linke T, Wilkening G, Sadeghlar F, Mozcall H, Bernardo K, Schuchman E, *et al*. Interfacial regulation of acid ceramidase activity. Stimulation of ceramide degradation by lysosomal lipids and sphingolipid activator proteins. *J Biol Chem* 2001; 276: 5760-8.