[Frontiers in Bioscience 11, 1924-1931, May 1, 2006]

Analysis of common gene expression patterns in four human tumor cell lines exposed to camptothecin using cDNA microarray: identification of topoisomerase-mediated DNA damage response pathways

XueQing Guo [1], JunPing Zhang [2], XuPing Fu [1], Qing Wei [1], YingHua Lu [1], Yao Li [1], Gang Yin [1], YuMin Mao [1], Yi Xie [1], YaoCheng Rui [2], and Kang Ying [1]

[1] State Key Laboratory of Genetic Engineering, Institute of Genetics, School of Life Science, Fudan University, Shanghai 200433, People's Republic of China, and [2] Department of Pharmacology, School of Pharmacy, Second Military Medical University, Shanghai 200433, People's Republic of China

TABLE OF CONTENTS

1. ABSTRACT

Camptothecin (CPT) is a potent inhibitor of DNA topoisomerase I with a wide spectrum of anti-tumor activity. Relatively little information is available regarding the relation of known topoisomerase-mediated DNA damage with other intracellular pathways. To gain an insight into the intracellular molecular mechanisms of Topoisomerase I inhibitor camptothecin-mediated DNA damage leading to cell death, we used a high-density cDNA microarray to assess sensitive early gene expression profiles in SGC7901 (gastric cancer), Hela (cervical adenocarcinoma), K562 (chronic myelogenous leukemia) and HL60 (promyelocytic leukemia) tumor cells stimulated with camptothecin for 1 h at the concentrations of GI50 (50 % growth inhibition after 24 h of treatment). Analysis of the differentially expressed genes obtained 29 response genes common to all four cell lines. Moreover, these cell lines also shared the direction of regulation. Most of these common response genes were functionally related to cell proliferation or apoptosis, and some of them were involved in ATM (ataxia-telangiectasia mutated) and ATR (ATM- and Rad3 related) checkpoint pathways, JNK (c-Jun N-terminal kinase) pathway, the survival phosphatidylinositol (PI) 3 kinase-Akt-dependent pathway, mitochondrial cell death pathway, endoplasmic reticulum (ER)-related cell death pathway, and to ubiquitin/proteasome dependent protein degradation pathway. The data provides evidence for a linkage between topoisomerase-mediated DNA damage and intracellular signaling events, which may facilitate our understanding of the camptothecin mediated molecular mechanisms of action.

2. INTRODUCTION

Camptothecin (CPT), a cytotoxic alkaloid isolated from *Camptotheca acuminata,* is a potent inhibitor of DNA topoisomerase I with a wide spectrum of anti-tumor activity. CPT binds and stabilizes the covalently linked topoisomerase I–DNA complexes, causing DNA damages to cell death(1). Evidence has been shown that regulatory pathways leading from topoisomerase-mediated DNA damage to cell death involve the proapoptotic Chk2, c-Abl and c-Jun N-terminal kinase/stress-activated protein kinases (JNK/SAPK) pathways, the survival PI3kinase-Akt-dependent pathway and the transcription factors p53 and NF-kappaB(2). These molecular responses can be cell-type specific and contribute to differential CPT sensitivity. It has been shown that DNA damage caused by CPT elicits a broad transcriptional changes, including p21/WAF1, cyclin B1, bcl-2, and bax, which can be either primary topoisomerase-mediated responses or secondary cellular outcome(3,4). Although a number of key regulatory pathways are now understood, relatively little information is available regarding the connectivity of known topoisomerase-mediated DNA damage with other intracellular pathways. Identification of changes in gene expression levels with cDNA microarray technology makes it possible to measure changes in the relative expression levels of thousands of genes and, therefore, to analyze multiple molecular events of drug response simultaneously and potentially identify novel pathways(5,6).

In this study, we used a high-density cDNA microarray to assess sensitive early gene expression profiles in four human tumor cell lines??SGC7901 (gastric cancer)??Hela (cervix adenocarcinoma), K562 (chronic myelogenous leukemia) and HL60 (promyelocytic leukemia), after brief CPT treatment at the 1h time-point. The four human tumor cell lines were chosen as more sensitive cells based on their responses to CPT treatment for 24 h to yield a half growth inhibition (GI50). To determine which genes are regulated rapidly by CPT and distinguish secondary gene changes that may involve protein/transcription factor neo-synthesis, we carried out experiments on early 1h time-point. We postulate that some molecular elements shared between different tumor cell lines might be central to and characteristic of the CPT-mediated cytotoxicity. Analysis of these common response genes may provide new insights into the molecular mechanisms of camptothecin.

3. MATERIALS AND METHODS

3.1. Cell culture and treatments

SGC7901, Hela, K562 and HL60 cells obtained from Shanghai Institute of Cell Biology and Biochemistry (Shanghai, China), were cultured in RPMI 1640 medium supplemented with 10 % fetal calf serum, penicillin100 units/ml and streptomycin 100 mg/ml. For treatment, cells in late log phase of growth were cultured in fresh medium at a concentration of 1×10^6/ml and incubated at 37 □ in a humidified atmosphere of 5% CO_2, 95% air. Camptothecin (Sigma, St. Louis, MO) dissolved in 0.2% dimethylsulfoxide (DMSO) at the concentration of GI50 was added to SGC7901 (13.16 μmol/L), Hela (74.12 μmol/L), K562 (23.84 μmol/L) and HL60 (18.09 μmol/L) and cultures were incubated for 1h. Control cells were incubated with an equal volume of drug solvent.

3.2. Gene expression analysis

Synthetic of fluorescent cDNA probes, hybridization with human 14,400 cDNA microarray, and signal analysis were conducted by United Gene Holdings, Ltd. (Shanghai, China) as described (7,8). The list of 14,400 genes is available at http://www.chinagenenet.com/Camptothecin_Microarray/. Approximately 1% of verified-sequence and expressed sequence tags presented in the microarray showed a more than 2.0-fold difference in signal intensity between the two channels, and the coefficient of variation of the United Gene cDNA microarray platform for differential expression is 8% to 12% across the entire signal range. In brief, RNA samples prepared from CPT-treated cells and from control cells were labeled with Cy5-dUTP and Cy3-dUTP respectively. The two cDNA probes were then mixed and hybridized to the human 14,400 cDNA microarray. The fluorescent intensities of arrays were scanned with a ScanArray 3000 (GSI Lumonics, Bellerica, Mass., USA) at two wavelengths to detect emission from both Cy3 and Cy5. The acquired images were analyzed using ImaGene 3.0 software (BioDiscovery, Los Angeles, Calif., USA). Overall intensities were normalized with a correction coefficient obtained using global normalization. To minimize artifacts arising from low expression values, only genes with raw intensity values for both Cy3 and Cy5 of more than 800 counts were chosen for differential analysis. Genes were identified as differentially expressed if the absolute value of the natural logarithm of the average ratios was above 0.69 (gene expression change more than 2-fold). Hybridizations were conducted in three separate experiments, from which only those genes whose expression level changed by at least 2-fold in all data sets were selected. Data represented average ratio of triplicate independent experiments.

To confirm some interesting genes regulated by CPT, Real-Time PCR was performed by using two pairs of CPT-treated and control Hela cell preparations isolated in independent experiments according to the protocol described by TaKaRa RT Reagents (TaKaRa Biotechnology, Dalian, China). Briefly, Hela cells were treated with CPT or vehicle as described in microarray experiments, total RNA samples were then extracted and reverse-transcribed. The resulting cDNA was used in subsequent real-time PCR reaction after ten times dilution. Real-time PCR was carried out using an ABI 7900HT sequence Detection System in 384-well reaction plates using ABSOLUTE™ QPCR SYBR GREEN LOW ROX MIXES (ABgene UK). All primers were designed using Prime Primer 5 software (PREMIER Biosoft International) (Table 1). Samples were analyzed in triplicate; the threshold cycle (Ct) method, as described in the manufacturer's protocol, was used to generate relative expression values. Results were expressed relative to GAPDH control.

4. RESULTS

4.1. Common response genes in human tumor cells treated with camptothecin

To access the sensitive early transcriptional response of tumor cell lines to camptothecin, we first

Table 1. Primer pairs used for real-time RT-PCR

Primer name	Forward primer (5'-3')	Reverse primer (5'-3')
ANT3	CGTGCAGGGCATCATCATCTA	TCACCACGATGTGCGTGTTC
DDB1	ATCATCCGGAATGGAATTGGAA	TCAGACCGCAGTGGCCATAA
GSP1	AAGACCCGCAGAATGCACCT	GGCCGCGTACTGTTCCAGAT
AKT1	GGCCCAGATGATCACCATCAC	CTATCGTCCAGCGCAGTCCA
MCM7	AGGTGGCTCTGTATGTGGACCTG	TCCATCATTAGCCGATGCTCAA
ZAK	GCTGACAGAGCAGTCCAACACC	GACATGACATCTCTGCACTGTTTGA
G3PDH	GCACCGTCAAGGCTGAGAAC	ATGGTGGTGAAGACGCCAGT

Table 2. Count number of the differential expressed genes of tumor cells in response to camptothecin.

Cell line	Count number of the differential expressed genes		
	Total	Up	Down
K562	242	106	136
Hela	538	166	372
SGC7901	539	33	506
HL60	1410	8	1402

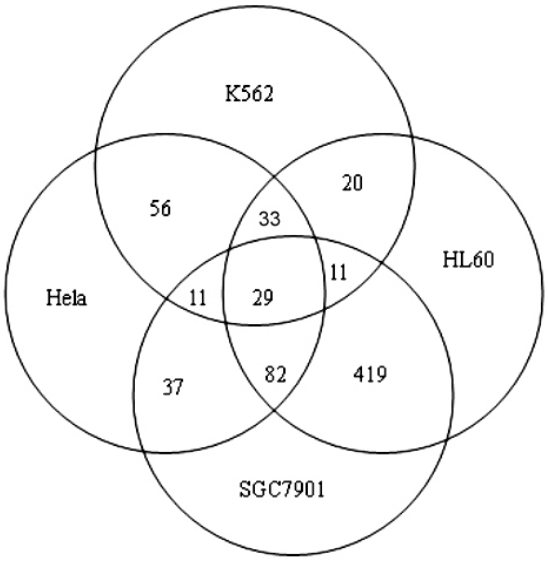

Figure 1. Venn diagram of the number of differential genes altered in HL60, K562, Hela, and SGC7901 cells treated with camptothecin.

analyzed gene expression patterns of SGC7901,Hela, K562 and HL60 cells upon addition of camptothecin at the concentrations of GI50 with United Gene cDNA microarray technology. Cells were treated for 1h, RNA isolated, and microarray analysis performed as described in "Materials and Methods". Of the genes on the array, about 80% gave measurable hybridization signals. Table 2 summarized the differential gene alternation in four cell lines. The complete gene expression profiles can be available at the website (http://www.chinagenenet.com/Camptothecin_Microarray/). Although the pharmacological effect (50% growth inhibition after 24 h of treatment) was the same for each cell line, the number of regulated genes differed markedly between experiments, with the strongest response in HL60 cells. Next, we compared common response genes among cell lines only with those sequences that were differentially altered in all experiments. As shown in Figure 1, the largest overlap was between HL60 cells and SGC7901 cells with 541 shared genes. A total of 166 drug response genes were found to overlap among three cell lines, and 29 genes were identified in all four cell lines (Table 3). Moreover, these common response genes also shared in the same direction. Most common genes were related functionally to cell proliferation or apoptosis as well as other important cellular functions such as the regulation of energy metabolism. In this study, we focused our attention on common response genes in all cell lines and examined their changes in specific pathways.

4.2. Multiple genes involved in DNA damage checkpoint mechanism

Several striking altered genes were identified to be involved with DNA damage checkpoint pathway. For example, DNA replication licensing factor MCM7(minichromosome maintenance deficient 7) and DDB1(damage-specific DNA binding protein 1), encoding enzymes functioned in DNA replication and repair were among the genes suppressed by CPT treatment. Gene for enzyme Protein kinase B(Akt1), a key survival factor in many cellular processes, was found to be down regulated in CPT-treated cells. In addition, expression of G protein pathway suppressor 1 (GPS1) and sterile alpha motif and leucine zipper containing kinase AZK (ZAK), two JNK pathway-related factors, was also shown to be repressed by CPT.

4.3. Camptothecin affects mitochondrial cell death pathway

Camptothecin exposure resulted in decreased expression of a cluster of genes that belong to mitochondrial cell death pathway. MRPL28 and MRPS18A, genes encoding mitochondrial ribosomal proteins, and pyruvate kinase and argininosuccinate lyase, genes encoding energy metabolism-related proteins, as well as ADP/ATP translocase 3 (ANT3), encoding for mitochondrial inner membrane protein were observed in all cells in response to CPT.

4.4. Camptothecin affects endoplasmic reticulum-related cell death pathway

CPT-treatment cells showed down-regulation of Ribophorin I and endoplasmic reticulum lumenal protein

Table 3. Common response genes in camptothecin-treated cell lines

Accession number	Gene name	Average Ratio (n=3)			
		K562	Hela	SGC7901	HL60
DNA replication and repair					
NM_005916	MCM7 minichromosome maintenance deficient 7 (MCF7)	0.480	0.389	0.323	0.174
NM_001923	damage-specific DNA binding protein 1(DDB1)	0.462	0.357	0.285	0.358
Translation					
NM_032378	eukaryotic translation elongation factor 1 delta (EEF1D)	0.325	0.200	0.251	0.160
NM_004461	phenylalanine-tRNA synthetase-like, alpha subunit(FARSLA)	0.498	0.361	0.345	0.289
RNA processing					
NM_015885	pre-mRNA cleavage complex II protein (PCF11)	0.477	0.399	0.317	0.438
NM_004966	heterogeneous nuclear ribonucleoprotein F(HNRPF)	0.480	0.302	0.369	0.321
NM_031266	heterogeneous nuclear ribonucleoprotein A/B(HNRPAB)	0.491	0.383	0.373	0.351
Signal tranduction					
NM_005163	Protein kinase B(Akt1)	0.481	0.424	0.410	0.388
NM_002708	protein phosphatase 1, catalytic subunit, alpha isoform(PPP1CA)	0.473	0.431	0.472	0.219
NM_133646	sterile alpha motif and leucine zipper containing kinase AZK	0.499	0.421	0.359	0.356
NM_004127	G protein pathway suppressor 1(GPS1)	0.463	0.308	0.351	0.272
Proteasome-related					
NM_002804	proteasome 26S subunit 3(PSMC3)	0.475	0.287	0.256	0.261
NM_007126	valosin-containing protein(VCP)	0.485	0.452	0.315	0.303
Mtabolism and mitochondrial					
NM_002654	pyruvate kinase, muscle(PKM2)	0.382	0.276	0.243	0.378
NM_000048	argininosuccinate lyase(ASL)	0.435	0.304	0.478	0.357
NM_006428	mitochondrial ribosomal protein L28(MRPL28)	0.460	0.352	0.417	0.411
NM_018135	mitochondrial ribosomal protein S18A(MRPS18A)	0.349	0.240	0.392	0.508
NM_001636	ADP/ATP translocase 3(ANT3)	0.271	0.145	0.268	0.218
ER-related					
NM_001662	ADP-ribosylation factor 5(ARF5)	0.455	0.319	0.255	0.400
NM_006817	endoplasmic reticulum lumenal protein ERp29	0.486	0.402	0.417	0.220
NM_002950	ribophorin I (RPN1)	0.493	0.383	0.344	0.536
Cell movement and Metastasis					
NM_033401	contactin associated protein-like 4 (CNTNAP4)	0.409	0.217	0.254	0.186
NM_020040	tubulin, beta polypeptide 4, member Q(TUBB4Q)	0.370	0.238	0.389	0.373
Growth factor					
NM_003288	tumor protein D52-like 2 (TPD52L2)	0.488	0.420	0.421	0.418
NM_002087	granulin (GRN)	0.405	0.344	0.314	0.307
Others					
NM_001288	chloride intracellular channel 1 (CLIC1)	0.478	0.528	0.271	0.160
BG283400	602407153F1 NIH_MGC_91 Homo sapiens cDNA clone IMAGE:4519381 5', mRNA sequence.	0.486	0.337	0.299	0.153
BC036583	Homo sapiens, clone IMAGE:4796818, mRNA	0.375	0.194	0.236	0.194
AK098686	Homo sapiens cDNA FLJ25820 fis, clone TST07642, highly similar to TUBULIN BETA-2 CHAIN	0.408	0.218	0.271	0.205

ERp29, which encode proteins associated with processing of secretory proteins within the endoplasmic reticulum (ER), and reduction of ADP-ribosylation factor 5 (hARF5), which gene family encode proteins involved in protein transport between the ER and Golgi compartments.

4.5. Camptothecin affects ubiquitin/proteasome-dependent protein degradation pathway

Proteasome 26S subunit 3 (PSMC3) and valosin-containing protein (VCP), which is involved in ubiquitin/proteasome dependent protein degradation pathway, were differentially regulated by CPT.

4.6. Camptothecin alters expression of other genes

In addition to the above-listed gene groups, some genes related to protein synthesis and RNA processing (e.g., eukaryotic translation elongation factor 1 delta EEF1D, phenylalanine-tRNA synthetase-like, alpha subunit FARSLA, heterogeneous nuclear ribonucleoprotein F, heterogeneous nuclear ribonucleoprotein A/B, pre-mRNA cleavage complex II protein Pcf11), tumor growth-related genes (eg. tumor protein D52-like 2, granulin), and others (eg. chloride intracellular channel, contactin associated protein-like 4) were also repressed by CPT treatment.

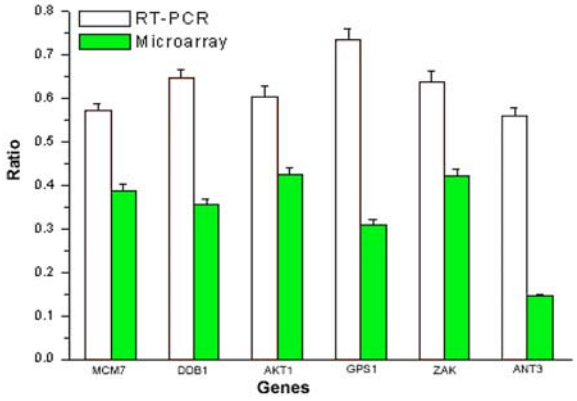

Figure 2. Effects of camptothecin on expression of MCM7, DDB1, AKT1, GPS1, ZAK and ANT3 in Hela cells. Hela cells were treated with camptothecin for 1 h and subjected to cDNA microarray and real time PCR as described in "Materials and Methods". Data are expressed as average ratio (n=3) relative to control.

4.7. Confirmation of gene changes by real-time RT-PCR

Six common response genes involved in several pathways were selected and subjected to real-time PCR analysis to confirm our microarrays results. As shown in Figure 2, the expression of MCM7, DDB1, Akt1, GPS1, AZK and ANT3 in Hela cells was down regulated after CPT treatment, which was consistent with the microarray results. The difference of expression levels between real-time PCR analysis and microarray signal intensity analysis might come from different data normalization method (GAPDH control vs global normalization).

5. DISCUSSION

In this study, we used cDNA microarray to examine the early gene alterations in four tumor cell lines with different genetic background in response to camptothecin. The cellular reaction to camptothecin involved a number of specific transcriptional evens. Interestingly, we identified 29 common response genes in all tested sensitive cells. Some interesting results were also confirmed by real-time RT-PCR. For the similar inhibitory effect induced by camptothecin in these cell lines after 24 h treatment, it is reasonable to speculate that these common response genes might be central to or responsible for the camptothecin-induced cytotoxicity. As expected, most of common response genes were functionally related to cell proliferation or apoptosis, which play important roles in multiple biochemical and regulatory pathways. Analysis of these genes might provide new insight into the molecular mechanisms of camptothecin.

Microarray showed striking changes after camptothecin treatment were the genes associated with several specific DNA damage checkpoint pathways. MCM7, which was found down regulation in all cell lines in this study, is linked to ATM (ataxia-telangiectasia mutated) and ATR (ATM-and Rad3related) checkpoint pathways through which multiple genotoxic responses are achieved, including cell cycle arrest and apoptosis. MCM7 is one of the highly conserved mini-chromosome maintenance proteins (MCMs) helicase complexes that function to initiate and elongate replication forks(9). Recently it has been found that ATR-interacting protein (ATRIP)-ATR interacts with MCM7 and reducing amount of MCM7 in cells would disrupt checkpoint signaling and cause an intra-S-phase checkpoint defect(10). DDB1, another notably down-regulated common response gene, encodes the large subunit of DNA damage-binding protein UV-DDB, which functions in global genomic nucleotide excision repair and cell cycle regulation. DDB1 has been implicated in the replication checkpoint pathway in S.pombe, whose defect activates checkpoint kinase Cds1 and shows delayed replication progression(11). Antisense inhibition of Chk2/hCds1, homolog of the yeast Cds1, is found to cause defective S-phase delay and enhance apoptosis following DNA damage induced by camptothecin or gamma-radiation(12). The activation of the c-Jun N-terminal kinase (JNK) pathway has been implicated in apoptotic responses to DNA damage, cell stress and cytotoxic drugs(13). In this study, expression of two JNK pathway-related factors GPS1 and ZAK was found suppressed by CPT. GPS1 is a signal transduction repressor, which might interfere with JNK activity by suppressing G-protein- and mitogen-activated protein kinase (MAPK)-mediated signal transduction(14). ZAK, a mixed lineage kinase-like protein, functions as mitogen-activated protein kinase kinase kinase in MAPK pathways by activating MKK7 leading to the activation of the JNK/stress-activated protein kinases (SAPK) pathway as well as the activation of transcription factor NF-kappaB. Moreover, overexpression of the ZAK gene induces tumor cell apoptosis(15). The induction of ZAK was found in other independent experiments in which Hela cells were treated with camptothecin at the tested concentration for 6 h sustained treatment (unpublished data). Why this gene expression was inhibited in the early response to camptothecin remains to be further study. In addition, AKT, or protein kinase B expression was also found in this study. The serine/threonine kinase AKT is a key survival factor, whose activation has been frequently observed in human cancers(16). Overexpresion of AKT has been associated with resistance to DNA damage-induced agents such as camptothecin(17). Specific inhibition of the activation of AKT by several small molecules sensitizes human cancer cells to chemotherapy-induced apoptosis or cell cycle arrest(18). Taken together, alternation of these early common response genes involved in several DNA damage checkpoint regular pathways by camptothecin might determine ultimate cellular fate.

The mitochondrial apoptotic pathway has been implicated the main pathways for DNA damage-induced cell death(19). We identified several mitochondrion related genes in this study. ADP/ATP translocase 3 (ANT3) is a bifunctional protein, the only mitochondrial translocase for the exchange of ATP for ADP through the inner mitochondrial membrane, and a core component of the so-called mitochondrial permeability transition pore (MPTP)(20). The involvement of MPTP in cell death has been reported by a number of groups(21). Opening of MPTP might cause egress of pro-apoptotic factors such as

Bcl-2, Cytochrome c to the cytosol to activate caspase cascade leading to ultimately cell death. Mitochondrial ribosomes MRPL28 and MRPS18A take part in protein synthesis within the mitochondrion, and mitochondrial ribosomal inaccuracy has been implicated in mitochondrial dysfunction and senescence(22). Pyruvate kinase and argininosuccinate lyase are key enzymes involved in energy production via tricarboxylic acid cycle and mitochondrial respiration. Decreased expression of these genes may induce defects in mitochondrial respiratory chain activity or Adenosine triphosphate (ATP) depletion leading to cell death. These finding suggested CPT-induced cell death might result from multiple mitochondrial dysfuctions.

In addition to mitochondrion, endoplasmic reticulum (ER) is another organelle coupled to apoptosis, which can also directly initiate pathways to caspase activation and apoptosis(23). ER stress induced by inhibitors of protein glycosylation and other ER stressors provokes accumulation of unfolded protein in the ER, as a result leading to cell death. Normal cells respond to ER stress by increasing transcription of genes encoding ER-resident chaperones such as GRP78/BiP, to facilitate protein folding. Our results showed CPT treatment down-regulated two genes associated with ER stress. Ribophorin I encodes a component of oligosaccharyl transferase complex, involving in protein glycosylation and peptide translocation across the membranes of ER(24), moreover, reduced expression of ribophorins has been found in apoptotic cells(25). ERp29, an ER-resident lunmental protein, is found to be associated with GRP78/BiP and this interaction is significantly enhanced by treatment with ER stressors(26). In addition to above two ER genes, expression of ADP-ribosylation factor 5 (ARF5) was also reduced upon CPT treatment. ARFs gene family encodes proteins involved in protein transport between the endoplasmic reticulum and Golgi compartments. It is widely known that ER stressor brefeldin A (BFA), targeting ADP-ribosylation factor-guanine nucleotide exchange factor, induces apoptosis and cell growth inhibition in various human cell lines, by the mechanism of blockade of protein transport between ER and the Golgi apparatus resulting in an aberrant accumulation of proteins in the ER(27). Our results suggest that changes in specific genes involved in ER stress may play an important role in camptothecin-mediated cell death.

A panel of studies has demonstrated that the inhibition of the ubiquitin/proteasome-dependent protein degradation pathway (Ub-Pr pathway) is associated with apoptosis induced by antitumor drugs(28). Previously Huang and others reports that camptothecin activation of NF-kappaB involves degradation of cytoplasmic IkappaBalpha by the ubiquitin-proteasome pathway(29). Here we showed PSMC3 and VCP associated with Ub-Pr pathway was down-regulated after camptothecin treatment, supporting the involvement of Ub-Pr pathway in camptothecin-mediated cytotoxicity. PSMC3 is a core component of the 19S regulatory particle of the 26S proteasome. The destruction of 26S proteasome-dependent protein degradation function caused by various stresses would accumulate mistaken protein leading to final cell death(28). VCP is a multiubiquitin chain-targeting factor required for the Ub-Pr pathway, which has been showed high express in many carcinomas(30,31). VCP modulates NF-kappaB activation by influencing the degradation process of Inhibitor IkappaBalpha(32). Our data suggested the alterations in these specific genes involved in Ub-Pr pathway may be responsible for camptothecin-induced cytotoxicity.

In conclusion, our present results provide a molecular description of early intracellular response to camptothecin in all four tumor cells. These common response genes were involved in ATM/ATR checkpoint pathways, JNK/SAPK pathways, the survival PI3kinase-Akt-dependent pathway, ER- and mitochondria-apoptotic pathway as well as ubiquitin/proteasome- dependent protein degradation pathway, which provides evidence for a linkage between topoisomerase-mediated DNA damage and intracellular signaling events leading to eventual cell death. Most of early common response genes discovered in this work, to our knowledge, had not previously been described in respect of camptothecin effects and thus represented potential novel molecular mechanisms.

6. ACKNOWLEDGEMENTS

This work was supported in part by grant 02dz19141 and 01dj19007 from Shanghai Science and Technology Development Foundation.

7. REFERENCES

1.Hentze H., M. Latta, G. Kunstle, S. Dhakshinamoorthy, A. G. Porter & A. Wendel: Topoisomerase inhibitor camptothecin sensitizes mouse hepatocytes in vitro and in vivo to TNF-mediated apoptosis. *Hepatology* 39 (5), 1311-20 (2004)

2. Sordet O., Q. A. Khan, K. W. Kohn & Y. Pommier: Apoptosis induced by topoisomerase inhibitors. *Curr Med Chem Anti-Canc Agents* 3 (4), 271-90 (2003)

3. Zhou Y., F. G. Gwadry, W. C. Reinhold, L. D. Miller, L. H. Smith, U. Scherf , E. T. Liu, K. W. Kohn, Y. Pommier & J. N. Weinstein: Transcriptional regulation of mitotic genes by camptothecin-induced DNA damage:microarray analysis of dose- and time-dependent effects. *Cancer Res.* 62 (6), 1688-95 (2002)

4. Ullmannova V. & C. Haskovec: Gene expression during camptothecin-induced apoptosis in human myeloid leukemia cell line ML-2. *Neoplasma* 51 (3), 175-80 (2004)

5. Schena M., D. Shalon, R. W. Davis & P. O. Brown: Quantitative monitoring of gene expression patterns with a complementary DNA microarray**.** *Science* 270 (5235), 467-70 (1995)

6. Brachat A., B. Pierrat, A. Xynos, K. Brecht, M. Simonen, A. Brungger & J. Heim: A microarray-based, integrated approach to identify novel regulators of cancer drug response and apoptosis**.** *Oncogene* 21 (54), 361-71 (2002)

7. Zhang J. P., K. Ying, Z. Y. Xiao, B. Zhou, Q. S. Huang, H. M. Wu, M. Yin, Y. Xie, Y. M. Mao & Y. C. Rui: Analysis of gene expression profiles in human HL-60 cell

exposed to cantharidin using cDNA microarray. *Int J Cancer* 108 (2), 212-8 (2004).
8. Wan Y. F. , X. Q. Guo, Z. H. Wang, K. Ying & M. H. Yao: Effects of paclitaxel on proliferation and apoptosis in human acute myeloid leukemia HL-60 cells**.** *Acta Pharmacol Sin.* 25 (3), 378-84 (2004)
9. Pacek M. & J. C. Walter: A requirement for MCM7 and Cdc45 in chromosome unwinding during eukaryotic DNA replication. *EMBO J.* 23 (18), 3667-76 (2004)
10. Cortez D., G. Glick & S. J. Elledge: Minichromosome maintenance proteins are direct targets of the ATM and ATR checkpoint kinases. *Proc Natl Acad Sci U S A*. 101 (27), 0078-83 (2004).
11. Bondar T., E. V. Mirkin, D. S. Ucker, W. E. Walden, S. M. Mirkin & P. Raychaudhuri: Schizosaccharomyces pombe Ddb1 is functionally linked to the replication checkpoint pathway. *J Biol Chem.* 278 (39), 37006-14 (2003)
12. Yu Q., J. H. Rose, H. Zhang & Y. Pommier: Antisense inhibition of Chk2/hCds1 expression attenuates DNA damage-induced S and G2 checkpoints and enhances apoptotic activity in HEK-293 cells. *FEBS Lett.* 505 (1), 7-12 (2001).
13. Crighton D. & K. M. Ryan: Splicing DNA-damage responses to tumour cell death. *Biochim Biophys Acta.* 1705 (1), 3-15 (2004).
14. Spain B. H., K. S. Bowdish, A. R. Pacal, S. F. Staub, D. Koo, C. Y. Chang, W. Xie & J. ColicelliJ: Two human cDNAs, including a homolog of Arabidopsis FUS6 (COP11), suppress G-protein- and mitogen-activated protein kinase-mediated signal transduction in yeast and mammalian cells. *Mol Cell Biol.* 16 (12), 6698-706 (1996).
15. Yang J. J: Mixed lineage kinase ZAK utilizing MKK7 and not MKK4 to activate the c-Jun N-terminal kinase and playing a role in the cell arrest. *Biochem Biophys Res Commun.* 297 (1), 105-10 (2002)
16. Nicholson K. M. & N. G. Anderson: The protein kinase B/Akt signalling pathway in human malignancy. *Cell Signal*. 14 (5), 381-95 (2002)
17. Reinhold W. C., H. Kouros-Mehr, K. W. Kohn, A. K. Maunakea, S. Lababidi, A. Roschke, K. Stover, J. Alexander, P. Pantazis, L. Miller, E. Liu, I. R. Kirsch, Y. Urasaki, Y. Pommier & J. N. Weinstein: Apoptotic susceptibility of cancer cells selected for camptothecin resistance. gene expression profiling, functional analysis, and molecular interaction mapping. *Cancer Res.* 63 (5), 1000-11 (2003).
18. Martelli A. M., P. L. Tazzari, G. Tabellini, R. Bortul, A. M. Billi, L. Manzoli, A. Ruggeri, R. Conte & L. Cocco: A new selective AKT pharmacological inhibitor reduces resistance to chemotherapeutic drugs, TRAIL, all-trans-retinoic acid, and ionizing radiation of human leukemia cells. *Leukemia.* 17 (9), 1794-805 (2003).
19. Kim R.: Recent advances in understanding the cell death pathways activated by anticancer therapy. *Cancer.* 103 (8), 1551-60 (2005).
20. Belzacq A. S., H. L. Vieira, G. Kroemer, & C. Brenner: The adenine nucleotide translocator in apoptosis. *Biochimie.* 84 (2-3), 167-76 (2002)
21. Kim J. S., L. He & J. J. Lemasters: Mitochondrial permeability transition. a common pathway to necrosis and apoptosis. *Biochem Biophys Res Commun.* 304 (3), 463-70 (2003)
22. Hipkiss A. R: Errors, mitochondrial dysfunction and ageing. *Biogerontology.* 4 (6), 397-400 (2003)
23. Rao R. V., H. M. Ellerby & D. E: Bredesen. Coupling endoplasmic reticulum stress to the cell death program. *Cell Death Differ.* 11 (4), 372-80 (2004)
24. Kelleher D. J., D. Karaoglu, E. C. Mandon & R. Gilmore: Oligosaccharyltransferase isoforms that contain different catalytic STT3 subunits have distinct enzymatic properties. *Mol Cell*. 12 (1), 101-11 (2003)
25. Sanjay A., J. Fu & G. Kreibich: DAD1 is required for the function and the structural integrity of the oligosaccharyltransferase complex. *J Biol Chem.* 273 (40), 26094-9 (1998).
26. Mkrtchian S., C. Fang, U. Hellman & M. Ingelman-Sundberg. A stress-inducible rat liver endoplasmic reticulum protein, ERp29. *Eur J Biochem.* 251 (1-2), 304-13 (1998)
27. Nebenfuhr A., C. Ritzenthaler, D. G. Robinson & A. Brefeldin: deciphering an enigmatic inhibitor of secretion. *Plant Physiol.* 130 (3), 1102-8 (2002).
28. Magill L., B. Walker & A. E. Irvine: The proteasome, a novel therapeutic target in haematopoietic malignancy. *Hematology.* 8 (5), 275-83 (2003)
29. Huang T. T., S. M. Wuerzberger-Davis, B. J. Seufzer, S. D. Shumway, T. Kurama, D. A. Boothman & S. Miyamoto: NF-kappaB activation by camptothecin. A linkage between nuclear DNA damage and cytoplasmic signaling events. *J Biol Chem.* 275 (13), 9501-9 (2000)
30. Yamamoto S., Y. Tomita, Y. Hoshida, M. Sakon, M. Kameyama, S. Imaoka, M. Sekimoto, S. Nakamori, M. Monden & K. Aozasa: Expression of valosin-containing protein in colorectal carcinomas as a predictor for disease recurrence and prognosis.*Clin Cancer Res* 10 (2), 651-7 (2004)
31. Tsujimoto Y., Y. Tomita, Y. Hoshida, T. Kono, T. Oka, S. Yamamoto, N. Nonomura, A. Okuyama & K. Aozasa: Elevated expression of valosin-containing protein (p97) is associated with poor prognosis of prostate cancer. *Clin Cancer Res.*10 (9), 3007-12 (2004)
32. Asai T., Y. Tomita, S. Nakatsuka, Y. Hoshida, A. Myoui, H. Yoshikawa & K. Aozasa: VCP (p97) regulates NFkappaB signaling pathway, which is important for metastasis of osteosarcoma cell line. *Jpn J Cancer Res.* 93 (3).296-304 (2003)

Abbreviations: ANT3: ADP/ATP translocase 3, ARF5: ADP-ribosylation factor 5, CPT: Camptothecin, DDB1: damage-specific DNA binding protein 1, EEF1D: eukaryotic translation elongation factor 1 delta, ERp29: endoplasmic reticulum lumenal protein 29 precursor, GAPDH: glyceraldehyde-3-phosphate dehydrogenase, GPS1: G protein pathway suppressor 1, Hela: human cervix adenocarcinoma, HL60: human promyelocytic leukemia, K562: human chronic myelogenous leukemia, MCM7: minichromosome maintenance deficient 7, MRPL28: mitochondrial ribosomal protein L28, MRPS18A: mitochondrial ribosomal protein S18A, PMSC3: proteasome 26S ATPase subunit 3, SGC7901: human gastric cancer, VCP: valosin-containing protein, ZAK:

sterile alpha motif and leucine zipper containing kinase AZK

Key words: Microarray, Gene Expression Pattern, Camptothecin

Send correspondence to: Dr Kang Ying, State Key Laboratory of Genetic Engineering, Institute of Genetics, School of Life Science, Fudan University, Shanghai 200433, People's Republic of China. Tel: 86-021-65989936, Fax: 86-21-65986530. E-mail: ying_kang@hotmail.com

http://www.bioscience.org/current/vol11.htm