Drug-sensitivity pattern analysis for study of functional relationship between gene products

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Abstract **We have developed a method that we call 'drug-sensitivity pattern analysis', or DSPA, for analysis of protein function. Cells are transfected with cDNA of the test molecule, followed by analysis of the sensitivity of the transfected cells to multiple growth-inhibitory drugs. If two cDNA products have similar functions, their transfected cells should show similar drug-sensitivity patterns. The cDNAs of some signaling molecules were transfected into NIH3T3 or Ha-*ras*-transformed NIH3T3 (ras-NIH) cells and stable transfectants, which expressed high amounts of the gene product, were isolated. Chemosensitivity of the transfected clone was compared with the parental cells by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide method using more than 40 drugs. The chemosensitivity changes caused by the transfected gene were calculated and expressed numerically as 'drug chemosensitivity index' (DCI). When the DCI values were analyzed by regression analysis, a significant positive relationship between IκBα superrepressor and dominant-negative IKKβ and an inverse relationship between p53 and Mdm2 were consistent with previous reports. Thus, the DSPA method is useful for identifying functional similarities between gene products.**

Key words: Protein function; Transfection; 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay; Signal transduction

1. Introduction

The ability to analyze the function of gene products may be one of the main challenges of biological research in the post-genomic era. The current methods for analyzing protein function are exemplified by yeast two-hybrid systems, GST pull-down assays and the TAP method [1] as a probe for binding proteins, and by differential display and DNA microarray to investigate changes in gene expression. Such methods are laborious, time-consuming, and expensive. Development of simple methods for analysis of protein function has been expected.

Functional analysis can also be studied in cultured cells by transfection followed by induction of the expression of the test cDNA. However, without remarkable changes in cell growth or morphology, one does not know how intracellular molecular events have been influenced by the transfected cDNA. We have reported that activated Ha-*ras*-transformed NIH3T3 (ras-NIH) cells have different sensitivities to anticancer drugs [2]. The patterns of sensitivity were similar to those of Ki-*ras*-transformed cells but different from v-*src*- or c-*erb*B2-transformed cells. We hypothesize that the changes in drug sensitivity of cells transfected by genes may produce a specific pattern, depending upon the function of the gene products. Analysis of the drug-sensitivity pattern (DSPA), following transfection by genes, may be useful for predicting the functional similarity of gene products.

2. Materials and methods

2.1. Cell culture

NIH3T3, ras-NIH and their transfected cells [3] were cultured in Dulbecco's modified Eagle's medium supplemented with 5% calf serum.

2.2. Transfection

The cDNAs were inserted into eukaryotic expression vectors pcDNA3, pRc-CMV (Invitrogen) or pMSG (Amersham Pharmacia) and transfected into NIH3T3 or ras-NIH cells using Lipofectamine reagent (Invitrogen) as previously described [4–6]. After culture in the presence of G418 (400 μg/ml) for 2 weeks, surviving colonies were isolated.

2.3. MTT assay

Five thousand cells per well were plated in 96-well plates in the presence of various concentrations of test drugs, and cultured for 3 days. The activity of mitochondrial succinic dehydrogenase was measured by incubation for 4 h in the presence of 0.5 mg/ml of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma). Absorbance was measured at 570 nm with a reference wavelength of 655 nm according to the method of Mosmann [7], using previously described methods [8]. Absorbance reflects the viable cell number and was expressed as a percentage of that of cells cultured in the absence of test drugs.

2.4. Preparation of cell extract and immunoblotting analysis

Cells were washed with phosphate-buffered saline three times and incubated in lysis buffer [0.5% Nonidet P-40, 20 mM Tris–HCl (pH 7.5), 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 50 μM leupeptin, 50 μM antipain, 50 μM pepstatin A and 50 μM *N*-acetyl-Leu-Leu-norleucinal (ALLN)] for 10 min at 4°C. The cell lysate was centrifuged at 13 000×*g* for 10 min and the resulting supernatant was lyophilized. Immunoblot analysis was carried out using Immunostar (Wako Pure Chemicals, Kyoto, Japan) as described previously [9].

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Table 1
List of drugs

Drug name	Primary activity or classification
Herbimycin A	Src, Abl kinase inhibitor
Geldanamycin	Src kinase inhibitor; hsp90 inhibitor
Erbstatin	EGFR inhibitor
Genistein	tyrosine kinase inhibitor, topoisomerase II inhibitor
Leflunomide	tyrosine kinase inhibitor
Staurosporine	PKC, cdk, MLCK inhibitor
K252a	CaM-kinase inhibitor
KN-93	CaM-kinase inhibitor
H-7	PKA, PKC, PKG inhibitor
H-8	PKG, PKA inhibitor
GF109203X	PKC inhibitor
ML-7	MLCK inhibitor
Y-27632	ROCK inhibitor
Olomoucine	cdk inhibitor
3-ATA	cdk4 inhibitor
Okadaic acid	PP1, PP2A inhibitor
Cantharidin	PP2A inhibitor
Sodium vanadate	tyrosine phosphatase inhibitor
Sodium arsenite	tyrosine phosphatase inhibitor
Mitomycin C	DNA cross-linker
5-Fluorouracil	thymidine synthetase inhibitor
Cisplatin	DNA cross-linker
Methotrexate	DHFR inhibitor
Mitoxantrone	DNA strand breaker
Aclarubicin	DNA intercalator
Ifosfamide	DNA alkylation
Hydroxyurea	ribonucleotide reductase inhibitor
6-Thioguanine	purine metabolism inhibitor
Camptothecin	topoisomerase I inhibitor
Peplomycin	DNA strand breaker
Vincristine	microtubule depolymerizer
Taxol	microtubule inhibitor
Etoposide	topoisomerase II inhibitor
Pifithrin	p53 inhibitor
BAY 11-7085	NF-κB inhibitor
Parthenolide	NF-κB inhibitor
HA14-1	Bcl-2 inhibitor
Ac-Leu-Leu-leucinal	calpain/proteasome inhibitor
ONO-3403	trypsin inhibitor
ONO-5046	elastase inhibitor
TPCK	chymotrypsin inhibitor
ICE inhibitor III	caspase inhibitor
Wortmannin	PI3K inhibitor
Quercetin	PI3K inhibitor
Manumycin	farnesyltransferase inhibitor
Cytochalasin D	actin polymerization inhibitor
Ouabain	Na^+,K^+-ATPase inhibitor
SAHA	histone deacetylase inhibitor
Sirtinol	deacetylase inhibitor
Telomerase inhibitor VIII	telomerase inhibitor
A23187	Ca^{2+} ionophore
BAPTA-AM	Ca^{2+} chelator
Thapsigargin	Ca^{2+}-ATPase inhibitor
Oligomycin	mitochondrial ATPase inhibitor
U73122	phospholipase C inhibitor
DFMO	ornithine decarboxylase inhibitor
SnPP	heme oxygenase inhibitor
Curcumin	5-lipoxygenase and cyclooxygenase inhibitor
NorNOHA	arginase inhibitor
5-AIQ	PARP inhibitor
Forskolin	adenylate cyclase activator
MDL-12,330A	adenylate cyclase inhibitor
NS2028	guanylate cyclase inhibitor
IBMX	phosphodiesterase inhibitor
Cycloheximide	protein synthesis inhibitor
Anisomycin	protein synthesis inhibitor
SNAP	NO donor
Resveratrol	COX1 inhibitor, ribonucleotide reductase inhibitor
Sulindac	COX inhibitor
Indomethacin	COX1/2 inhibitor, phospholipase A2 inhibitor
Sodium azide	cytochrome a/a_3 binding
Rapamycin	immunosuppressant
Betulinic acid	anti-HIV
C_8-ceramide	apoptosis inducer

The antibody used was phospho-specific extracellular signal-regulated kinase (ERK) antibody (Santa Cruz Biotechnology).

3. Results and discussion

3.1. Competence of MTT assay for analysis of chemosensitivity

The cDNAs were constructed in eukaryotic expression vectors and transfected into NIH3T3 or ras-NIH cells using Lipofectamine. Transfected cells were selected by culture in the presence of 400 μg/ml of G418 for 2 weeks. Clones that expressed the highest level of the cDNA-encoded protein, as analyzed by immunoblotting, were used in the MTT assay.

Sensitivities to at least 40 drugs (Table 1) were examined by Mosmann's MTT assay [7]. Because the MTT assay was highly reproducible, duplicate assays were sufficient to determine the sensitivity. The sensitivity of ras-NIH cells to wortmannin was almost the same when examined on different days (Fig. 1a). A similar overlap in the sensitivity to cisplatin between ras-NIH and STAT1-transfected ras-NIH cells was also observed (Fig. 1b). High expression of STAT1 caused no apparent effect on the sensitivity to cisplatin. In contrast, transfection of wild-type p53 into ras-NIH cells reduced the sensitivity to sodium arsenite (Fig. 1c).

A concentration of drug that gave 40% inhibition, the turning point for most of the sigmoid curves, was used to quantitate the difference of the chemosensitivity from the results of MTT assays (Fig. 1c). Then, the ratios of the drug concentrations giving 40% inhibition in a test cDNA-introduced clone (IC_{40}test) versus those in the parental cells (IC_{40}parent) were calculated. The logarithmic values of the ratios ($\log(IC_{40}\text{test}/IC_{40}\text{parent})$), designated drug chemosensitivity index (DCI), were entered into the database, partly shown in Table 2. The DSPA is based on comparison of the regression analysis of the DCI values of transfected cells by conventional statistical software. If significant correlation was observed for any two transfectants, it is likely that the gene products of the transfected cDNA were functionally similar.

3.2. DSPA for cells transfected with cDNAs encoding both functionally similar and functionally different proteins

Fig. 2a shows the distribution pattern between p53- and p21/cip1-transfected ras-NIH cells. Each point represents the DCI value of a single drug in the transfected cells shown on the ordinate and abscissa. The correlation coefficient (r) was 0.56 ($P < 0.001$), indicating that DCI values of p53- and p21-transfected cells were significantly correlated. These data are consistent with the previous report that p53 transactivates p21 and thereby suppresses cell proliferation [10]. The DCI values of p53-transfected cells were negatively correlated with those of wild-type Mdm2-transfected cells, but positively correlated with those of mutant Mdm2-transfected cells (Fig. 2b,c). The mutant Mdm2 was unable to associate with p53 [11]. Mdm2 is induced by activated p53, but the high expression of Mdm2 stimulates degradation of p53 protein [12,13]. Because Mdm2

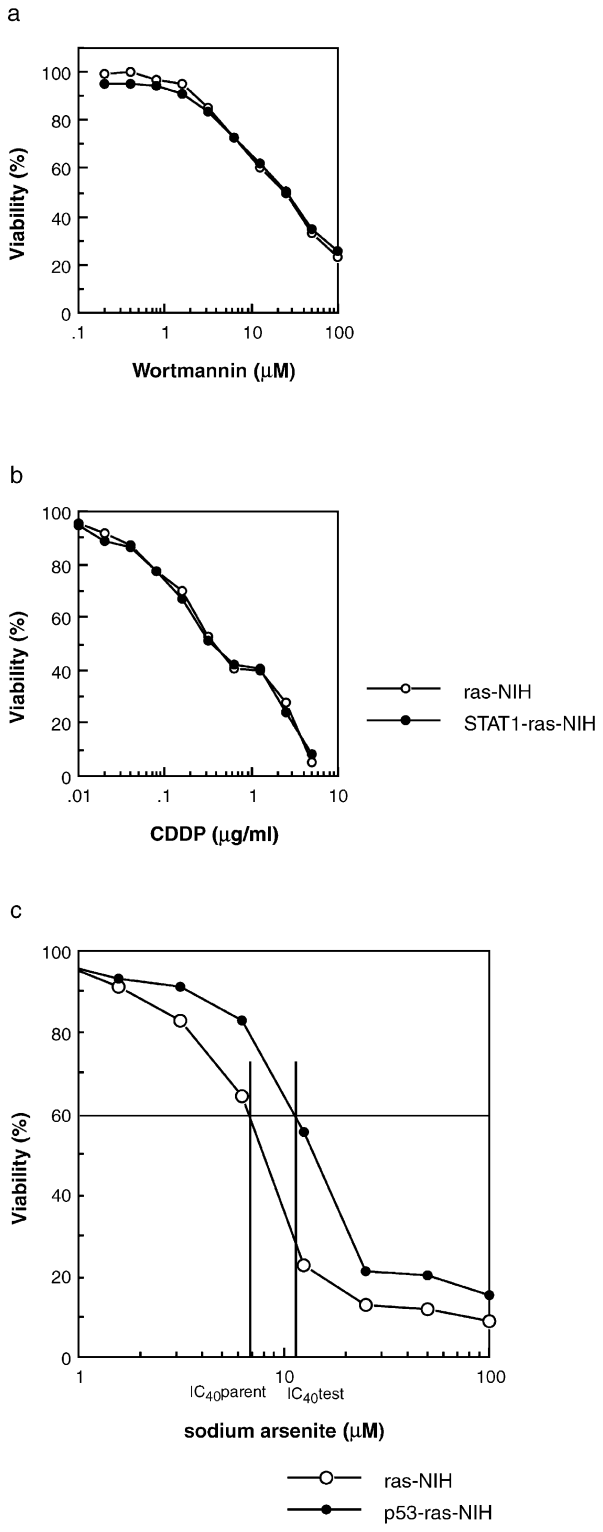

Fig. 1. MTT assay. Cells were cultured for 3 days in the presence of various concentrations of drugs, and the relative cell viability was measured by the MTT assay. The concentration of drug is shown on the abscissa and the absorbance of the formazan is shown on the ordinate. a: ras-NIH cells were examined twice for chemosensitivity to wortmannin on different days. b: Sensitivity of ras-NIH (○) and STAT1-transfected ras-NIH cells (●) to cisplatin. c: Sensitivity of ras-NIH (○) and p53-transfected ras-NIH cells (●) to sodium arsenite. The positions of drug concentrations giving 40% inhibition in p53-transfected cells (IC_{40}test) and in the parental cells (IC_{40}parent) are shown.

Table 2
The DCIs of transfected cells

	Staurosporine	K252a	H-7	Y-27632	HMA	Genistein	Erbstatin	Okadaic acid	Cantharidin	ALLN	ONO-3403	ONO-5046	U73122	BAPTA-AM	A23187	$NaAsO_2$
p53	0.07	0.05	0.00	0.30	−0.22	0.26	0.17	−0.05	0.30	0.18	0.04	0.09	0.11	0.00	0.30	0.20
p21/Cip1	0.00	−0.11	0.22	0.00	−0.30	0.02	0.06	−0.15	0.00	0.18	0.04	−0.14	0.08	0.00	0.04	0.30
Mdm2-wt	0.02	−0.17	0.02	−0.10	−0.08	−0.06	0.03	−0.02	0.08	−0.08	0.00	0.03	0.18	0.08	0.14	0.02
Mdm2-mutant	−0.16	−0.09	0.05	0.16	−0.17	−0.14	0.03	−0.09	−0.11	0.38	0.05	−0.45	0.00	−0.03	0.00	−0.33
IκB-SR	0.32	0.00	0.30	0.00	−0.14	−0.05	0.60	0.25	0.24	0.10	0.47	0.07	0.00	0.00	0.20	0.14
IKK-DN	0.32	0.02	0.25	0.21	−0.18	0.25	0.63	0.15	0.30	0.00	0.17	0.02	−0.09	0.00	0.00	0.05
p16/INK4A	−0.22	−0.22	0.08	0.16	0.41	0.40	0.51	0.06	0.16	0.09	0.29	0.22	0.28	0.20	0.00	0.16
HSP40	0.30	0.07	0.15	0.22	0.00	0.00	0.00	−0.02	−0.16	0.18	0.17	−0.15	−0.20	0.05	−0.16	−0.02
HSP90	0.22	0.56	−0.03	0.76	0.40	0.41	0.26	0.07	−0.20	0.16	0.36	0.52	0.18	0.60	0.28	0.10
Hsdj	0.50	0.16	0.45	0.32	0.24	0.03	0.23	0.12	0.17	0.12	0.40	0.00	−0.24	0.21	0.46	0.03
TERT-WT	0.00	0.00	0.09	0.36	0.08	−0.15	−0.05	0.03	0.00	0.37	−0.37	−0.32	0.03	0.32	0.11	−0.05
TERT-DN	0.56	0.00	0.28	0.30	0.62	0.18	0.17	−0.16	0.14	0.03	0.07	0.32	0.06	0.84	0.59	0.34
Ha-ras	0.30	0.65	0.23	0.31	−0.35	−0.35	0.08	0.11	−0.40	0.15	−0.22	0.00	−0.19	−0.20	−0.28	0.04
Ki-ras	0.38	0.15	0.28	0.45	−0.14	0.00	0.04	0.04	−0.46	0.11	−0.30	0.51	−0.60	−0.15	0.06	0.08
N-ras	0.24	−0.16	0.30	0.00	0.43	0.42	−0.22	0.00	−0.21	0.17	−0.17	0.23	−0.54	0.00	0.20	−0.19
Calpastatin	0.04	0.10	−0.14	−0.22	−0.14	0.13	−0.20	−0.04	−0.04	0.10	0.00	−0.10	−0.31	0.00	−0.12	0.00
ERK-DN	0.06	0.05	0.14	0.00	0.00	0.00	0.13	0.00	−0.05	0.00	0.10	0.00	0.00	0.12	0.00	0.16
JNK-DN	0.14	−0.28	0.09	0.13	−0.14	−0.10	0.11	−0.07	−0.04	−0.24	0.22	−0.09	−0.07	0.06	−0.08	−0.07
p38-DN	−0.05	−0.15	0.10	0.00	−0.05	0.00	0.06	0.12	0.00	0.04	0.14	−0.04	0.00	0.00	−0.12	0.00
Calpastatin-AS	−0.15	0.00	0.00	−0.12	−0.10	0.00	0.06	0.13	−0.18	0.00	0.00	−0.06	−0.10	0.06	−0.36	0.02

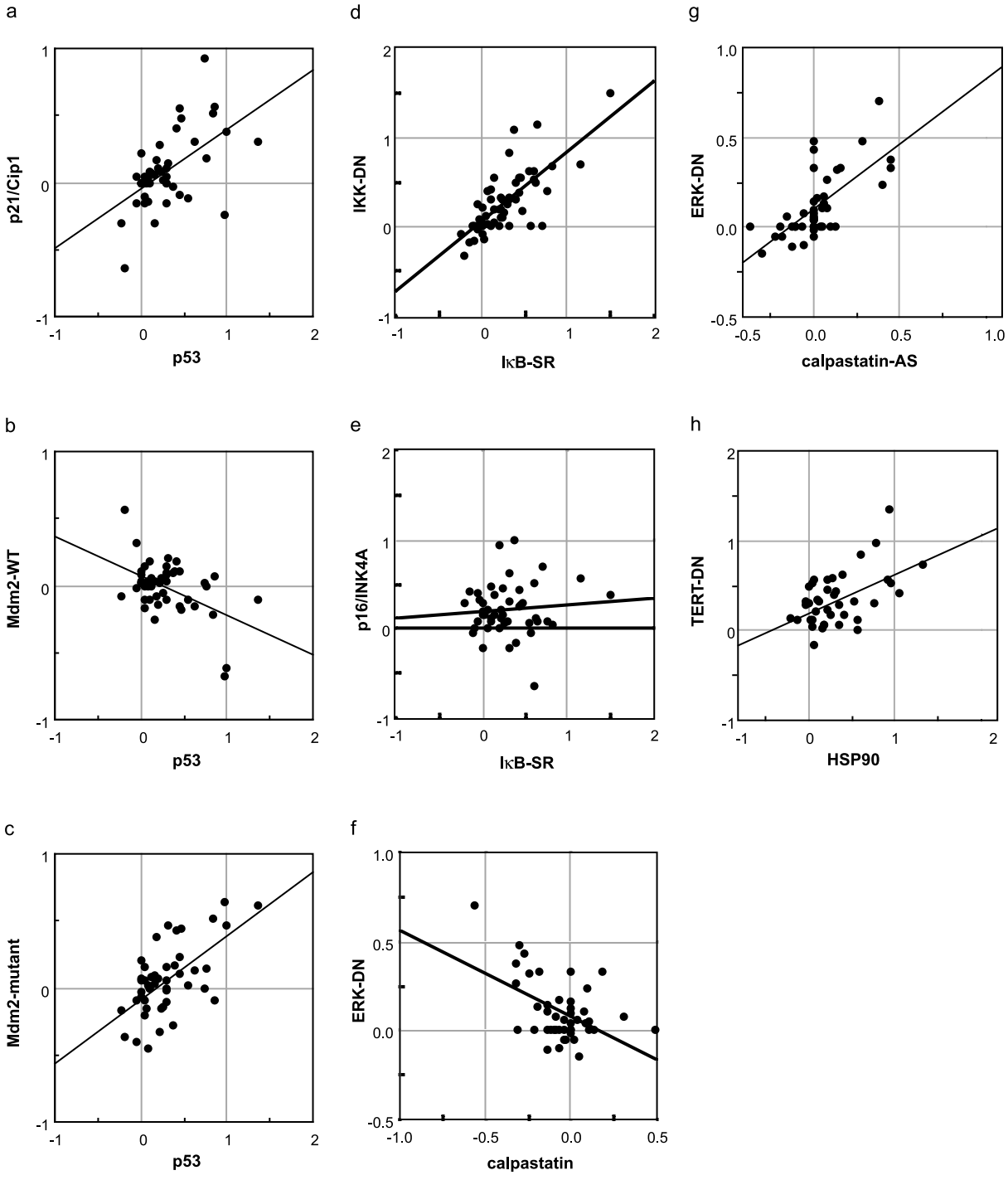

Fig. 2. Regression analysis of the DCI values of transfected cells. The DCI values, shown in Table 2, were plotted for two transfected cells. Transfected cDNAs were (a) p53 and p21, (b) p53 and Mdm2-WT, (c) p53 and Mdm2-mutant, (d) IκB-SR and IKK-DN, (e) IκB-SR and p16, (f) calpastatin and ERK-DN, (g) calpastatin-antisense (AS) and ERK-DN, and (h) HSP90 and TERT-DN.

and p53 cause opposite effects on cell proliferation by direct interaction, these data are compatible with the expectations of DSPA.

The activity of transcription factor NF-κB is suppressed by the binding protein IκB [14]. Phosphorylation of IκB by IKK results in proteolytic degradation of IκB by proteasome [15]. Thus, a non-phosphorylated IκBα superrepressor mutant, IκB-SR, and a dominant-negative IKKβ mutant, IKK-DN, can inhibit the activity of NF-κB [16]. The DCI values of IκB-SR- and IKK-DN-transfected cells were highly correlated with each other ($r = 0.75$, $P < 0.001$) (Fig. 2d). On the other hand, the drug-sensitivity profiles of IκB-SR-transfected cells were totally unrelated to those of p16/INK4A-transfected cells (Fig. 2e). The fact that there is no evidence for interaction between p16 and IκB is also consistent with our data. The DSPA method can therefore discriminate functional unrelatedness between two gene products.

We have recently reported that calpastatin, an endogenous calpain inhibitor, induces anchorage-independent transformation of NIH3T3 cells [17]. Furthermore, the transformed phe-

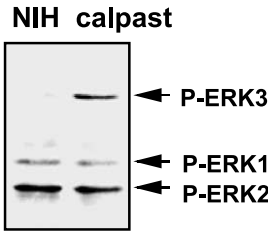

Fig. 3. Expression of activated ERKs in calpastatin-transfected NIH3T3 cells. Cytoplasmic cell extracts of NIH3T3 (NIH) and its calpastatin transfectant (calpast) were analyzed by immunoblotting using phospho-specific ERK antibody which recognized activated ERKs. Arrows indicate the positions of activated ERKs (P-ERK1, P-ERK2 and P-ERK3).

notype of ras-NIH cells was reversed by transfection with calpastatin antisense (calpastatin-AS) cDNA. The DSPA showed that the DCI values of calpastatin-transfected NIH3T3 cells were negatively correlated with those of dominant-negative ERK (ERK-DN)-transfected ras-NIH cells ($r = -0.49$, $P < 0.001$; Fig. 2f) and that DCIs of calpastatin-AS-transfected ras-NIH cells were positively correlated with those of ERK-DN transfectants ($r = 0.68$, $P < 0.001$; Fig. 2g). This suggests that calpastatin expression caused similar effects to those caused by ERK. When the activated ERKs were examined by immunoblotting using phospho-specific ERK antibody, activated ERK3 in addition to ERK1 and ERK2 was detected in calpastatin-transfected cells (Fig. 3). Thus, DSPA analysis also appears to be useful for predicting the signaling pathway of the cDNA gene product.

A recent report showed that there is a stable association between HSP90 and telomerase reverse transcriptase (TERT) [18]. The good correlation of the DCI values of HSP90-transfected ras-NIH cells with those of dominant-negative TERT-transfected ras-NIH cells ($r = 0.53$, $P < 0.001$) confirmed this interaction (Fig. 2h). This results may imply opposite roles between HSP90 and wild-type TERT.

Correlation coefficients (r) are summarized in Table 3. When the DCI values were obtained by the analysis of 40 drugs, r values higher than 0.4 or lower than -0.4 represent statistical significance ($P < 0.01$). r values higher than 0.5 or lower than -0.5 show even higher significance ($P < 0.001$). These significant relations are differentially shown in color. It may be impossible to confirm all of the results of Table 3 at present. However, there are no data thus far to contradict the findings of DSPA. Based on the DSPA results, some of

Table 3
Correlation of DCI values of transfected cells by DSPA

	p53	p21	Md-W	Md-m	IkB	IKK	p16	HS40	HS90	Hsdj	TR-W	TR-D	Hras	Kras	Nras	CS	ERK	JNK	p38	CS-A
p53																				
p21	0.56																			
Md-W	-0.50	-0.10																		
Md-m	0.62	0.39	-0.51																	
IkB	0.52	0.44	-0.18	0.33																
IKK	0.45	0.62	-0.19	0.33	0.75															
p16	0.41	0.21	-0.35	0.45	0.09	0.16														
HS40	0.00	0.17	0.21	0.20	0.04	0.25	-0.20													
HS90	0.42	0.15	-0.43	0.54	0.23	0.28	0.46	0.21												
Hsdj	0.16	0.34	-0.10	0.38	0.29	0.41	-0.04	0.34	0.33											
TR-W	0.04	-0.08	-0.17	0.02	-0.13	-0.15	-0.06	-0.02	-0.19	-0.18										
TR-D	0.44	0.36	-0.60	0.43	0.12	0.24	0.34	-0.15	0.53	0.34	0.06									
Hras	-0.32	-0.56	0.09	-0.36	-0.23	-0.57	-0.38	-0.16	-0.25	-0.34	0.25	-0.31								
Kras	-0.32	-0.37	0.07	-0.32	-0.28	-0.49	-0.23	-0.13	-0.10	-0.05	0.20	-0.13	0.70							
Nras	-0.34	-0.37	0.13	-0.37	-0.12	-0.35	-0.18	-0.33	-0.32	-0.18	-0.01	-0.14	0.55	0.56						
CS	-0.36	-0.28	0.38	-0.48	-0.06	-0.20	-0.44	0.00	-0.57	-0.15	0.00	-0.49	0.33	0.30	0.38					
ERK	0.45	0.39	-0.31	0.53	0.22	0.40	0.50	0.27	0.66	0.24	-0.15	0.29	-0.41	-0.34	-0.51	-0.49				
JNK	0.21	0.37	0.03	0.08	0.19	0.31	0.02	0.21	0.09	0.14	-0.13	0.03	-0.22	-0.20	-0.28	-0.25	0.37			
p38	0.28	0.46	0.08	0.24	0.23	0.40	0.18	0.48	0.22	0.07	-0.03	-0.15	-0.32	-0.30	-0.35	-0.19	0.63	0.54		
CS-A	0.35	0.17	-0.15	0.43	0.11	0.21	0.44	0.38	0.51	0.11	0.00	-0.02	-0.22	-0.17	-0.45	-0.19	0.68	0.06	0.60	

Abbreviations
p53: wild-type p53
p21: p21/Cip1
Md-W: wild-tyep Mdm2
Md-m: mutant Mdm2
IkB: IκB-superrepressor
IKK: IKK-dominant negative
p16: p16/INK4A
HS40: HSP40
HS90: HSP90
Hsdj: Hsdj
TR-W: wild-typeTERT
TR-D: TERT-dominant negative
Hras: Ha-ras
Kras: Ki-ras
Nras: N-ras
CS: calpastatin
ERK: ERK-dominant negative
JNK: JNK-dominant negative
p38: p38-dominant negative
CS-A: calpastatin-antisense

$r = 1.00$
$r > 0.05$
$0.05 > r > 0.04$
$0.04 > r > -0.04$
$-0.04 > r > -0.05$
$-0.05 > r$

Correlation coefficients (r) obtained by DSPA are summarized. Significant relations are shown in color as indicated.

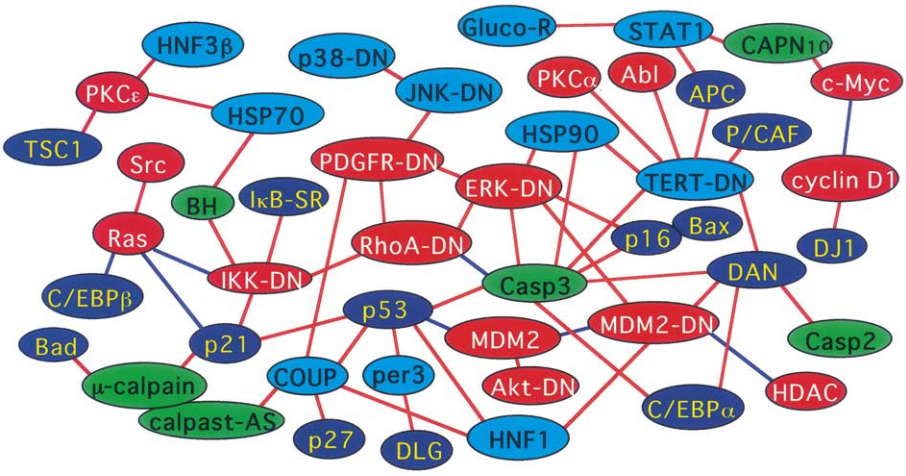

Fig. 4. Functional relationships between p53 and other molecules. Functional relationships suggested by DSPA are shown. Red lines represent positive relationships between two molecules and negative relationships are shown in blue.

the functional molecular interactions around p53 are shown in Fig. 4. It allowed us to predict the signaling network centered by p53.

3.3. Advantages and limitations of DSPA

DSPA is a new method for comprehensive analysis of gene products with many advantages. (i) Functional similarities between the test cDNA and all other genes listed in the database have been revealed only by addition of the DCI values of the test-cDNA-transfected cells. (ii) By using the DSPA method, functional similarity of gene products can be predicted in the absence of protein manipulation. (iii) The estimation of the functional similarity does not simply reflect characteristics associated with cell growth or survival. (iv) There are technical advantages to DSPA. Specifically, the experimental periods for analysis are short. The inexpensive and technically simple MTT assay can be finished within 2 weeks and requires no special equipment. Routine protocols produce reproducible results. The data are not influenced by operator bias. (v) The DSPA is available for analysis of post-translational modifiers such as kinases and proteases. The relationship of signaling pathways affected by the transfected genes can also be studied; the DSPA points to functional similarities among proteins of the same gene family (e.g. Ha-*ras*, Ki-*ras* and N-*ras*), binding proteins (p53 and Mdm2, HSP90 and TERT), transcription factors and their target genes (p53 and p21), and enzymes and the substrates.

The DSPA also has some limitations. (i) DSPA data might be meaningful only in the cell system that is being used. For example, the results of our study in mouse fibroblasts may not be reliably extrapolated to other cell systems. With a large database that incorporates a variety of cells, such as embryonic, epidermal, neuronal, and hematopoietic, utility could be dramatically expanded. (ii) Another problem would exist if the cDNA gene products are not effective in regulation of cell viability. It may also be difficult to discern the functional relationships of the molecules that are not directly involved in cell growth or survival. However, most of signaling pathways are interrelated, and direct, as well as indirect, effects on branch pathways may impact cell viability. (iii) The DSPA system may, in some cases, require analysis of multiple, rather than single, independent transfectants. For example, most of the cells transfected with growth-inhibitory molecules were unable to form G418-resistant colonies. Nevertheless, some clones grew because of secondary changes that suppressed the signaling. These secondary changes which may account for the clonal variation could be related to the transfected gene, and also be identified by DSPA. (iv) A significant inverse correlation is not necessarily observed between the wild-type and dominant-negative mutant forms of a molecule. The expression of the dominant-negative form may not cause effects, particularly when the endogenous activity of the wild-type is low. On the other hand, if the endogenous activity is already high, overexpression of the wild-type molecule may have little effect.

As a further application, DSPA can potentially be used to study the mechanism of action of bioactive chemical compounds. Transfection with dominant-negative mutants produces significant results as shown in Fig. 2c,d,h. If hypothetical drug X can inhibit hypothetical enzyme Y, the cells transfected with the dominant-negative form of the enzyme will have the same DCI values as the cells treated with drug X. Thus, the drug-treated cells can also be used instead of transfected cells. This application may be particularly important because it will facilitate recognition of unexpected side effects of newly developed drugs.

These data, taken together, indicate that molecular relationships suggested by DSPA have enormous potential for future application and provide direction for new investigations. The DSPA method is a potentially useful primary strategy that is rapid, simple and convenient for study of functional relationship between gene products.

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