Experimental Lung Research, 38, 463–474, 2012

ISSN: 0190-2148 print / 1521-0499 online
DOI: 10.3109/01902148.2012.731625

informa
healthcare

TMEM158 and *FBLP1* as novel marker genes of cisplatin sensitivity in non-small cell lung cancer cells

Ahmed El Sayed Mohammed,[1,4,5,8] Hidetaka Eguchi,[1,4,5] Satoru Wada,[1,4,5] Nobuyuki Koyama,[2] Michio Shimizu,[3] Keiko Otani,[6] Megu Ohtaki,[6] Keiji Tanimoto,[7] Keiko Hiyama,[7] Mohammed Soliman Gaber,[8] and Masahiko Nishiyama[1,4,5,9]

[1]*Translational Research Center, Saitama Medical University International Medical Center, Hidaka, Saitama, Japan*

[2]*Department of Respiratory Medicine, Saitama Medical University International Medical Center, Hidaka, Saitama, Japan*

[3]*Department of Pathology, Saitama Medical University International Medical Center, Hidaka, Saitama, Japan*

[4]*Divisions of Disease Control and Project, Research Center for Genomic Medicine, Saitama Medical University, Hidaka, Saitama, Japan*

[5]*Frontier Medical Development Center, Saitama Medical University, Hidaka, Saitama, Japan*

[6]*Department of Environmetrics and Biometrics, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan*

[7]*Radiation Medicine, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan*

[8]*Department of Clinical and Radiation Oncology, Faculty of Medicine, Sohag University, Sohag, Egypt*

[9]*Department of Molecular Pharmacology and Oncology, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan*

ABSTRACT

Even after development of molecular targeting therapies, platinum-based chemotherapy is still a standard care for treatment of locally advanced non-small cell lung cancer (NSCLC). So far, critical molecular markers capable to predict the therapeutic response in NSCLC patients remain undetermined. We here attempted to identify novel biomarker genes for cisplatin (CDDP) for a tailored therapy. Initial screening to explorer association of IC_{50} values of CDDP obtained by MTT assay and gene expression levels measured with oligonucleotide microarray and real-time RT-PCR provided 6 candidate genes, namely, *NUBPL, C9orf30, ZNF12, TMEM158, GSK3B,* and *FBLP1* using 9 lung cancer cells consisting of 3 small and 6 NSCLC cells. These 6 genes together with 5 reported biomarkers, i.e., *GSTP1, ERCC1, BRCA1, FRAP1*, and *RRM1,* were subjected to a linear regression analysis using 12 NSCLC cell lines including 6 additional NSCLC cells: only *FBLP1* and *TMEM158* genes showed positive associations with statistical significances ($P = .016$ and .026, respectively). The biological significance of these genes was explored by in vitro experiments: Knockdown experiments in PC-9/CDDP cells revealed that the reduced expression of *TMEM158* significantly decreased the chemo-resistance against CDDP ($P < .0001$), while 2 transformants of PC-6 cells stably over-expressing *FBLP1* resulted in an enhanced resistance to CDDP ($P = .004$ and $P = .001$). Furthermore, a stepwise multiple regression analysis demonstrated the best prediction formula could be fixed when we used expression data of *TMEM158* and *FBLP1* ($R^2 = 0.755$, $P = .0018$). *TMEM158* and *FBLP1* may be powerful predictive biomarkers for CDDP therapy in NSCLC.

KEYWORDS cisplatin-chemotherapy, microarray, NSCLC, predictive marker

Received 30 April 2012; accepted 15 September 2012.

We thank Ms. Oda and Ms. Kumazawa for their enormous efforts and skillful technical assistance. This work was supported in part by Research Grant of The Ministry of Health Labor and Welfare (3rd Term Comprehensive 10-year Strategy for Cancer Control) and a grant of Strategic Research Foundation Grant-aided Project for Private Universities from Ministry of Education, Culture, Sport, Science, and Technology, Japan (MEXT) (S0801004).
Address correspondence to Prof. Masahiko Nishiyama, Department of Molecular Pharmacology and Oncology, Gunma University Graduate School of Medicine, 3-39-22, Showa-machi, Maebashi, Gunma, 371-8511, Japan. E-mail: m.nishiyama@gunma-u.ac.jp

INTRODUCTION

In advanced non-small cell lung cancer (NSCLC), the advent of new agents such as pemetrexed, gefitinib, erlotinib, and crizotinib, offers hope for improving patient outcomes, and the pharmacogenomic studies are increasing the potential of individual optimization of NSCLC molecular targeted therapy [1]: Striking examples established in clinical practice include epidermal growth factor receptor (EGFR) inhibitors in EGFR-mutated NSCLC and anaplastic lymphoma kinase (ALK) inhibitors in NSCLC with translocation for ALK, EML4-ALK fusion. Three randomized trials of gefitinib versus chemotherapy (IPASS, WJTOG3405, NEJ002) in stage IV NSCLC have consistently demonstrated better response rate (RR) and progression-free survival (PFS) for EGFR-mutated NSCLCs treated with gefitinib. Crizotinib (PF02341066), an ALK tyrosine kinase inhibitor (TKI), has shown impressive activity against ALK translocated NSCLC in an expanded cohort of a phase I trial (NCT00585195) [2, 3].

Even so, advanced NSCLC remains a fatal disease [1–5]. Most NSCLC cases reveals advanced or metastatic disease at the time of diagnosis, but the front-line chemotherapy is still palliative. Current standard of care for first-line therapy of these patients is considered as platinum-based doublets with third generation agents, reporting a response rate (RR) racing from 20% to 35% with a median survival time (MST) of about 10 months [1–2, 6–8]. The 5-year estimated survival rate is dismal, at less than 4%, and further the therapeutic outcome varies significantly among patients [5, 6, 9]. Numerous patients undergo a regimen without benefit. The limited therapeutic benefit highlights the need to identify potent predictive markers of response to platinum-based chemotherapy for optimal selection of the regimen for each individual.

Up to date, a number of candidate markers have been suggested to be of predictive benefit [10–17]: Various nucleotide excision repair enzymes and phase II detoxification enzymes, such as the glutathione-S-transferases (GSTs), appear to be putative determinants for platinum resistance. And among numerous genes involved in the altered DNA repair mechanisms, the excision repair cross complementing 1 (*ERCC1*) gene, the x-ray cross complementing group 1 (*XRCC1*) gene, the xeroderma pigmentosum Group D (*XPD* or *ERCC2*) gene and the x-ray cross complementing group 3 (*XRCC3*) gene are known to play important roles in DNA repair. Nevertheless, none of these factors alone is consistently critical in response to platinum-based chemotherapy.

Drug action and metabolism pathways are too ingenious to be entirely understood: Multiple factors are involved in the pathways and they have a complex interplay with each other [14–17]. We are still restricted to narrow limits in the prediction of drug response using these hypothesis-driven markers alone. High-throughput arrays enable us to identify such markers from tens of thousands of genes or polymorphisms, no definitive way to identify drug response determinants from a huge number of candidates has yet been established. Most of these studies were statistically underpowered to find robust novel candidates [17, 18].

In this study, we first attempted to select further promising biomarkers for CDDP response genome-wide without considering their functional roles in CDDP action. To screen candidate predictive genes from a relatively small number of samples (a total of 9 lung cancer cell lines), we employed a unique statistical method, a two-dimensional (2D) mixed normal model proposed by Otaki et al [19]. The candidate genes were then subjected to a linear regression analysis using 12 NSCLC cell lines. We identified two powerful candidates, namely *TMEM158* and *FBLP1*. In vitro experiments have shown that both of these genes were directly connected to the molecular action of CDDP in human NSCLC cells.

METHODS

Chemicals

Cisplatin (Cis-diamminedichloroplatinum, CDDP) was purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals were analytic grade and were purchased from Wako Pure Chemicals (Osaka, Japan), Nacalai Tesque (Kyoto, Japan), and Sigma.

Cell Lines

Lung adenocarcinoma cell lines PC-9 and PC-14, and their variant cells PC-9/CDDP and PC-14/CDDP that are resistant to cisplatin [20], were generously provided by Dr. K. Nishio, Kinki University School of Medicine, Osaka, Japan. A549 (adenocarcinoma) and LC-S (squamous cell carcinoma) cells were from Dr. Y. Yamaguchi, Kawasaki Medical University Hospital, Kurashiki, Japan. PC-6 (small cell carcinoma), DQ2–2 (CPT-11-resistant variant from PC-6) and SN2–5 (SN-38-resistant variant from PC-6) cells were kindly provided by Dr. A. Tohgo, Daiichi Pharmaceutical Col Ltd, Tokyo, Japan. ABC-1 (adenocarcinoma), RERF-LC-Ad2 (adenocarcinoma), RERF-LC-KJ (adenocarcinoma),

RERF-LC-OK (adenocarcinoma), RERF-LC-Sq1 (squamous cell carcinoma), and RERF-LC-MS (adenocarcinoma) were generously provided by the Department of Radiobiology/Molecular Epidemiology, Radiation Effects Research Foundation (Hiroshima, Japan).

Cells were cultured in RPMI 1640 medium supplemented with L-glutamine and phenol red containing 10% heat-inactivated fetal bovine serum (FBS) (Biowest, Nuaillé, France) and 50 μg/mL kanamycin-sulfate. Cells were grown at 37°C in an incubator with humidified atmosphere of 5% CO_2 and maintained in continuous exponential growth.

EXTRACTION AND PURIFICATION OF RNA

For gene expression analysis, exponentially grown cultured cells were collected and total RNA was prepared using NucleoSpin RNA II Purification kit (Macherey-Nagel, Düren, Germany). The quality of the RNA was evaluated using Agilent Technologies 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA).

MTT Assay

Drug-induced cytotoxicity was evaluated by conventional 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide dye reduction assay as essentially described previously [21]. Cells were counted with hemocytometer and seeded in 96-microwell plates (Corning) at a density of 4×10^3 otherwise not specified per well in RPMI 1640 with 10% FBS. After 24-hour incubation, the medium was replaced and cells were exposed to the indicated drug concentrations for 72 hours, after which 100 μL of PBS without EDTA was added to each well, then aspirated and 10 μL of 0.4% 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide reagent and 0.1 mol/L sodium succinate were added to each well. After 1–3-hour incubation, 150 μL dimethyl sulfoxide (DMSO) was added to dissolve the purple formazan precipitate. The formazan dye was measured spectrophotometrically (570–650 nm) using Multiskan JX Microplate Reader (Thermo Fisher Scientific, Waltham, MA). The cytotoxic effect of each treatment was assessed by IC_{50} (drug concentration of 50% absorbance of control). Data are expressed as the percentage of viable cells in treated relative to nontreated conditions.

Screening of Candidate Genes Using Data of Comprehensive Gene Expression Analyses

The statistical significance was evaluated with *P*-value obtained from the Monte Carlo method by generating null distribution under the hypothesis that there was no correlation between any two sets of measurements. Two dimensional (2D) mixed normal model is a statistical method proposed by Ohtaki et al, which can effectively adjust the microarray data to facilitate comparisons through eliminating systemic biases in the measured expression levels, referred to as normalization, and identify differentially expressed genes between two cells showing different biological behaviors based on the functional status of the genes [19]. The probability of the gene being differentially expressed between the query and the reference samples, i.e., the status of the gene is ("on," "off") or ("off." "on") between them, was obtained as a posterior probability. The terms "on" and "off" are used to express the functional status of a gene. If a gene actually expressed yielding its product (i.e., "mRNA") as the true signal, the status is "on"; otherwise (i.e., mRNA is not in the sample), it is "off." When the status of a gene is "off," the observed measurement reflects only the amount of systematic error and measurement error.

The rank correlation coefficient (Spearman's correlation coefficient) is known as a robust statistical index for quantifying degrees of correlation between ranks of two sets of measurements; it is useful even when data are contaminated with certain outliers. The statistical significance was evaluated with *p*-value obtained from the Monte Carlo method by generating null distribution under the hypothesis that there was no correlation between any two sets of measurements.

REAL-TIME REVERSE TRANSCRIPTION-PCR

Total RNA (1 μg) was reverse-transcribed using ReverTra Ace Kit (TOYOBO Co, Ltd, Osaka, Japan). Aliquot (1/200) of the cDNA (equivalent to 5 ng of total RNA) was subjected to real-time reverse transcription-PCR (RT-PCR). Real-time RT-PCR was conducted using specific primer sets, Universal ProbeLibrary (Roche Diagnostics, Basel, Switzerland), and qPCR QuickGoldStar Mastermix Plus (Eurogenetec, Hampshire, UK) or ABsolute QPCR ROX Mix (Thermo Fisher Scientific). Each reaction was carried out in triplicate for each cell line using ABI Prism 7900HT Sequence Detection System (Applied Biosystems), using serially diluted fragments of the target fragments cloned into a

plasmid pTA2 (TOYOBO) as standards. These triplicate measurements were averaged, and relative gene expression levels were calculated as a ratio to hypoxanthine phosphoribosyltransferase 1 (*HPRT1*) expression level.

Construction of Plasmid

PCR reaction was carried out using KOD-Plus or KOD FX (TOYOBO) as a DNA polymerase, according to the manufacturer's protocol. Oligonucleotides HA_tag_F01: ACG TAA GCT TCA CGT GGC GGC CGC TCT AGA ATG GCT TAC CCA TAC GAT GTT C, and HA_tag_R01: ACG TGG GCC CTC AAG CGT AAT CTG GAA CAT CGT ATG were annealed and digested with restriction enzymes *Hin*dIII and *Apa*I of which recognition sites were indicated by underlines and the resultant fragment was inserted into *Hin*dIII and *Apa*I site of the expression vector pRc/CMV (Invitrogen) to construct pRc/CMV-HA. Coding region of *FBLP1* gene was amplified using specific primers FBLP1-F01: GCC ACC ATG GCC TCA AAG CCT GAG AAG, and FBLP1-R01: ACG TTC TAG AGC CAG GAT GAT CTC GAT CTC, and was digested with *Xba*I and ligated into cloning sites of pRc/CMV-HA digested with *Eco*72I and *Xba*I. Ligation high Ver.2 (TOYOBO) according to the manufacturer's manual and transformed into competent *E. coli* DH5α (TOYOBO). All the constructs were confirmed by DNA sequencing using BigDye® Terminator 3.1 Cycle sequencing kit and 3130 Genetic Analyzer (Life Technologies, Tokyo, Japan).

Transfection and Selection of Stable Transformants

The plasmid expressing each gene was linearized by a single cut with a restriction enzyme *Sca* I and then transfected into lung carcinoma cell lines, using HilyMax transfection reagent (Dojindo Molecular Technologies, Inc., Osaka, Japan) according to the manufacturer's manual. Transfected cells were cultured in RPMI-1640 medium with 10% FBS containing 200–1,000 μg/mL of G418, from 24 hours after transfection for approximately one month to select stable transfected clones. Expression levels of mRNA of each gene in transfected cells were measured by real time RT-PCR.

Knockdown Experiments Using siRNA

Specific siRNAs (Stealth Select RNAi©) for *TMEM158* (HSS146605, HSS146606, and HSS146607) and for *NUBPL* (HSS129499, HSS129500, HSS129501) and negative control siRNA (Stealth RNAi™ siRNA Negative control siRNA) were purchased from Invitrogen. After validation of knockdown efficiencies using two different cell lines, we selected HSS146607 for *TMEM158* and HSS129499 for *NUBPL*, respectively, as the most potent siRNAs and thus were used for subsequent experiments.

For *TMEM158*, the siRNAs were transfected into PC-9/CDDP cells overexpressing the selected genes using Lipofectamine RNAiMax and according to the manufacturer's manual. A mixture of 4×10^3 cells in RMPI/10% FBS without antibiotics, 60 pmoles of siRNA, and 10 μL of LipofectamineTM RNAiMax in total volume of 1 mL and serial dilutions of CDDP were seeded in 96-microplates and incubated at 37°C in a humidified 5% CO_2 incubator for 72 hours, and then cytotoxicity was measured by MTT assay.

For *NUBPL*, siRNAs were delivered to PC-9 cells via electoroporation method using a device CUY21Pro-Vitro (NEPA GENE, Ichikawa Japan) according to the manufacturer's recommendation. Briefly, 15 pmoles of siRNA and 1×10^6 cells were mixed in total of 100 μL of Opti-MEM medium (Invitrogen) and the mixture was transferred into EC-002 electoroporation cuvettes (NEPA GENE). The electroporated cells were seeded in 96-microplates at a density of 2×10^3 cells and incubated at 37°C in a humidified 5% CO_2 incubator for 72 hours, and then cytotoxicity was measured by MTT assay.

Efficacy of siRNA-mediated knockdown of mRNA of selected genes was evaluated in the cells exposed to siRNA for 72 hours in the absence of CDDP using real-time RT-PCR.

STATISTICAL ANALYSIS

Multiple linear regression analysis for the initial screening has been conducted essentially as described previously [22]. We used a statistical language, R for calculation of stepwise multiple regression analysis [23]. The value of AIC (Akaike's information criterion) was used for evaluation of the fitness of tested model. Statistical analyses were conducted using a software JMP 9.0 (SAS Institute, Cary, NC) otherwise specified.

RESULTS

Genomewide Screening of Candidate Marker Genes Using Lung Cancer Cell lines

To select marker genes that predict efficacy of CDDP, we first performed comprehensive gene expression

analysis in 9 lung cancer cell lines, A549, PC-9, PC-14, PC-9/CDDP, PC-14/CDDP, LC-S, PC-6, DQ2–2 and SN2–5. In the microarray analysis, we used not only NSCLC cells but also 3 small cell lung cancer cells PC-6, DQ2–2, and SN2–5, in order to explore various genes that may be useful for prediction of resistance/sensitivity of CDDP from a variety of gene expression patterns. We measured IC_{50} values for CDDP in these cell lines, and then analyzed associations between each gene expression levels and the IC_{50} values among these cell lines by means of two statistical methods, namely 2D mixed normal model and rank correlation. In total, 210 probes fulfilled the following criteria: *P*-values less than .001 for 2D mixed normal model and less than .025 in rank correlation analysis. One hundred and eighty-one probes out of the 210 have been assigned official gene symbols. Among these, we selected 23 genes of interest as initial candidates for further analysis by referring to the values of correlation coefficients provided by the two statistical methods (Table 1). On the other hand, well-known marker genes *GSTP1*, *ERCC1*, *BRCA1*, *FRAP1*, and *RRM1* were not found in the top 210 probes list.

To validate the associations obtained from a microarray analysis, expression levels of these genes were quantitatively measured by a real-time RT-PCR. We then analyzed associations of those and IC_{50} values for CDDP among the 9 cells using a linear regression. Six genes, namely *NUBPL, ZNF12, C9orf30, FBLP1, GSK3B,* and *TMEM158*, out of the 23 genes showed statistically significant levels of associations in the 9 lung cancer cells (Table 1).

Selected Candidates and Known Marker Genes

Since the candidate 6 genes were screened from both NSCLC and small cell lung cancer cells, we next conducted a real-time RT-PCR analysis using only 12 cell lines derived from NSCLC: 2 squamous cell carcinoma cells, LC-S and RERF-LC-Sq1; 10 adenocarcinoma, A549, PC-9, PC-14, PC-9/CDDP, PC-14/CDDP, ABC-1, RERF-LC-Ad2, RERF-LC-KJ, RERF-LC-MS, and RERF-LC-OK. Among these, 6 cells, namely, RERF-LC-Sq1, ABC-1, RERF-LC-Ad2, RERF-LC-KJ, RERF-LC-MS, and RERF-LC-OK were not included in the initial screening.

Together with these 6 genes, we also analyzed expression levels of 5 genes well known as possible biomarkers for CDDP, namely *GSTP1*, *ERCC1*,

TABLE 1 Initial Marker Candidates: Correlations Between Gene Expression Levels and Sensitivity to Cisplatin in 9 Lung Cancer Cells

	Microarray				Real-time RT-PCR	
	2D-mixed normal model		Rank-correlation		Linear regression	
Gene symbol	R	P	R	P	R	P
NUBPL	−0.96	0.00001	−0.89	<0.005	−0.77	0.035
ZNF12	−0.95	0.00002	−0.905	<0.005	−0.61	0.016
C9orf30	0.95	0.00002	0.833	<0.005	0.67	0.007
FBLP1	0.93	0.00006	0.91	<0.005	0.82	0.050
GSK3B	0.88	0.00051	0.90	<0.005	0.58	0.025
TMEM158	0.88	0.00053	0.70	<0.005	0.73	0.0005
SREBF2	−0.98	<0.00001	−0.91	<0.005	−0.42	0.27
TRIM52	−0.97	<0.00001	−0.83	<0.005	−0.46	0.22
RAB3A	0.96	0.00001	0.77	<0.025	−0.40	0.29
HTGN29	−0.96	0.00001	−0.88	<0.005	−0.51	0.16
KIAA0195	−0.96	0.00001	−0.87	<0.005	−0.53	0.14
RERE	−0.95	0.00003	−0.96	<0.005	−0.37	0.33
CDK3	−0.94	0.00004	−0.85	<0.005	−0.61	0.08
ZAK	0.94	0.00005	0.93	<0.005	−0.34	0.37
PMI	0.94	0.00005	0.87	<0.005	−0.37	0.33
RALY	0.93	0.00008	0.84	<0.005	−0.33	0.39
BAG2	0.92	0.00016	0.87	<0.005	0.27	0.47
NAPB	0.91	0.00024	0.79	<0.025	NA*	NA*
DNCLI1	0.89	0.00036	0.82	<0.005	0.38	0.31
NCOR1	0.90	0.00033	0.85	<0.005	0.03	0.93
FOSL1	0.89	0.0004	0.86	<0.005	0.47	0.21
H41	0.89	0.0004	0.85	<0.005	0.22	0.57
EDR2	0.86	0.00099	0.82	<0.005	0.35	0.36
S100A2	0.60	0.00043	0.83	<0.025	0.58	0.10

TABLE 2 Linear Regression Analyses of the Candidate Genes Expression Levels and Sensitivity to Cisplatin Assessed with Real-time RT-PCR Analyses in 12 NSCLC Cells

	Gene symbol	R^2	P
Newly selected	*FBLP1*	0.456	0.016
	TMEM158	0.405	0.026
	NUBPL	0.270	0.083
	ZNF12	0.118	0.275
	C9orf30	0.029	0.596
	GSK3B	0.024	0.630
Reported	*GSTP1*	0.187	0.160
	RRM1	0.113	0.285
	ERCC1	0.106	0.301
	FRAP1	0.078	0.378
	BRCA1	0.047	0.498

BRCA1, *FRAP1*, and *RRM1* [10–17]. We then performed a linear regression analysis of gene expression levels and IC_{50} values in the 12 NSCLC cells (Table 2). Among the tested 11 genes, *FBLP1 and TMEM158* genes showed statistically significant positive-associations ($P = .016$ and $P = .026$, respectively; Figure 1A & 1B). *NUBPL* tended to reveal an inverse-association with a marginal significance in a linear regression analysis ($P = .083$) (Figure 1C). Even so, when divided the NSCLC cells into two groups by median of *NUBPL* expression levels, those cells with higher *NUBPL* expression showed significantly lower IC_{50} values as compared with those with low expression ($P = .0066$, *t*-test with unequal variances) (Figure 2). On the other hand, other tested genes including all the 5 reported genes provided no statistical significance at all in the linear regression analysis (Table 2).

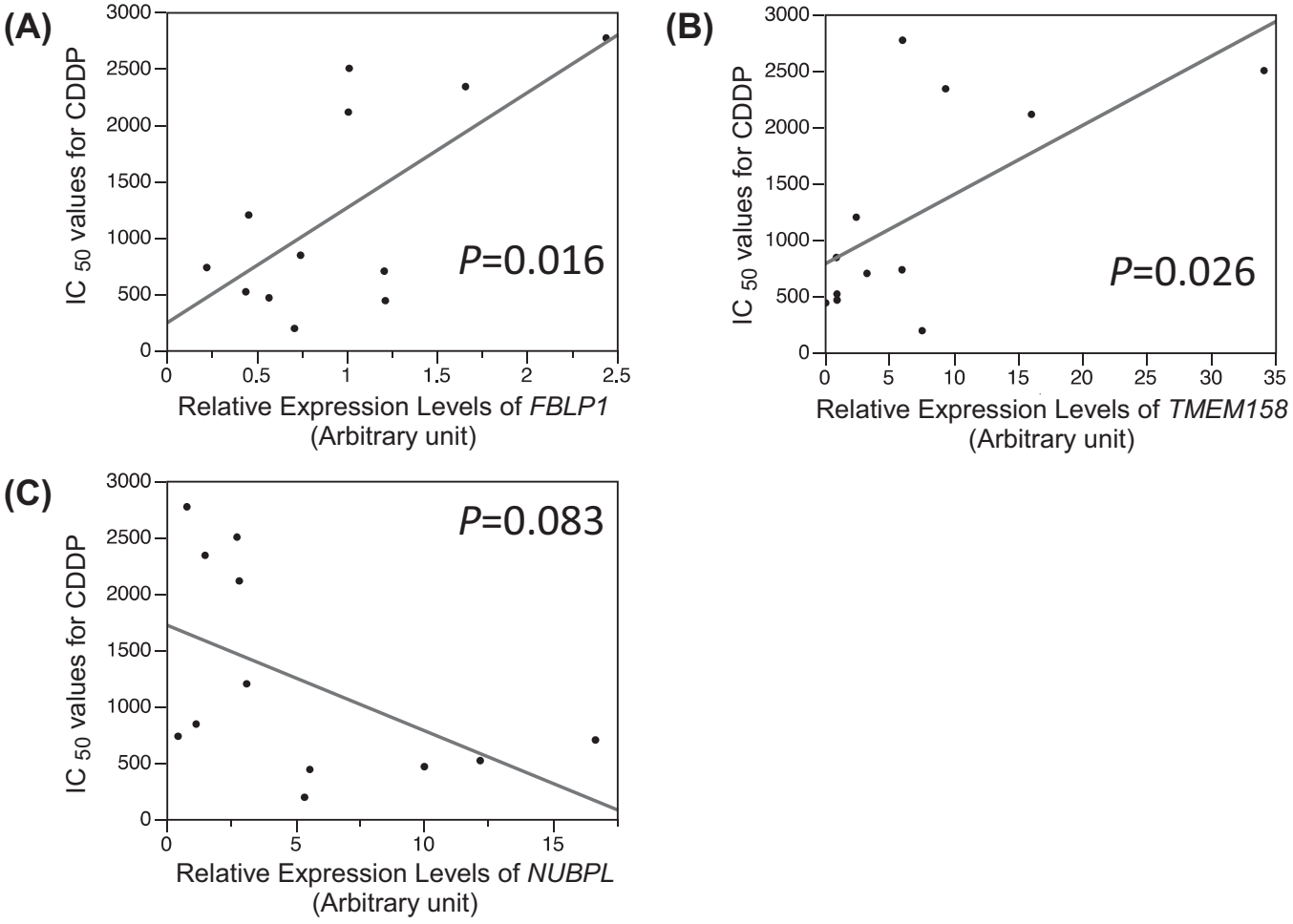

FIGURE 1 Correlation between CDDP sensitivity and expression of selected genes as potent response predictors. In 12 human non-small cell lung cancer (NSCLC) cell lines, the expression levels of *FBLP1* (A) and *TMEM158* (B) were positively correlated with IC_{50} values for cisplatin (CDDP) with statistical significances ($P = .016$ and $P = .026$, respectively), while expression of *NUBPL* (C) was tended to show inverse correlation with CDDP resistance with a marginal significance ($P = .083$).

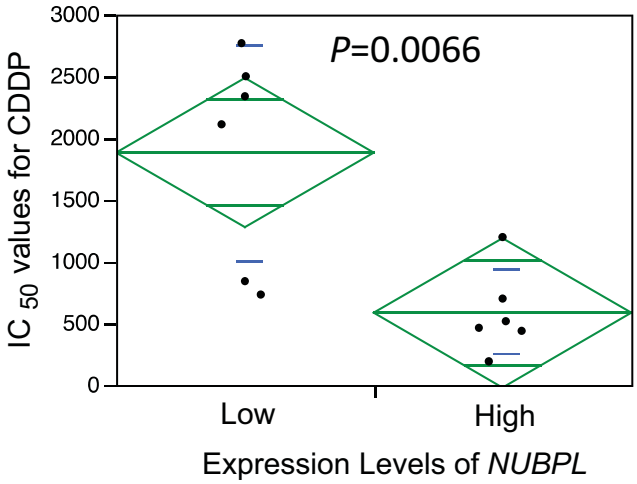

FIGURE 2 Expression of *NUBPL* as a possible CDDP sensitivity marker. When NSCLC cells were divided into high and low *NUBPL* expression groups at the median expression level, *NUBPL* high expression group was significantly sensitive to CDDP (with lower IC_{50} values) as compared with low expression group ($P = .0066$, Welch's *t*-test).

Knockdown Experiments to Validate Biological Significance of TMEM158 and NUBPL Genes as CDDP Resistant Markers

Since we selected these genes simply based on associations of their expression levels and CDDP drug sensitivity in NSCLC cells, biological significances of these genes with respect to CDDP sensitivity are unknown. We then explored whether altered expressions of these genes affect cell sensitivity to CDDP.

We first conducted a knockdown experiment using siRNA for *TMEM158* to test whether decreased *TMEM158* affects the CDDP sensitivity. We treated CDDP resistant-cell line PC-9/CDDP with a *TMEM158* siRNA, HSS146607, for 24 hours and the gene expression levels were quantified. Gene expression level of *TMEM158* resulted in 83.7% reduction as compared with those in cells treated with negative control (Figure 3A). We then compared IC_{50} values with CDDP-resistant PC-9/CDDP cells treated with *TMEM158*-specific siRNA and those with negative control. Treatment of the cells with *TMEM158* siRNA significantly reduced IC_{50} value by 59.4% as compared with control ($P < .0001$, Student's *t*-test) (Figure 3B), in good agreement with our observation that *TMEM158* gene expression levels positively associated with IC_{50} values for CDDP in NSCLC cells.

We also knocked down *NUBPL* gene in PC-9 cells and assessed its effects on sensitivity to the CDDP. When treated PC-9 cells with a *NUBPL* specific siRNA, HSS129499 for 24 hours, the gene expression level of *NUBPL* resulted in 82.5% reduction as compared with those in cells treated with negative control (Figure 4A). Knockdown of *NUBPL* gene using the specific siRNA significantly increased IC_{50} value as compared with those treated with negative control ($P < .005$, Student's *t*-test) (Figure 4B).

Forced Expression Experiments to Validate Biological Significance of FBLP1 Genes as CDDP Sensitive Markers

We then aimed to establish stable transformants of PC-6 cells harbouring expression vector of *FBLP1*

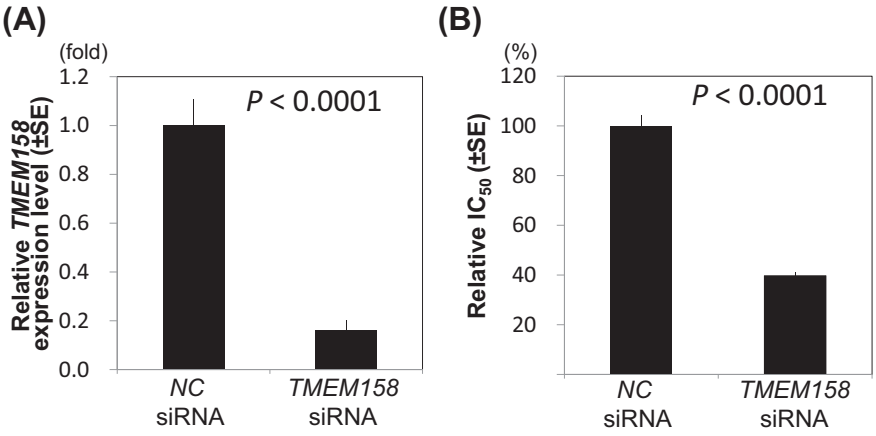

FIGURE 3 Effect of *TMEM158* knockdown by siRNA on the CDDP sensitivity in PC-9/CDDP cells. Treatment with siRNA specific for *TMEM158* significantly decreased the relative expression levels of the *TMEM158* gene normalized against *HPRT1* in PC-9/CDDP cells ($P < .0001$) (A), and attenuated the resistance of PC-9/CDDP cells to CDDP ($P < .0001$)) (B). NC indicates negative control. Relative IC_{50} values of CDDP were expressed as taking the mean value in PC-9/CDDP cells treated with NC siRNA to be 100%.

driven under CMV promoter. PC-6 cells were used as an experimental model, since *FBLP1* expression levels were extremely low in the cells. After selection with G418, the isolated clones were subjected to quantification of mRNA and protein of FBLP1.

PC-6 stable clones #8 and #10 showed approximately 2,000 and 500 folds overexpression of *FBLP1* mRNA, respectively, as compared with PC-6 cells with vector only (Figure 5A). This was confirmed by the Western blot analysis: Stable clones #8 and #10 demonstrated enhanced expression of FBLP1 protein (Figure 5B). These two clones exhibited significantly increased resistance against CDDP treatment ($P = .0041$ and $P = .001$, respectively, Dunnett test) (Figure 5C).

CONSTRUCTION OF MULTIPLE REGRESSION MODEL FOR IC50 VALUES BY GENE EXPRESSION LEVELS IN NSCLC CELLS

FBLP1, TMEM158, and *NUBPL* may be powerful predictive genes of CDDP response in the univariate analysis. Since several genes/signals may be involved in the determination of CDDP resistance/sensitivity, we thought that prediction using multiple genes would improve accuracy, and then conducted a stepwise multiple regression analysis, using the expression data of these selected genes together with known 6 genes, and IC_{50} values to compose prediction models for the NSCLC cells. The linear regression analyses provided the best prediction formula that showed the highest fitness, when we used expression data of 2 genes—*FBLP1* and *TMEM158*—($R^2 = 0.755$, $P = .0018$, AIC = 193.8). Taking these results into consideration, we then finally select *FBLP1 and TMEM158* genes as candidate biomarkers for NSCLC in vitro.

DISCUSSION

The development of predictive biomarkers of cisplatin-based chemotherapy is a high priority in lung cancer research. At present the excision repair cross-complementation group 1 (*ERCC1*) gene is the most putative predictive markers of CDDP response in NSCLC [10–17]. Multiple studies have shown that upregulation of *ERCC1* is associated with CDDP resistance and response and survival of patients with advanced NSCLC were improved in the presence of low *ERCC1* expression [24, 25]. Nevertheless, the role of *ERCC1* expression as a predictive marker, the biological significance on NSCLC patients, is still controversial. Many published results suggested that low expression of *ERCC1* might predict increased sensitivity to platinum-based chemotherapy, but high expression of *ERCC1* might be a positive prognostic factor. Recent meta-analysis study confirmed these incontinent results [26], and distinct mechanisms of chemotherapeutic response have also been suggested among histological subclasses of NSCLC. ERCC1 could predict clinical response of cisplatin-based chemotherapy in squamous cell carcinoma but not of adenocarcinoma. [27]. *ERCC1* is a putative predictive marker gene of CDDP response in NSCLC, but identification of a better prediction marker is keenly warranted.

We searched for more powerful biomarkers of CDDP drug-response in NSCLC, applying the data sets of the array expression and drug-sensitivity to 2D mixed normal model, and found 6 novel genes-*NUBPL, ZNF12, C9orf30, FBLP1, GSK3B,* and *TMEM158*- as first candidates [28–50].All the 6 candidate genes were closely related to cellular sensitivities to CDDP also in the quantified expression levels, and more correlative than 5 possible genes—*GSTP1, RRM1, BRCA1, FRAP1,* and further *ERCC1*—which were widely recognized as being of key importance among a variety of drug sensitivity genes for CDDP even when used alone [10–17]. Among the 6 genes, *NUBPL* might be the more, and *FBLP1* and *TMEM158* may be the most possible predictive markers [28–30, 35–38, 47–50]. *NUBPL, FBLP1, and TMEM158* demonstrated its functional significance as a CDDP-sensitivity determinant through the transfection analyses or siRNA-mediated knock-down experiments.

Multiple genes are involved in the mechanisms and they have complex interplay with each other. Despite still being in the investigational phase, attempts to predict tumor response using expression profiles of multiple key genes have also been intensively performed for various malignancies including lung cancer [21, 51–53]. Our additional attempt to predict in vitro response of CDDP using expression data of a set of all selected marker genes revealed that expressions of *FBLP1* and *TMEM158* were of key importance in the prediction formulae for CDDP. The multiple regression analyses provided the best prediction formula that showed the highest fitness when we used expression data of 2 genes—*FBLP1* and *TMEM158.* These multiple-gene approaches, we believe, will probably predict drug response more accurately than that when we use single predictor alone, and we finally propose *FBLP1* and *TMEM158* as a most putative novel predictive markers for CDDP response. The potent predictive value of the fixed formulae indicates that we probably succeeded in selecting

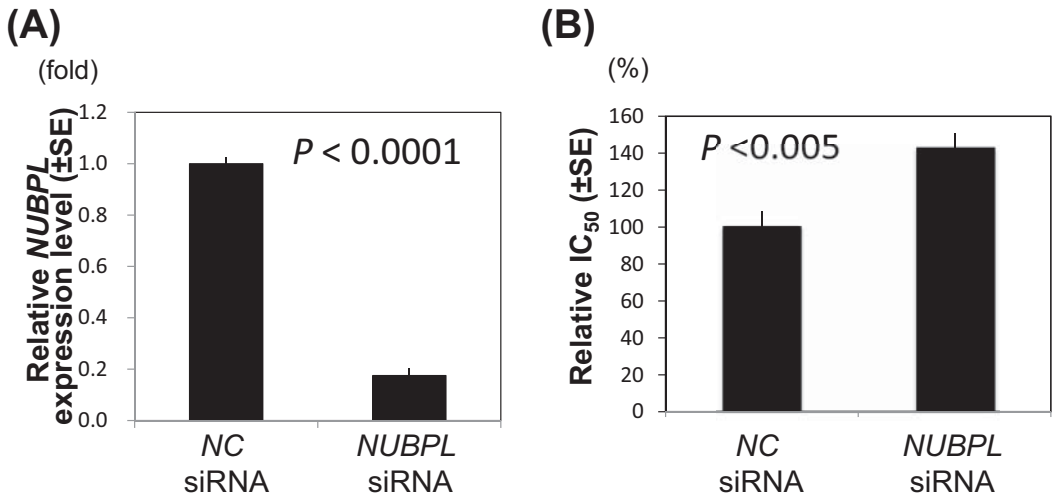

FIGURE 4 **Effect of *NUBPL* knockdown by siRNA on the CDDP sensitivity in PC-9 cell.** Treatment with siRNA specific for *NUBPL* significantly decreased relative expression levels of the *NUBPL* gene normalized against *HPRT1* in PC-9 cells to CDDP ($P < .0001$) (A), and enhanced the resistance of PC-9 cells to CDDP ($P < .005$) (B). NC indicates negative control. Relative IC_{50} values of CDDP were expressed as taking the mean value in PC-9 cells treated with NC siRNA to be 100%.

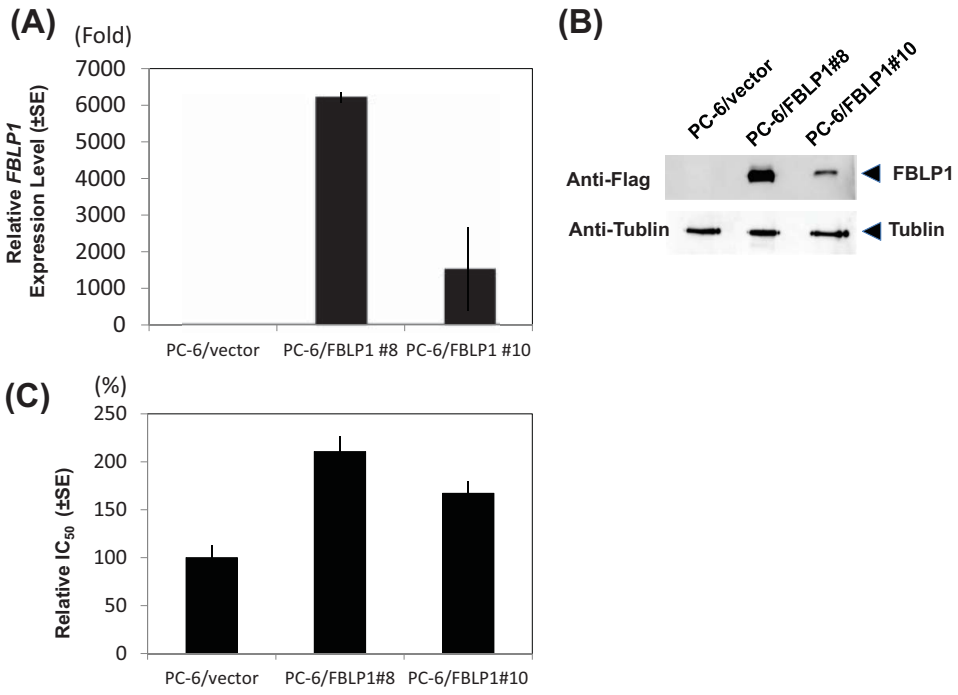

FIGURE 5 **Effect of forced expression of *FBLP1* on the CDDP sensitivity in PC-6 cells.** Comparison of (A) gene expression, (B) protein expression and (C) relative IC_{50} for CDDP in *FBLP1* stable transfectants #8 and #10 along with control PC-6/vector cells. Stable transformants #8 and #10 overexpressing FBLP1 showed much higher IC_{50} values for CDDP than PC-6/vector cells ($P = .0041$ and $P = .001$, respectively, Dunnett test). Relative IC_{50} values of CDDP were expressed as taking the mean IC_{50} value in PC-6/vector cells to be 100%.

the better prediction marker genes and precisely estimating their interaction in the expression levels.

Their functions remain little known, but various results suggest their possible roles in drug sensitivity and/or cancer outcome: *FBLP1* (Filamin-binding LIM protein 1) is a splice variant of migfilingene, which shares the N-terminal region. Migifilin was recently identified, as a widely expressed component of actin-cytoskeleton membrane junctions that is emerging as a key regulator of a variety of fundamental cellular processes, including shape modulation, motility, and differentiation [35–37]. *FBLP1* lacks the third LIM domain of migfilin, therefore, lacking the ability to localize to cell-ECM or cell–cell adhesions mediated by Mig-2-binding activity; predominantly it associates with the actin filaments. Recently, it has been shown that migfilin functions as an important activator of *Src*, linking cell-ECM adhesion to *Src* activation and survival signaling; and this migfilin-mediated signaling pathway is dysfunctional in multiple types of carcinoma cells, which likely contributes to aberrant *Src* activation and anoikis resistance in these cancerous cells [38]. *TMEM158*/*RIS1* (Ras-induced senescence 1) gene is a transmembrane protein 158 upregulated in response to activation of the Ras pathway [47–50]; it was isolated in a screen for genes specifically upregulated in Ras-senescent human fibroblasts. *TMEM158*/*RIS1* has been proposed to be a tumor-suppressor gene and a target gene in the mutator pathway, but the role as drug sensitivity marker is still poorly understood. The 3 genes probably play some important roles in cell proliferation, cell viability, and/or cell death, and thus possibly in cancer outcome and/or drug sensitivity. Molecular mechanisms—how *TMEM158* and *FBLP1* get involved in the action of CDDP—should be elucidated in the near future.

In the current analysis, *GSTP1, RRM1, BRCA1, FRAP1,* and *ERCC1* did not show any statistical significance in association studies of IC_{50} values with gene expression levels measured with microarray and real-time RT-PCR. Shimizu J et al. also reported that no correlation was observed between RRM1 or ERCC1 mRNA levels and IC_{50} values of CDDP in 20 lung cancer cell lines (7 adenocarcinoma, 5 squamous cell, 3 large cell, and 5 small cell lung cancers) [54], inconsistent with our observation. Possible explanations for the observed discrepancy between the mRNA expression levels of these known genes and IC_{50} levels of CDDP are as following: (1) Post-translational regulation, e.g., phosphorylation of GSTP1 mediated by PKCα and EGFR, may be involved in the determination of CDDP resistance [55,56]; (2) post-transcriptional regulation, e.g., specific microRNAs effects on protein expression levels of GSTP1 and ERCC1, may play some roles [57, 58]; (3) regulation of mTORC1 complex consisting of FRAP1 (also called mTOR), Raptor and mLST8 by the growth factor/PI3K/Akt signaling pathway may modulate the response [59]; (4) polymorphisms of amino acid sequence of the protein such as GSTP1 Ala114Val may cause alteration of CDDP resistance [60]; and (5) in sufficient numbers of cell lines tested to cover various signals relative to CDDP resistance/sensitivity.

Despite the limited amount of data and unknown detailed functions, all of the observed data in this study lead to the proposal that the selected 3 genes, especially for *FBLP1* and *TMEM158*, are possibly more powerful candidates for prediction markers of CDDP than current hypothesis-driven ones including *ERCC1*, and a two-dimensional mixed normal model could work well to identify novel marker genes from numerous candidates. Our first application of a two-dimensional mixed normal model to the selection of drug response marker was for TXL/CDDP therapy in ovarian cancer patients, and the attempt suggested its significant potential [21, 61]. The statistical method may identify differentially expressed genes between two cell samples with different biological behaviors based on the functional status of the genes.

Unbiased screening will generate more hypotheses to be tested, and with increasing understanding of tumor biology, more hypothesis-driven markers will be evaluated [18]. We believe that our approach may provide a step forward for tailoring CDDP treatment in NSCLC patients maximizing the benefit from cisplatin therapy for them, although the practical usefulness needs to be clearly evaluated by a larger prospective clinical study. We are now planning such a prospective clinical study, and will continue our search for the functional roles of the selected 2 genes in CDDP sensitivity.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

[1] Bareschino MA, Schettino C, Rossi A, Maione P, Sacco PC, Zeppa R, Gridelli C: Treatment of advanced non small cell lung cancer. J Thorac Dis. 2011;3:122–133.

[2] Gaughan EM, Costa DB: Genotype-driven therapies for non-small cell lung cancer: focus on EGFR, KRAS and ALK gene abnormalities. Ther Adv Med Oncol. 2011;3:113–125.

[3] Varughese S, Jahangir KS, Simpson CE, Boulmay BC, Lopez F: A paradigm shift in the treatment of advanced non-small cell lung cancer. Am J Med Sci. 2012;344:147–150.

[4] Jemal A, Siegel R, Xu J, Ward E: Cancer statistics. CA Cancer J Clin. 2010;60:277–300.

[5] Youlden DR, Cramb SM, Baade PD: The international epidemiology of lung cancer: geographical distribution and secular trends. J Thorac Oncol. 2008;3:819–831.

[6] Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, Zhu J, Johnson DH: Eastern Cooperative Oncology Group: Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med. 2002;346:92–98.

[7] NSCLC Meta-Analysis Collaborative Group: Chemotherapy in addition to supportive care improves survival in advanced non-small-cell lung cancer: a systematic review and meta-analysis of individual patient data from 16 randomized controlled trials. J Clin Oncol. 2008;26:4617–4625.

[8] Ettinger DS, Akerley W, Bepler G, Blum MG, Chang A, Cheney RT, Chirieac LR, D'Amico TA, Demmy TL, Ganti AK, Govindan R, Grannis FW Jr, Jahan T, Jahanzeb M, Johnson DH, Kessinger A, Komaki R, Kong FM, Kris MG, Krug LM, Le QT, Lennes IT, Martins R, O'Malley J, Osarogiagbon RU, Otterson GA, Patel JD, Pisters KM, Reckamp K, Riely GJ, Rohren E, Simon GR, Swanson SJ, Wood DE, Yang SC; NCCN Non-Small Cell Lung Cancer Panel Members: Non-small cell lung cancer. J Natl Compr Canc Netw. 2010;8:740–801.

[9] Azzoli CG, Temin S, Aliff T, Baker S Jr, Brahmer J, Johnson DH, Laskin JL, Masters G, Milton D, Nordquist L, Pao W, Pfister DG, Piantadosi S, Schiller JH, Smith R, Smith TJ, Strawn JR, Trent D, Giaccone G: 2011 Focused update of 2009 American society of clinical oncology clinical practice guideline update on chemotherapy for stage IV non-small-cell lung cancer. J Clin Oncol. 2011;29:3825–3831.

[10] Evans WE, Relling MV: Pharmacogenomics: translating functional genomics into rational therapeutics. Science. 1999;286:487–491.

[11] McLeod HL, Evans WE: Pharmacogenomics: unlocking the human genome for better drug therapy. Ann Rev Pharmacol Toxicol. 2001;41:101–121.

[12] Gibbs JB: Mechanism-based target identification and drug discovery in cancer research. Science. 2000;287: 1969–1973.

[13] Nishiyama M, Eguchi H: Recent advances in cancer chemotherapy: current strategies, pharmacokinetics, and pharmacogenomics. Adv Drug Deliv Rev. 2009;6:367–368.

[14] Langer CJ: Individualized therapy for patients with non-small cell lung cancer: emerging trends and challenges. Crit Rev Oncol Hematol. 2012;83:130–144.

[15] Pérez-Soler R: Individualized therapy in non-small-cell lung cancer: future versus current clinical practice. Oncogene. 2009;28 (Suppl 1):S38–S45.

[16] Hildebrandt MA, Gu J, Wu X: Pharmacogenomics of platinum-based chemotherapy in NSCLC. Expert Opin Drug Metab Toxicol. 2009;5:745–55.

[17] Danesi R, Pasqualetti G, Giovannetti E, Crea F, Altavilla G, Del Tacca M, Rosell R: Pharmacogenomics in non-small-cell lung cancer chemotherapy. Adv Drug Deliv Rev. 2009;61:408–417.

[18] Walther A, Johnstone E, Swanton C, Midgley R, Tomlinson I, Kerr D: Genetic prognostic and predictive markers in colorectal cancer. Nat Rev Cancer. 2009;9:489–499.

[19] Ohtaki M, Otani K, Satoh K, Kawamura T, Hiyama K Nishiyama M: Model-based analysis of microarray data: exploration of differentially expressed genes between two cell types based on a two-dimensional mixed normal model. Jpn J Biometrics. 2005;26:31–48.

[20] Hong WS, Saijo N, Sasaki Y, Minato K, Nakano H, Nakagawa K, Fujiwara Y, Nomura K, Twentyman PR: Establishment and characterization of cisplatin-resistant sublines of human lung cancer cell lines. Int J Cancer. 1988;41:462–467.

[21] Komatsu M, Hiyama K, Tanimoto K, Yunokawa M, Otani K, Ohtaki M, Hiyama E, Kigawa J, Ohwada M, Suzuki M, Nagai N, Kudo Y, Nishiyama M: Prediction of individual response to platinum/paclitaxel combination using novel marker genes in ovarian cancers. Mol Cancer Ther. 2006;5:767–775.

[22] Rousseau PJ: Least median of squares regression. J Am Stat Assoc. 1984;79:871–880.

[23] R Development Core Team: R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2010ISBN 3–900051-07–0, URL http://www.R-project.org/.

[24] Olaussen KA, Dunant A, Fouret P, Brambilla E, André F, Haddad V, Taranchon E, Filipits M, Pirker R, Popper HH, Stahel R, Sabatier L, Pignon JP, Tursz T, Le Chevalier T, Soria JC: DNA repair by ERCC1 in nonsmall-cell lung cancer and cisplatin-based adjuvant chemotherapy. N Engl J Med. 2006;355:983–991.

[25] Postel-Vinay S, Vanhecke E, Olaussen KA, Lord CJ, Ashworth A, Soria JC: The potential of exploiting DNA-repair defects for optimizing lung cancer treatment. Nat Rev Clin Oncol. 2012;9:144–155.

[26] Jiang J, Liang X, Zhou X, Huang R, Chu Z, Zhan Q: ERCC1 expression as a prognostic and predictive factor in patients with non-small cell lung cancer: a meta-analysis. Mol Biol Rep. 2012;39:6933–6942.

[27] Pierceall WE, Olaussen KA, Rousseau V, Brambilla E, Sprott KM, Andre F, Pignon JP, Le Chevalier T, Pirker R, Jiang C, Filipits M, Chen Y, Kutok JL, Weaver DT, Ward BE, Soria JC: Cisplatin benefit is predicted by immunohistochemical analysis of DNA repair proteins in squamous cell carcinoma but not adenocarcinoma: theranostic modeling by NSCLC constituent histological subclasses. Ann Oncol. 2012;23:2245–2252.

[28] Calvo SE, Tucker EJ, Compton AG, Kirby DM, Crawford G, Burtt NP, Rivas M, Guiducci C, Bruno DL, Goldberger OA, Redman MC, Wiltshire E, Wilson CJ, Altshuler D, Gabriel SB, Daly MJ, Thorburn DR, Mootha VK: High-throughput, pooled sequencing identifies mutations in NUBPL and FOXRED1 in human complex I deficiency. Nat Genet. 2010;42:851–858.

[29] Sheftel AD, Stehling O, Pierik AJ, Netz DJ, Kerscher S, Elsässer HP, Wittig I, Balk J, Brandt U, Lill R: Human ind1, an iron-sulfur cluster assembly factor for respiratory complex I. Mol Cell Biol. 2009;29:6059–6073.

[30] Bych K, Kerscher S, Netz DJ, Pierik AJ, Zwicker K, Huynen MA, Lill R, Brandt U, Balk J: The iron-sulphur protein Ind1 is required for effective complex I assembly. EMBO J. 2008;27:1736–1746.

[31] Basit S, Ali G, Wasif N, Ansar M, Ahmad W: Genetic mapping of a novel hypotrichosis locus to chromosome 7p21.3-p22.3 in a Pakistani family and screening of the candidate genes. Hum Genet. 2010;128:213–220.

[32] Huang YC, Schmitt M, Yang Z, Que LG, Stewart JC, Frampton MW, Devlin RB: Gene expression profile in circulating mononuclear cells after exposure to ultrafine carbon particles. Inhal Toxicol. 2010;22:835–846.

[33] Zhao Y, Zhou L, Liu B, Deng Y, Wang Y, Wang Y, Huang W, Yuan W, Wang Z, Zhu C, Liu M, Wu X, Li Y: ZNF325, a novel human zinc finger protein with a RBaK-like RB-binding domain, inhibits AP-1- and SRE-mediated transcriptional activity. Biochem Biophys Res Commun. 2006;346:1191–1199.

[34] Song Q, Jing H, Wu H, Zhou G, Kajiyama T, Kambara H: Gene expression analysis on a photodiode array-based bioluminescence analyzer by using sensitivity-improved SRPP. Analyst. 2010;135:1315–1319.

[35] Takafuta T, Saeki M, Fujimoto TT, Fujimura K, Shapiro SS: A new member of the LIM protein family binds to filamin B and localizes at stress fibers. J Biol Chem. 2003;278:12175–12181.

[36] Tu Y, Wu S, Shi X, Chen K, Wu C: Migfilin and Mig-2 link focal adhesions to filamin and the actin cytoskeleton and function in cell shape modulation. Cell. 2003;113:37–47.
[37] Wu C: Migfilin and its binding partners: from cell biology to human diseases. J Cell Sci. 2005;118(Pt 4):659–664.
[38] Zhao J, Zhang Y, Ithychanda SS, Tu Y, Chen K, Qin J, Wu C: Migfilin interacts with Src and contributes to cell-matrix adhesion-mediated survival signaling. J Biol Chem. 2009;284:34308–34320.
[39] Chou CH, Chou AK, Lin CC, Chen WJ, Wei CC, Yang MC, Hsu CM, Lung FW, Loh JK, Howng SL, Hong YR: GSK3β regulates Bcl2L12 and Bcl2L12A anti-apoptosis signaling in glioblastoma and is inhibited by LiCl. Cell Cycle. 2012;11:532–542.
[40] Doble BW, Woodgett JR: GSK-3: tricks of the trade for a multi-tasking kinase. J. Cell Sci. 2003;116:1175–1186.
[41] Grimes CA, Jope RS: The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling. Pro Neurobiol. 2001;65:391–426.
[42] Zhang D, Guo M, Zhang W, Lu XY: Adiponectin stimulates proliferation of adult hippocampal neural stem/progenitor cells through activation of p38 mitogen-activated protein kinase (p38MAPK)/glycogen synthase kinase 3β (GSK-3β)/β-catenin signaling cascade. J Biol Chem. 2011;286:44913–44920.
[43] Beurel E, Kornprobst M, Blivet-Van Eggelpoël MJ, Ruiz-Ruiz C, Cadoret A, Capeau J, Desbois-Mouthon C: GSK-3beta inhibition by lithium confers resistance to chemotherapy-induced apoptosis through the repression of CD95 (Fas/APO-1) expression. Exp Cell Res. 2004;300:354–364.
[44] Dong J, Peng J, Zhang H, Mondesire WH, Jian W, Mills GB, Hung MC, Meric-Bernstam F: Role of glycogen synthase kinase 3beta in rapamycin-mediated cell cycle regulation and chemosensitivity. Cancer Res. 2005;65:1961–1972.
[45] Ding Q, He X, Hsu JM, Xia W, Chen CT, Li LY, Lee DF, Liu JC, Zhong Q, Wang X, Hung MC: Degradation of Mcl-1 by beta-TrCP mediates glycogen synthase kinase 3-induced tumor suppression and chemosensitization. Mol Cell Biol. 2007;27:4006–4017.
[46] Kwok JB, Loy CT, Hamilton G, Lau E, Hallupp M, Williams J, Owen MJ, Broe GA, Tang N, Lam L, Powell JF, Lovestone S, Schofield PR: Glycogen synthase kinase-3beta and tau genes interact in Alzheimer's disease. Ann Neurol. 2008;64:446–454.
[47] Barradas M, Gonos ES, Zebedee Z, Kolettas E, Petropoulou C, Delgado MD, León J, Hara E, Serrano M. Marta Barradas, Efstathios S. Gonos: Identification of a candidate tumor-suppressor gene specifically activated during Ras-induced senescence. Exp Cell Res. 2002;273:127–137.
[48] Silva J, Silva JM, Barradas M, García JM, Domínguez G, García V, Peña C, Gallego I, Espinosa R, Serrano M, Bonilla F: Analysis of the candidate tumor suppressor Ris-1 in primary human breast carcinomas. Mutat Res. 2006;594:78–85.
[49] Birch AH, Quinn MC, Filali-Mouhim A, Provencher DM, Mes-Masson AM, Tonin PN: Transcriptome analysis of serous ovarian cancers identifies differentially expressed chromosome 3 genes. Mol Carcinog. 2008;47:56–65.
[50] Iglesias D, Fernández-Peralta AM, Nejda N, Daimiel L, Azcoita MM, Oliart S, González-Aguilera JJ: RIS1, a gene with trinucleotide repeats, is a target in the mutator pathway of colorectal carcinogenesis. Cancer Genet Cytogenet. 2006;167:138–144.
[51] Gurubhagavatula S, Liu G, Park S, Zhou W, Su L, Wain JC, Lynch TJ, Neuberg DS, Christiani DC: XPD and XRCC1 genetic polymorphisms are prognostic factors in advanced non-small-cell lung cancer patients treated with platinum chemotherapy. J Clin Oncol. 2004;22:2594–2601.
[52] Tanaka T, Tanimoto K, Otani K, Satoh K, Ohtaki M, Yoshida K, Toge T, Yahata H, Tanaka S, Chayama K, Okazaki Y, Hayashizaki Y, Hiyama K, Nishiyama M: Concise prediction models of anticancer efficacy of 8 drugs using expression data from 12 selected genes. Int J Cancer. 2004;111:617–626.
[53] Ichikawa W, Takahashi T, Suto K, Shirota Y, Nihei Z, Shimizu M, Sasaki Y, Hirayama R: Simple combinations of 5-FU pathway genes predict the outcome of metastatic gastric cancer patients treated by S-1. Int J Cancer. 2006;119:1927–1933.
[54] Shimizu J, Horio Y, Osada H, Hida T, Hasegawa Y, Shimokata K, Takahashi T, Sekido Y, Yatabe Y: mRNA expression of RRM1, ERCC1 and ERCC2 is not associated with chemosensitivity to cisplatin, carboplatin and gemcitabine in human lung cancer cell lines. Respirology. 2008;13:510–517.
[55] Singh S, Okamura T, Ali-Osman F: Serine phosphorylation of glutathione S-transferase P1 (GSTP1) by PKCα enhances GSTP1-dependent cisplatin metabolism and resistance in human glioma cells. Biochem Pharmacol. 2010;80:1343–1355.
[56] Okamura T, Singh S, Buolamwini J, Haystead T, Friedman H, Bigner D, Ali-Osman F: Tyrosine phosphorylation of the human glutathione S-transferase P1 by epidermal growth factor receptor. J Biol Chem. 2009;284:16979–16989.
[57] Zhang X, Zhu J, Xing R, Tie Y, Fu H, Zheng X, Yu B: miR-513a-3p sensitizes human lung adenocarcinoma cells to chemotherapy by targeting GSTP1. Lung Cancer. 2012;77:488–494.
[58] Wang Q, Zhong M, Liu W, Li J, Huang J, Zheng L: Alterations of microRNAs in cisplatin-resistant human non-small cell lung cancer cells (A549/DDP). Exp Lung Res. 2011;37:427–434.
[59] Ballou LM, Lin RZ: Rapamycin and mTOR kinase inhibitors. J Chem Biol. 2008;1:27–36.
[60] Moyer AM, Salavaggione OE, Wu TY, Moon I, Eckloff BW, Hildebrandt MA, Schaid DJ, Wieben ED, Weinshilboum RM: Glutathione s-transferase p1: gene sequence variation and functional genomic studies. Cancer Res. 2008;68:4791–4801.
[61] Fumoto S, Shimokuni T, Tanimoto K, Hiyama K, Otani K, Ohtaki M, Hihara J, Yoshida K, Hiyama E, Noguchi T, Nishiyama M: Selection of a novel drug-response predictor in esophageal cancer: a novel screening method using microarray and identification of IFITM1 as a potent marker gene of CDDP response. Int J Oncol. 2008;32:413–423.