Targeted sequencing reveals genetic variants associated with sensitivity of 79 human cancer xenografts to anticancer drugs

CHIHIRO UDAGAWA[1,2], YASUSHI SASAKI[3], HIROSHI SUEMIZU[4], YASUYUKI OHNISHI[4], HIROSHI OHNISHI[1], TAKASHI TOKINO[3] and HITOSHI ZEMBUTSU[1,2]

[1]Liquid Biopsy Development Group, Project for Development of Liquid Biopsy Diagnosis, Cancer Precision Medicine Center, Japanese Foundation for Cancer Research, Tokyo 135-8550; [2]Division of Genetics, National Cancer Center Research Institute, Tokyo 104-0045; [3]Department of Medical Genome Science, Research Institute for Frontier Medicine, Sapporo Medical University, School of Medicine, Hokkaido 060-8556; [4]Laboratory Animal Research Department, Central Institute for Experimental Animals, Kawasaki, 210-0821, Japan

Abstract. Although there has been progress moving from a 'one-size-fits-all' cytotoxic approach to personalized molecular medicine, the majority of patients with cancer receive chemotherapy using cytotoxic anticancer drugs. The sequencing analysis of 409 genes associated with cancer was conducted in the present study using 59 DNA sequences extracted from human cancer xenografts implanted into nude mice, of which sensitivity to 9 cytotoxic anticancer drugs [5-fluorouracil, nimustine, adriamycin, cyclophosphamide, cisplatin, mitomycin C (MMC), methotrexate, vincristine (VCR), and vinblastine] was examined. The present study investigated the association between the sensitivities of the xenografts to the 9 anticancer drugs and the frequency of single nucleotide variants (SNV). The correlation between the expression level of the genes and sensitivities to the 9 drugs in the above xenografts was also estimated. In the screening study using 59 xenografts, 3 SNVs (rs1805321, rs62456182 in PMS1 Homolog 2, Mismatch Repair System Component and rs13382825 in LDL Receptor Related Protein 1B), were associated with sensitivity to VCR and MMC, respectively (P<0.001). A replication study of 596 SNVs was subsequently performed, which indicated P<0.05 in the screening study using independent samples of 20 xenografts. A combined result of the screening and replication studies indicated that 35 SNVs were potentially associated with sensitivities to one or more of the nine anticancer drugs

($P_{combined}$=0.0011-0.035). Of the 35 SNVs, rs16903989 and rs201432181 in Leukemia Inhibitory Factor Receptor α and Adhesion G Protein-Coupled Receptor A2 were commonly associated with sensitivity to 2 or 4 anticancer drugs, respectively. These findings provide novel insights which may benefit the development of personalized anticancer therapy for patients with cancer in the future.

Introduction

Over the past decade, the understanding of human cancer and development of molecular targeted therapies have benefitted from genomic technologies (1). A large proportion of patients with cancer suffer adverse effects from molecular targeted or cytotoxic agents while exhibiting no effective response in terms of tumor shrinkage (2). Although molecular targeted therapy is a standard cancer treatment, anticancer therapies using cytotoxic drugs remain a gold standard approach for cancer treatment (3-5). The efficacy of cytotoxic anticancer drugs varies among individual patients (6-8). Although a number of recent studies have attempted to establish a diagnostic method for predicting chemosensitivity (9-12), to the best of our knowledge, no clinically applicable genetic markers for the prediction of sensitivity or resistance to cytotoxic anticancer drugs have been developed. In order to distinguish which patients may respond to certain drugs from those who may not, prior to initiating treatment, to offer a 'cancer precision medicine' program of more effective chemotherapy and also to relieve patients from severe adverse events, a larger set of genetic variants in tumors must be identified to serve as accurate predictive markers for each anticancer drug.

The development of next generation sequencing technologies has revolutionized cancer genomic research because it provides a comprehensive method of detecting genomic alterations (somatic mutations) in cancer cells (13-15). A number of studies have reported an association between clinical outcomes and variant allele frequencies (VAFs) in tumors (16-20). As the properties of cancer cells may be influenced by complicated interactions among genes associated

with cancer, such as oncogenes or tumor suppressor genes expressed in cancer cells (21-23), the present study hypothesized that the genetic variants of these genes within the tumors may serve important roles in determining the response to cytotoxic anticancer drugs.

In the current study, to identify genetic markers for sensitivity or resistance to 9 cytotoxic anticancer drugs, all exons of 409 genes associated with cancer from 79 cancer xenografts in mice that had been established from 12 different human organs were sequenced. The association between single nucleotide variants (SNVs) detected in the xenografts and sensitivities to the 9 cytotoxic anticancer drugs were then investigated using a nonparametric approach. The present study identifies the genes associated with cancer that may also be associated with sensitivity to ≥1 of the 9 anticancer drugs examined. The results of the current study may help to elucidate the mechanism that causes the different clinical responses to chemotherapy among patients and may be applicable in the development of a prediction system to optimize treatment.

Materials and methods

Xenografts, anticancer drugs and examination of xenografts for sensitivity to anticancer drugs. A total of 79 human cancer xenografts, including 12 breast cancers, 12 gastric cancers, 10 neuroblastomas, 10 non-small-cell lung cancers, 7 gliomas, 6 pancreatic cancers, 5 colon cancers, 5 choriocarcinomas, 4 small-cell lung cancers, 4 hematopoietic cancers, 3 ovarian cancers and 1 osteosarcoma were transplanted to athymic BALB/c-*nu/nu* mice (weight, 26.3±1.8 g; age, 8-10 weeks) and maintained by serial subcutaneous transplantation of 2x2x2 mm fragments into the flank once a month as described previously (24). A total of 7,900 mice were purchased from Japan CLEA Inc. (Tokyo, Japan) and housed in a controlled temperature of 23±1˚C and relative humidity 50-70%, with *ad libitum* access to food and water. Mice were divided into 10 groups of 6 mice, per xenograft. A total of 79 human tumor tissues from 79 patients were obtained aseptically during surgery or autopsy at 13 hospitals. Mitomycin C (MMC), adriamycin (ADR; both Kyowa Hakko Bio Co., Ltd., Tokyo, Japan), cyclophosphamide (CPM), vincristine (VCR), vinblastine (VLB; all Shionogi & Co. Ltd., Osaka, Japan), nimustine (ACNU; Daiichi Sankyo Co., Ltd., Tokyo, Japan), cisplatin (DDP), 5-fluorouracil (5FU; both Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and methotrexate (MTX; Wyeth Lederle Japan, Ltd., Tokyo, Japan) were dissolved in sterile 0.85% NaCl containing 1% mannitol (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The maximum tolerated dose for these drugs in mice was determined as described previously [MMC: 6.7 mg/kg, CPM: 260 mg/kg, ACNU: 48 mg/kg, DDP: 10 mg/kg, ADR: 12 mg/kg, VCR: 1.6 mg/kg, VLB: 11 mg/kg, 5-FU: 19 mg/kg (x5), MTX: 15 mg/kg (x5)] (24). Each anticancer drug was administered individually, at the maximum tolerated dose, to nude mice bearing human cancer xenografts (in groups of 6). Administration route was intravenous infusion in all cases. 5-FU and MTX were administered for 5 days and all other drugs in a single dose. The control group did not receive any treatment (6 mice per xenograft). Chemosensitivity was calculated as the relative tumor volume of treated mice (T) with respect to control (C) using the mean values for the treatment and control groups on day 14, as described previously [T/C (%)] (25,26). All animal studies were approved by the institutional committee of Central Institute for Experimental Animals, and conducted according to previously described protocols (27). Mice were sacrificed 21 days after drug administration.

Gene expression analysis. Total RNA was extracted from xenograft untreated tissues using ISOGEN (Nippon Gene Co., Ltd., Toyama, Japan) according to the manufacturer's protocol. To eliminate genomic DNA contamination, samples were treated with Recombinant DNase (RNase-free; Takara Bio, Inc., Otsu, Japan) following the manufacturer's protocol. cDNA was prepared from 5 μg total RNA using SuperScript III reverse transcriptase (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Firstly, 5 μg total RNA, 1 μl oligo dT primers (Invitrogen; Thermo Fisher Scientific, Inc.) and diethyl pyrocarbonate (DEPC) water were mixed to a total volume of 16 μl. This mixture was incubated at 70˚C for 10 min and then chilled on ice for 5 min. The following components were added: 5 μl 5X first strand buffer (Invitrogen; Thermo Fisher Scientific, Inc.), 1 μl 25 mM dNTP (Wako Pure Chemical Industries, Ltd.), 2.5 μl 100 mM DTT (Invitrogen; Thermo Fisher Scientific, Inc.) and 0.5 μl Recombinant RNase Inhibitor (Takara Bio, Inc.), followed by 1.5 μl SuperScript III Reverse Transcriptase. This reaction mixture was incubated at 42˚C for 50 min and terminated by heating to 70˚C for 15 min. The cDNA products were stored at -20˚C until required. mRNA expression profiles were obtained from an in-house cDNA microarray consisting of 23,040 genes, as described previously (25,26). For the 69 genes (Table I) whose expression was not available in the aforementioned profile, reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was completed, using the SYBR Green Real-Time PCR system (Thermo Fisher Scientific, Inc.) and the StepOnePlus and 7900HT Fast Real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.), following the manufacturer's protocols. Each PCR reaction mixture contained 5 μl Fast SYBR Green Master Mix (2x) (Applied Biosystems; Thermo Fisher Scientific, Inc.), 0.2 μl of each primer (10 pmol/μl) (Sigma-Aldrich; Merck KGaA), 1 μl cDNA and DEPC water (Ambion; Thermo Fisher Scientific, Inc.), for a total volume of 10 μl. The reaction was performed at 95˚C for 20 sec, 40 cycles of 95˚C for 3 sec and 60˚C for 30 sec, 95˚C for 15 sec, 60˚C for 1 min and 95˚C for 15 sec. The sequences of the primers are shown in Table I. The level of mRNA was assessed using the relative standard curve method, relative to β-actin reference gene (28).

Sample preparation and targeted next-generation sequencing. Tumor genomic DNA was extracted from 79 xenografts using the QIAmp DNA Mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. In the screening study, targeted next generation sequencing was performed in 59 xenografts (12 breast cancers, 12 gastric cancers, 10 neuroblastomas, 10 non-small-cell lung cancers, 7 gliomas, 6 pancreatic cancers, 1 ovarian cancer and 1 osteosarcoma) using the Ion AmpliSeq Comprehensive Cancer Panel (CCP;

Table I. Sequences of primers used for qRT-PCR.

Gene	Primer sequence
MTRR	Forward 5'-AGCCTACTCCAAAGACTGCA-3'
	Reverse 5'-CAGGTATATGCTGGGGTAAGGT-3'
ADAMTS20	Forward 5'-GGAAATTACTGTGTGGGCCG-3'
	Reverse 5'-GCACTGCTTCTCTCGAAAGT-3'
ASXL1	Forward 5'-TTCACGCTCAAGAAGGATGC-3'
	Reverse 5'-GGCTTCATTAGACCCACAGC-3'
ADGRA2/	Forward 5'-AGAAGGTGGAGATCGTGGTG-3'
GPR124	Reverse 5'-AGGACTGGTAGGCTGTGATG-3'
ADGRB3/	Forward 5'-AACGGGCGAAGAAGTGAGAA-3'
BAI3	Reverse 5'-GTGGCATTCAGGGGACATTG-3'
AKT1	Forward 5'-TCAACAACTTCTCTGTGGCG-3'
	Reverse 5'-GAAGGTGCGTTCGATGACAG-3'
AMER1/	Forward 5'-GGGTATCTGTACTCTGCCTAGTT-3'
FAM123B	Reverse 5'-CTTGCTGAGACCTTTCTTGGAG-3'
ATR	Forward 5'-TGTAAATGTGAGTGGAAGCCA-3'
	Reverse 5'-AATGACAGGAGGGAGTTGCT-3'
BCL3	Forward 5'-AACCTGCCTACACCCCTATAC-3'
	Reverse 5'-CACCACAGCAATATGGAGAGG-3'
BCL6	Forward 5'-ACGGCTATGTACCTGCAGAT-3'
	Reverse 5'-TCTTCACGAGGAGGCTTGAT-3'
BRIP1/	Forward 5'-CCACTCTGGCTGCAAAGTTA-3'
FANCJ	Reverse 5'-TCTGTTCCAAAGCAATGACGT-3'
CDH1	Forward 5'-ATTTTTCCCTCGACACCCGAT-3'
	Reverse 5'-TCCCAGGCGTAGACCAAGA-3'
CRBN	Forward5'-TCCTTGAGCTAAGAACACAGTCA-3'
	Reverse 5'-AAGGCAACACACATTCGGGAA-3'
CRTC1	Forward 5'-GGTCCCCGGAATCAACATCT-3'
	Reverse 5'-AGTGGATGTTGGTCAGGTCG-3'
CDKN2A	Forward 5'-CCTCAGACATCCCCGATTGA-3'
	Reverse 5'-GAAAGCGGGGTGGGTTGT-3'
CMPK1	Forward 5'-ATGGATGGGAAGGCAGATGT-3'
	Reverse 5'-TCCAAGCTCTCTCTGTTGTCA-3'
CYP2C19	Forward 5'-GTATTTTGGCCTGGAACGCA-3'
	Reverse 5'-CAGTGGGAAATGGCCTCTTC-3'
CYP2D6	Forward 5'-ACCAGGCTCACATGCCCTA-3'
	Reverse 5'-TTCGATGTCACGGGATGTCAT-3'
DDIT3	Forward 5'-TGTTAAAGATGAGCGGGTGG-3'
	Reverse 5'-TGCTTTCAGGTGTGGTGATG-3'
EP300	Forward 5'-AAATGGCCGAGAATGTGGTG-3'
	Reverse 5'-TGGTAAGTCGTGCTCCAAGT-3'
ERBB3	Forward 5'-CAACTCTCAGGCAGTGTGTC-3'
	Reverse 5'-CATCACCACCTCACACCTCT-3'
ERCC1	Forward 5'-ACCCAGACTACATCCATGGG-3'
	Reverse 5'-TCTTAGCCAGCTCCTTGAGG-3'
FANCD2	Forward 5'-GGGATTATTGGTGCTGTGACC-3'
	Reverse 5'-GCTCAGGTTGGCTCTCTCTT-3'
FAS	Forward 5'-GATGAACCAGACTGCGTGC-3'
	Reverse 5'-TCACACAATCTACATCTTCTGCA-3'
FLCN	Forward 5'-GAGGCAGAGCAGTTTGGATG-3'
	Reverse 5'-CACTTGTCAGCGATGTCAGC-3'
FH	Forward 5'-TGTTAGGAGGTGAACTTGGCA-3'
	Reverse 5'-ATGTGCATTGCTGTGGGAAA-3'
GNA11	Forward 5'-TACGAGCAGAACAAGGCCAA-3'
	Reverse 5'-GTCGTAGCATTCCTGGATGC-3'
HNF1A	Forward 5'-GCTGATTGAAGAGCCCACAG-3'
	Reverse 5'-CTCTCGCTCCTCCTTGCTAG-3'
IKBKE	Forward 5'-GAGAAGTTCGTCTCGGTCTATGG-3'
	Reverse 5'-TGCATGGTACAAGGTCACTCC-3'
ITGA10	Forward 5'-ACTTAGGTGACTACCAACTGGG-3'

Table I. Continued.

Gene	Primer sequence
	Reverse 5'-CCACAAGCACGAGACCAGA-3'
IL2	Forward 5'-AACTCCTGTCTTGCATTGCAC-3'
	Reverse 5'-GCTCCAGTTGTAGCTGTGTTT-3'
IL21R	Forward 5'-CTTCATGGCCGACGACATTT-3'
	Reverse 5'-GGAGAAAGCTGCCACACTC-3'
KEAP1	Forward 5'-TGGCCACATCTATGCCGTC-3'
	Reverse 5'-ATCCTTCGTGTCAGCATTGG-3'
KDR	Forward 5'-GGCCCAATAATCAGAGTGGCA-3'
	Reverse 5'-CCAGTGTCATTTCCGATCACTTT-3'
KIT	Forward 5'-CGTTCTGCTCCTACTGCTTCG-3'
	Reverse 5'-CCCACGCGGACTATTAAGTCT-3'
LRP1B	Forward 5'-CCAACGGTTCTGTATGTGTCA-3'
	Reverse 5'-GCGACATTCCCGTAGTCAGTAAA-3'
KAT6B	Forward 5'-CACCTCAGTATCCCAGTGCA-3'
	Reverse 5'-ATTGGAATGGGATCAGCACG-3'
KDM6A	Forward 5'-TACAGGCTCAGTTGTGTAACCT-3'
	Reverse 5'-CTGCGGGAATTGGTAGGCTC-3'
MALT1	Forward 5'-AAGGTTGCACAGTCACAGAA-3'
	Reverse 5'-ACTGCCTTTGACTCTGGGTT-3'
MDM4	Forward 5'-TGATTGTCGAAGAACCATTTCGG-3'
	Reverse5'-TGCAGGGATCAAAAAGTTTGGAG-3'
MEN1	Forward 5'-CAACCCTTCCATTGACCTGC-3'
	Reverse 5'-GCTCCTCTAGATCTGCCAGG-3'
MPL	Forward 5'-CTGAAGTGTTTCTCCCGAACAT-3'
	Reverse 5'-GCGGGTAGGCATACAGCAG-3'
MSH2	Forward 5'-AGAGCTGGAAATAAGGCATCC-3'
	Reverse 5'-AACACCCACAACACCAATGG-3'
MYH11	Forward 5'-GGATGAGAGGGACAGAGCTG-3'
	Reverse 5'-GCTTCCAAGGCCTCTTCAAG-3'
NTRK1	Forward 5'-TCAACAACGGCAACTACACG-3'
	Reverse 5'-CTCGGGGTTGAACTCGAAAG-3'
NOTCH1	Forward 5'-TGGACCAGATTGGGGAGTTC-3'
	Reverse 5'-GCACACTCGTCTGTGTTGAC-3'
NUMA1	Forward 5'-GGGCTAAACCTTAATGAGGACC-3'
	Reverse 5'-AGGAAGCGAATCTCCCTCTTG-3'
PAX3	Forward 5'-AGCCGCATCCTGAGAAGTAA-3'
	Reverse 5'-CTTCATCTGATTGGGGTGCT-3'
PAX7	Forward 5'-CAATGGAATGGCAGGGACAC-3'
	Reverse 5'-GATCACACAGCGGTACTTGC-3'
PALB2	Forward 5'-GGAAAGCTCTGGATGCTTGG-3'
	Reverse 5'-CCCAAAGCTACACACACGAG-3'
PIK3CD	Forward 5'-CTGGGGAATTTCAAGACCAAGT-3'
	Reverse 5'-CCCTGCTGAATCACATGGAC-3'
PIK3CG	Forward 5'-AGTATGACGTCAGTTCCCAAGT-3'
	Reverse 5'-GGAACTCTAAAGCTTTCGGGG-3'
PIK3C2B	Forward 5'-CTGGCTATGTCTGGAGTGCT-3'
	Reverse 5'-CAGTGGAGGAACAGTTGCAG-3'
PLAG1	Forward 5'-AAACTTTTGAAAGCACGGGAGT-3'
	Reverse 5'-GGCGATCACAATGTTCGCAC-3'
PDGFRB	Forward 5'-TGATGCCGAGGAACTATTCATCT-3'
	Reverse 5'-TTTCTTCTCGTGCAGTGTCAC-3'
PDGFB	Forward 5'-ACTCGATCCGCTCCTTTGAT-3'
	Reverse 5'-GGGTCATGTTCAGGTCCAAC-3'
PKHD1	Forward 5'-GCTCCGCTTCTTTCCTTCAC-3'
	Reverse 5'-AGAGTGGTGCCAGTGACATT-3'
PRDM1	Forward 5'-TAAAGCAACCGAGCACTGAGA-3'
	Reverse 5'-ACGGTAGAGGTCCTTTCCTTTG-3'
PTGS2	Forward 5'-TCCCTTCCTTCGAAATGCAA-3'
	Reverse 5'-GAGGTTAGAGAAGGCTTCCCA-3'

Table I. Continued.

Gene	Primer sequence
PTPRT	Forward 5'-CAATGGAATGGCAGGGACAC-3'
	Reverse 5'-GATCACACAGCGGTACTTGC-3'
RECQL4	Forward 5'-CCCTGCTGTCACTCATGGAT-3'
	Reverse 5'-GACAGATTCCCGTTGCTTCC-3'
REL	Forward 5'-TCCTCCTGTTGTCTCGAACC-3'
	Reverse 5'-CCTCCTCTGACACTTCCACA-3'
RUNX1	Forward 5'-CATCGCTTTCAAGGTGGTGG-3'
	Reverse 5'-GTTCTTCATGGCTGCGGTAG-3'
SMO	Forward 5'-TCGAATCGCTACCCTGCTG-3'
	Reverse 5'-CAAGCCTCATGGTGCCATCT-3'
SAMD9	Forward5'-ATGGCAAAGCAACTTAACCTTCC-3'
	Reverse 5'-CCATTCACGTCTTGTTCAGTCA-3'
TAF1L	Forward 5'-TCCCTCAGTACGTCTCGAGA-3'
	Reverse 5'-TCTGGAGTGGCAGTGGAAAT-3'
TET1	Forward 5'-CATCAGTCAAGACTTTAAGCCCT-3'
	Reverse 5'-CGGGTGGTTTAGGTTCTGTTT-3'
TNFAIP3	Forward 5'-ACCCCATTGTTCTCGGCTAT-3'
	Reverse 5'-AATCTTCCCCGGTCTCTGTT-3'
TCF12	Forward 5'-CTCCTGACCATACCAGCAGT-3'
	Reverse 5'-CTTGGGGATGAAGGTGCTTG-3'
β-actin	Forward 5'-GAATGATGAGCCTTCGTGCC-3'
	Reverse 5'-GGTCTCAAGTCAGTGTACAGG-3'

Thermo Fisher Scientific, Inc.), which targets the exons of 409 tumor suppressor genes and frequently cited and mutated oncogenes. DNA concentrations were determined using the TaqMan RNase P Detection Reagents kit (Thermo Fisher Scientific, Inc.). Barcoded amplicon libraries for individual DNA samples were prepared using the Ion Xpress Barcode Adapters and the Ion AmpliSeq Library kit 2.0 (Thermo Fisher Scientific, Inc.) following the manufacturer's protocol. Pooled barcoded libraries were subsequently conjugated with sequencing beads by emulsion PCR and enriched using the Ion PI Hi-Q Chef kit and Ion Chef (Thermo Fisher Scientific, Inc.) according to the Ion Torrent protocol (Thermo Fisher Scientific, Inc.). Sequencing of templates was performed with 8-10 samples per Ion PI Chip V3 using the Ion Proton system (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocols. Sequencing reads generated were aligned with the human genome build 19 (hg19) and mouse genome build 38 (mm10). Reads with an alignment score where mm10 ≥hg19 were considered as reads derived from the mouse genome and subsequently removed. The Variant Caller plugin (version 5.0.2.1; Thermo Fisher Scientific, Inc.) was used to identify variations from the reference sequence (hg19). In the replication study, targeted sequencing was performed in 20 xenografts, including 5 colon cancers, 5 choriocarcinomas, 4 small-cell lung cancers, 4 hematopoietic cancers and 2 ovarian cancers. PolyPhen2 (genetics. bwh.harvard.edu/pph2/) and SIFT (sift.jcvi.org/) were used for the computational prediction of the functional changes that amino acid substitutions may have on protein function. Variants were predicted to be 'benign', 'possibly damaging' or 'probably damaging' by Polyphen2, and 'tolerated' or 'damaging' by SIFT.

Statistical analysis. Xenografts were classified into three groups according to variant allele frequencies (VAFs), low (<10%), middle (10-90%) and high (>90%), and the difference of sensitivity to each anticancer drug (T/C (%)) among the groups was examined using a nonparametric approach (Mann-Whitney U-test for two groups or Kruskal-Wallis test for three groups). To identify genes, which may distinguish patients who may respond to the anticancer drugs, from those who may not, SNVs of which the difference between the maximum and the minimum VAF was <50% were removed from further analysis. $P<8.39 \times 10^{-5}$ (0.05/596) was determined to indicate a statistically significant difference in the replication study for the adjustment of multiple testing by the strict Bonferroni correction. A Pearson correlation coefficient was performed to estimate the association between the gene expression and sensitivity to each anticancer drug. Combination effects were investigated by totaling the score of each VAF group.

Results

Identification of the candidate SNVs associated with chemosensitivity. To identify genetic variants significantly associated with the efficacy of one or more of nine anticancer drugs (5FU, ACNU, ADR, CPM, DDP, MMC, MTX, VCR and VLB) examined in the current nude mice system, all exons of 409 genes associated with cancer using 59 xenografts derived from breast cancer, gastric cancer, neuroblastoma, non-small-cell lung cancer, glioma, pancreatic cancer, ovarian cancer and osteosarcoma at the screening stage were sequenced. A total of 5,494 SNVs were identified in the sequence analysis of the 59 xenografts, and the median number of SNVs called in one sample was 988. A total of 2,206 SNVs with a difference between the maximum and the minimum VAF <50% were removed from further analysis, and 2,087, 2,134, 2,134, 2,134, 2,134, 2,134, 1,944, 2,124 and 2,124 SNVs were assessed for sensitivity to 5FU, ACNU, ADR, CPM, DDP, MMC, MTX, VCR and VLB, respectively. The xenografts were classified into three groups, low (<10%), middle (10-90%) and high (>90%) VAF, and the association between the VAF group and sensitivities to cytotoxic anticancer drugs was assessed using the Kruskal-Wallis test or Mann-Whitney U-test. Chemosensitivity was calculated as T/C and the variants whose allele frequency was higher in xenografts with lower T/C as were defined as 'chemosensitive variants' and variants whose allele frequency were higher in xenografts with higher T/C as 'chemoresistant variants'. As presented in Table II, when 59 xenografts were analyzed in a screening study, 43-98 SNVs exhibited a potential association with sensitivity to the aforementioned 9 drugs. The top 10 variants that revealed the smallest P-values are displayed in Tables III-XI .

In the screening study using 59 xenografts, three SNVs were observed to exhibit associations (P<0.001) with the associated genes; rs1805321 (P=0.00018; Table X) and rs62456182 (P=0.00054; Table X) in PMS1 Homolog 2, Mismatch Repair System Component, and rs13382825 (P=0.00092; Table VIII) in LDL Receptor Related Protein 1B. The three SNVs were associated with sensitivity to MMC and VCR (no. 1 and 2 in Table X), respectively (Tables VIII

Table II. Number of SNVs exhibiting a potential association with sensitivity to 9 anticancer drugs in a screening study of 59 xenografts.

Anticancer drug	SNVs
5FU	61
ACNU	64
ADR	76
CPM	65
DDP	59
MMC	98
MTX	43
VCR	85
VLB	45

SNV, single nucleotide variant; 5FU, 5-fluorouracil; ACNU, nimustine; ADR, adriamycin; CPM, cyclophosphamide; DDP, cisplatin; MMC, mitomycin C; MTX, methotrexate; VCR, vincristine; VLB, vinblastine.

and X). The xenografts with higher VAFs of rs1805321 and rs62456182 demonstrated an increased response to VCR compared with those that exhibited a lower variant allele frequency of the two SNVs (Table X). By contrast, xenografts with higher VAFs of rs13382825 exhibited a decreased response to MMC compared with those that presented with lower variant allele frequencies (Table VIII), suggesting that this genetic variant is associated with resistance to MMC.

Replication study using additional xenografts. To further validate the result of the screening-stage analysis, a replication study was performed, using 596 SNVs showing P<0.05 in ≥1 anticancer drugs in the screening set using independent samples of 20 xenografts. No SNVs revealed significant levels of association in the replication study following Bonferroni correction, including rs1805321, rs62456182 and rs13382825, which demonstrated an association (P<0.001) with VCR (no. 1 and 2; Table X) and MMC (no. 1; Table VIII) in the screening study.

A combined result of the screening and replication studies suggested potential associations of 35 SNVs, which exhibited a stronger association in the combined study than those in screening study, with sensitivity to ≥1 anticancer drugs (Table XII). However, significant association was not observed in these SNVs (0.0011<$P_{combined}$<0.035 in Table XII) following Bonferroni correction. The SNV which revealed the lowest P-value in the combined study was rs79555258 (no. 1 in Table XII) in Activin A Receptor Type 2A (*ACVR2A*; P=0.0011). As presented in Fig. 1, xenografts with more variant alleles of rs79555258 in the three studies (screening, replication and combined) exhibited a lower response to CPM than those with less variant alleles, suggesting that this variant may be associated with resistance to CPM.

Identification of SNVs associated with multi-drug sensitivity. Of the 35 SNVs, that demonstrated a potential association with sensitivity to ≥1 anticancer drugs examined, rs16903989 and rs201432181 (no. 16 and 3, respectively; Table XII) were commonly associated with sensitivity to 2 (VCR and CPM)

and 4 (ACNU, MMC, VLB and ADR) drugs, respectively. Xenografts with more variant alleles in rs16903989, which is located in intron 9 of Leukemia Inhibitory Factor Receptor Alpha (*LIFR*), exhibited a higher response to VCR and CPM ($P_{combined}$=0.0098 and 0.026, respectively; Table XII). The correlation analysis between gene expression and drug sensitivity demonstrated a significantly positive correlation between the expression level of *LIFR* and sensitivity to VCR (*r*=0.42, P=0.00031) and CPM (*r*=0.36, P=0.0020) as presented in Table XII (no. 16). The xenografts with more variant alleles in rs201432181, which is located in exon 19 of *GPR124*, demonstrated a higher response to ACNU, MMC, VLB and ADR ($P_{combined}$=0.0013, 0.0040, 0.017 and 0.029, respectively; no. 3 Table XII), however, no significant association was observed between the expression level of *GPR124* and sensitivity to these 4 cytotoxic anticancer drugs in the present study (ACNU, MMC, VLB and ADR; Table XII).

Combination analysis with markedly associated SNVs with chemosensitivity. A combined effect of markedly associated SNVs with chemosensitivity was investigated ($P_{combined}$<0.01) on sensitivities to ADR, 5FU, ACNU and CPM (Table XII). The xenografts were scored 0, 1 and 2 based on the allele frequency of the chemosensitive variants ($P_{combined}$<0.01) as low (<10%), middle (10-90%), and high (>90%), respectively. Furthermore, the xenografts were scored 2, 1 and 0 depending on the allele frequency of the chemoresistant variants ($P_{combined}$<0.01) as low (<10%), middle (10-90%), and high (>90%), respectively. The xenografts were then classified into 4-6 groups according to the sum of the scores. The combination analysis using rs4589708, rs113962761 and rs1050171 revealed a cumulative effect on sensitivity to ADR (P=0.000012; Fig. 2). Similarly, combination analysis using strongly associated SNVs with sensitivity to 5FU, ACNU and CPM (P<0.01), also revealed a cumulative effect on sensitivity to them (P=0.00025, P=0.000076 and P=0.00021, respectively, data not shown).

Discussion

The present study conducted two-step association studies between frequencies of SNVs in 409 genes (three VAF groups; <10%, 10-90%, >90%) and the sensitivities to 9 cytotoxic anticancer drugs using 79 human cancer xenografts, and identified 35 SNVs with potential associations to sensitivity or resistance to ≥1 cytotoxic anticancer drugs in a combined study. The SNV demonstrating the lowest P-value in the combined study, rs79555258, is located in intron 9 of the *ACVR2A* gene, and tumors with more variant alleles of rs79555258 were demonstrated to be more likely to be resistant to CPM. *ACVR2A* is a receptor for activin A, which is a member of the transforming growth factor-β superfamily of cytokines and a putative tumor suppressor gene that is frequently mutated in microsatellite-unstable colon cancer (29,30). Activin participates in the regulation of cell proliferation, differentiation and migration, DNA damage repair and apoptosis (29,31-33). Although the functional relevance of *ACVR2A* to the sensitivity to CPM remains to be elucidated, the single nucleotide variant (rs79555258) of this gene may be a predictive marker for sensitivity to CPM.

Table III. Single nucleotide variants potentially associated with sensitivity to 5-fluorouracil.

No.	Chr	SNP ID	Position	Gene	Allele Ref./Variant	Sensitivity	Study set	Variant allele frequency			P-value
								<10%	10-90%	>90%	
1	1	rs11121691	11181327	MTOR	C/T	Sensitive	Screening	52	3	1	0.00536
							Replication	19	1	0	NA
							Combined	71	4	1	0.03340
2	14	rs8020503	51239067	NIN	C/G	Sensitive	Screening	25	0	31	0.00668
							Replication	5	1	14	0.67994
							Combined	30	1	45	0.01397
3	2	rs1128919	148657117	ACVR2A	G/A	Sensitive	Screening	15	28	13	0.01129
							Replication	3	6	11	0.23334
							Combined	18	34	24	0.11270
4	7	rs3802064	92731586	SAMD9	A/G	Resistant	Screening	46	8	2	0.01191
							Replication	17	2	1	0.83228
							Combined	63	10	3	0.02524
5	18	-	22642739	ZNF521	A/G	Sensitive	Screening	43	13	0	0.01218
							Replication	19	1	0	NA
							Combined	62	14	0	0.00564
6	7	rs78644495	98552958	TRRAP	G/A	Resistant	Screening	46	10	0	0.01244
							Replication	15	5	0	0.51253
							Combined	61	15	0	0.10691
7	10	rs2435352	43600689	RET	A/G	Resistant	Screening	34	16	6	0.01268
							Replication	11	4	5	0.78343
							Combined	45	20	11	0.02998
8	10	rs11574851	104160959	NFKB2	C/T	Sensitive	Screening	46	9	1	0.01305
							Replication	15	1	4	0.17591
							Combined	61	10	5	0.01631
9	22	rs3818120	41523770	EP300	G/A	Resistant	Screening	47	9	0	0.01354
							Replication	16	3	1	0.04714
							Combined	63	12	1	0.41164
10	22	rs20554	41553259	EP300	G/A	Resistant	Screening	47	9	0	0.01354
							Replication	16	3	1	0.04714
							Combined	63	12	1	0.41164

The top 10 variants that revealed the smallest P-values in the screening study. Chr, chromosome; SNP, single nucleotide polymorphism; SNP ID, rs ID from the NCBI database of genetic variation (dbSNP). '-', this variant is not identified in dbSNP; Ref., reference; NA, not available.

Table IV. Single nucleotide variants potentially associated with sensitivity to nimustine.

No.	Chr	SNP ID	Position	Gene	Allele Ref./Variant	Sensitivity	Study set	Variant allele frequency			P-value
								<10%	10-90%	>90%	
1	19	rs3218066	30,312,874	CCNE1	C/T	Sensitive	Screening	38	18	1	0.00224
							Replication	16	3	1	0.92463
							Combined	54	21	2	0.00379
2	19	rs3218068	30,313,344	CCNE1	T/C	Sensitive	Screening	38	18	1	0.00224
							Replication	16	3	1	0.92463
							Combined	54	21	2	0.00379
3	4	rs7688174	40,244,982	RHOH	C/G	Resistant	Screening	53	1	3	0.00828
							Replication	19	1	0	NA
							Combined	72	2	3	0.02986
4	5	rs6962	256,509	SDHA	G/A	Resistant	Screening	51	6	0	0.01003
							Replication	19	1	0	NA
							Combined	70	7	0	0.00849
5	11	rs5030171	32,449,417	WT1	C/G	Resistant	Screening	12	17	28	0.01085
							Replication	2	6	12	0.92759
							Combined	14	23	40	0.01873
6	11	rs5030170	32,449,420	WT1	C/A	Resistant	Screening	12	17	28	0.01085
							Replication	2	6	12	0.92759
							Combined	14	23	40	0.01873
7	5	rs10039029	251,469	SDHA	G/A	Resistant	Screening	49	7	1	0.01148
							Replication	19	1	0	NA
							Combined	68	8	1	0.01366
8	1	rs76717731	193,107,192	CDC73	C/T	Resistant	Screening	52	5	0	0.01303
							Replication	16	3	1	0.53863
							Combined	68	8	1	0.08246
9	11	rs74662318	4,150,239	RRM1	T/G	Resistant	Screening	48	9	0	0.01422
							Replication	17	3	0	0.56000
							Combined	65	12	0	0.02893
10	5	rs28363396	138,148,036	CTNNA1	A/G	Sensitive	Screening	51	6	0	0.01557
							Replication	18	2	0	0.16531
							Combined	69	8	0	0.20441

The top 10 variants that revealed the smallest P-values in the screening study. Chr, chromosome; SNP, single nucleotide polymorphism; SNP ID, rs ID from the NCBI database of genetic variation (dbSNP). '-', this variant is not identified in dbSNP; Ref., reference; NA, not available.

Table V. Single nucleotide variants potentially associated with sensitivity to adriamycin.

No.	Chr	SNP ID	Position	Gene	Allele Ref./Variant	Sensitivity	Study set	Variant allele frequency			P-value
								<10%	10-90%	>90%	
1	11	rs77233576	44,130,665	EXT2	A/C	Resistant	Screening	51	5	1	0.00115
							Replication	14	5	1	0.43313
							Combined	65	10	2	0.01565
2	9	rs464826	136,913,355	BRD3	T/C	Resistant	Screening	15	15	27	0.00131
							Replication	5	7	8	0.88274
							Combined	20	22	35	0.03060
3	2	rs117225004	141,259,253	LRP1B	T/C	Resistant	Screening	53	3	1	0.00315
							Replication	20	0	0	NA
							Combined	73	3	1	0.00355
4	15	rs2229765	99,478,225	IGF1R	G/A	Resistant	Screening	26	25	6	0.00363
							Replication	10	7	3	0.58702
							Combined	36	32	9	0.01565
5	15	rs2293117	99,478,713	IGF1R	T/C	Resistant	Screening	26	25	6	0.00363
							Replication	10	7	3	0.58702
							Combined	36	32	9	0.01565
6	7	rs113962761	50,450,446	IKZF1	C/T	Resistant	Screening	47	10	0	0.00365
							Replication	19	1	0	NA
							Combined	66	11	0	0.00147
7	5	rs16903089	38,504,303	LIFR	A/T	Sensitive	Screening	28	23	6	0.00509
							Replication	14	5	1	0.59174
							Combined	42	28	7	0.03189
8	1	rs138622243	47,691,061	TAL1	G/T	Sensitive	Screening	54	2	1	0.00591
							Replication	19	0	1	NA
							Combined	73	2	2	0.00764
9	22	rs180812	23,657,735	BCR	G/A	Resistant	Screening	30	3	24	0.00662
							Replication	8	1	11	0.33467
							Combined	38	4	35	0.01663
10	6	rs12196767	51,776,535	PKHD1	T/C	Resistant	Screening	41	15	1	0.00950
							Replication	14	6	0	0.59174
							Combined	55	21	1	0.01484

The top 10 variants that revealed the smallest P-values in the screening study. Chr, chromosome; SNP, single nucleotide polymorphisms; SNP ID, rs ID from the NCBI database of genetic variation (dbSNP). '-', this variant is not identified in dbSNP; Ref., reference; NA, not available.

Table VI. Single nucleotide variants potentially associated with sensitivity to cyclophosphamide.

No.	Chr	SNP ID	Position	Gene	Allele Ref./Variant	Sensitivity	Study set	Variant allele frequency			P-value
								<10%	10-90%	>90%	
1	6	rs4331993	152,793,572	SYNE1	T/A	Resistant	Screening	48	4	7	0.00119
							Replication	16	4	0	0.60273
							Combined	64	8	7	0.00139
2	6	rs1024195	56,507,135	DST	T/C	Sensitive	Screening	24	23	12	0.00188
							Replication	10	6	4	0.27755
							Combined	34	29	16	0.14291
3	2	rs79555258	148,680,526	ACVR2A	T/C	Resistant	Screening	55	3	1	0.00312
							Replication	18	0	2	0.02313
							Combined	73	3	3	0.00109
4	12	rs3217786	4,383,158	CCND2	T/C	Resistant	Screening	24	3	32	0.00378
							Replication	5	0	15	0.12606
							Combined	29	3	47	0.00247
5	14	rs8020503	51,239,067	NIN	C/G	Resistant	Screening	27	0	32	0.00602
							Replication	5	1	14	0.09672
							Combined	32	1	46	0.07844
6	5	rs28363396	138,148,036	CTNNA1	A/G	Sensitive	Screening	53	6	0	0.00675
							Replication	18	2	0	0.84988
							Combined	71	8	0	0.02011
7	18	-	22,642,750	ZNF521	G/C	Resistant	Screening	55	4	0	0.00796
							Replication	19	1	0	NA
							Combined	74	5	0	0.10716
8	7	rs2360885	151,971,043	MLL3	T/C	Resistant	Screening	22	37	0	0.00844
							Replication	0	20	0	NA
							Combined	22	57	0	0.03116
9	3	-	128,202,753	GATA2	G/A	Resistant	Screening	10	49	0	0.00862
							Replication	0	20	0	NA
							Combined	10	69	0	0.01830
10	14	rs1152783	99,642,360	BCL11B	C/G	Resistant	Screening	52	6	1	0.00895
							Replication	16	3	1	0.92461
							Combined	68	9	2	0.12611

The top 10 variants that revealed the smallest P-values in the screening study. Chr, chromosome; SNP, single nucleotide polymorphisms; SNP ID, rs ID from the NCBI database of genetic variation (dbSNP). '-', this variant is not identified in dbSNP; Ref., reference; NA, not available.

Table VII. Single nucleotide variants potentially associated with sensitivity to cisplatin.

No.	Chr	SNP ID	Position	Gene	Allele Ref./Variant	Sensitivity	Study set	Variant allele frequency			P-value
								<10%	10-90%	>90%	
1	18	-	22,642,741	ZNF521	A/G	Resistant	Screening	34	23	0	0.00331
							Replication	11	9	0	0.51842
							Combined	45	32	0	0.02421
2	6	rs2228480	152,420,095	ESR1	G/A	Resistant	Screening	44	8	5	0.00403
							Replication	15	3	2	0.58609
							Combined	59	11	7	0.00419
3	17	rs11653832	5,424,906	NLRP1	C/G	Sensitive	Screening	54	1	2	0.00774
							Replication	19	0	1	NA
							Combined	73	1	3	0.11849
4	17	rs11653580	5,424,991	NLRP1	G/A	Sensitive	Screening	54	1	2	0.00774
							Replication	19	0	1	NA
							Combined	73	1	3	0.11849
5	17	rs56872041	5,433,841	NLRP1	A/G	Sensitive	Screening	54	1	2	0.00774
							Replication	19	0	1	NA
							Combined	73	1	3	0.11849
6	17	rs35596958	5,433,966	NLRP1	T/C	Sensitive	Screening	54	1	2	0.00774
							Replication	19	0	1	NA
							Combined	73	1	3	0.11849
7	17	rs34733791	5,437,285	NLRP1	G/A	Sensitive	Screening	54	1	2	0.00774
							Replication	19	0	1	NA
							Combined	73	1	3	0.11849
8	18	rs79073678	56,414,592	MALT1	T/C	Sensitive	Screening	43	6	8	0.00953
							Replication	15	3	2	0.81174
							Combined	58	9	10	0.02702
9	1	rs1318056	179,112,145	ABL2	C/G	Sensitive	Screening	54	1	2	0.01006
							Replication	18	2	0	0.61429
							Combined	72	3	2	0.05767
10	10	rs755793	123,310,871	FGFR2	A/G	Sensitive	Screening	52	3	2	0.01078
							Replication	18	2	0	0.89974
							Combined	70	5	2	0.03628

The top 10 variants that revealed the smallest P-values in the screening study. Chr, chromosome; SNP, single nucleotide polymorphism; SNP ID, rs ID from the NCBI database of genetic variation (dbSNP). '-', this variant is not identified in dbSNP; Ref., reference; NA, not available.

Table VIII. Single nucleotide variants potentially associated with sensitivity to mitomycin C.

No.	Chr	SNP ID	Position	Gene	Allele Ref./Variant	Sensitivity	Study set	Variant allele frequency			P-value
								<10%	10-90%	>90%	
1	2	rs13382825	141,528,435	LRP1B	T/C	Resistant	Screening	49	9	1	0.00092
							Replication	17	1	2	0.25630
							Combined	66	10	3	0.00793
2	7	rs2230585	100,410,597	EPHB4	G/A	Resistant	Screening	34	14	11	0.00266
							Replication	8	8	4	0.01083
							Combined	42	22	15	0.01284
3	5	rs216123	149,460,553	CSF1R	A/G	Sensitive	Screening	42	13	4	0.00310
							Replication	13	3	4	0.24816
							Combined	55	16	8	0.00728
4	11	rs2295081	32,439,038	WT1	T/C	Resistant	Screening	15	20	24	0.00431
							Replication	3	4	13	0.98644
							Combined	18	24	37	0.04518
5	9	rs686346	135,978,378	RALGDS	T/C	Resistant	Screening	33	16	10	0.00591
							Replication	7	8	5	0.05342
							Combined	40	24	15	0.00397
6	11	rs16754	32,417,945	WT1	T/C	Resistant	Screening	16	20	23	0.00723
							Replication	20	0	0	NA
							Combined	36	20	23	0.01174
7	14	rs17111401	81,528,412	TSHR	T/A	Sensitive	Screening	42	9	8	0.00758
							Replication	17	1	2	0.63346
							Combined	59	10	10	0.04605
8	18	-	22,642,750	ZNF521	G/C	Resistant	Screening	55	4	0	0.00794
							Replication	19	1	0	NA
							Combined	74	5	0	0.09450
9	7	rs56173078	100,420,155	EPHB4	A/G	Sensitive	Screening	55	3	1	0.00907
							Replication	20	0	0	NA
							Combined	75	3	1	0.01301
10	5	rs2229992	112,162,854	APC	T/C	Resistant	Screening	6	19	34	0.00954
							Replication	2	6	12	0.48368
							Combined	8	25	46	0.06409

The top 10 variants that revealed the smallest P-values in the screening study. Chr, chromosome; SNPs, single nucleotide polymorphism; SNP ID, rs ID from the NCBI database of genetic variation (dbSNP). '-', this variant is not identified in dbSNP; Ref., reference; NA, not available.

Table IX. Single nucleotide variants possibly associated with sensitivity to methotrexate.

No.	Chr	SNP ID	Position	Gene	Allele Ref./Variant	Sensitivity	Study set	Variant allele frequency			P-value
								<10%	10-90%	>90%	
1	2	rs62154469	100,209,627	AFF3	C/T	Sensitive	Screening	38	10	2	0.00146
							Replication	14	3	1	0.07029
							Combined	52	13	3	0.15022
2	18	-	22,642,744	ZNF521	A/G	Resistant	Screening	28	22	0	0.00317
							Replication	11	7	0	0.68283
							Combined	39	29	0	0.01315
3	9	rs4489420	139,418,260	NOTCH1	A/G	Sensitive	Screening	2	9	39	0.00457
							Replication	2	0	16	0.20492
							Combined	4	9	55	0.02555
4	19	rs1048290	10,600,442	KEAP1	G/C	Sensitive	Screening	22	11	17	0.00778
							Replication	5	4	9	0.01099
							Combined	27	15	26	0.03367
5	1	rs4870	2,488,153	TNFRSF14	A/G	Sensitive	Screening	35	11	4	0.00822
							Replication	9	5	4	0.06420
							Combined	44	16	8	0.04414
6	6	rs7747060	56,476,262	DST	T/C	Resistant	Screening	28	17	5	0.01127
							Replication	12	3	3	0.80779
							Combined	40	20	8	0.04457
7	6	rs17215781	152,570,274	SYNE1	A/G	Sensitive	Screening	47	3	0	0.01305
							Replication	18	0	0	NA
							Combined	65	3	0	0.02790
8	19	rs273269	18,279,638	PIK3R2	T/C	Sensitive	Screening	1	2	47	0.01342
							Replication	0	0	18	NA
							Combined	1	2	65	0.01020
9	5	rs75732095	149,495,537	PDGFRB	G/A	Sensitive	Screening	28	15	7	0.01376
							Replication	12	3	3	0.94673
							Combined	40	18	10	0.07319
10	15	rs316618	41,796,498	LTK	T/A	Resistant	Screening	47	3	0	0.01383
							Replication	17	0	1	NA
							Combined	64	3	1	0.00644

The top 10 variants that revealed the smallest P-values in the screening study. Chr, chromosome; SNP, single nucleotide polymorphism; SNP ID, rs ID from the NCBI database of genetic variation (dbSNP). '-', this variant is not identified in dbSNP; Ref., reference; NA, not available.

Table X. Single nucleotide variants potentially associated with sensitivity to vincristine.

No.	Chr	SNP ID	Position	Gene	Allele Ref./Variant	Sensitivity	Study set	Variant allele frequency			P-value
								<10%	10-90%	>90%	
1	7	rs1805321	6,026,988	PMS2	G/A	Sensitive	Screening	24	15	17	0.00018
							Replication	10	6	4	0.93002
							Combined	34	21	21	0.00172
2	7	rs62456182	6,038,722	PMS2	T/C	Sensitive	Screening	22	18	16	0.00054
							Replication	10	6	4	0.93002
							Combined	32	24	20	0.00372
3	1	rs2453056	120,477,998	NOTCH2	C/A	Resistant	Screening	52	3	1	0.00293
							Replication	20	0	0	NA
							Combined	72	3	1	0.00437
4	17	rs1136201	37,879,588	ERBB2	A/G	Resistant	Screening	47	8	1	0.00386
							Replication	14	4	2	0.21126
							Combined	61	12	3	0.01309
5	7	rs2228006	6,026,775	PMS2	T/C	Sensitive	Screening	1	4	51	0.00508
							Replication	1	3	16	0.29807
							Combined	2	7	67	0.57955
6	3	rs3732565	134,968,232	EPHB1	C/T	Sensitive	Screening	49	7	0	0.00927
							Replication	18	1	1	0.84994
							Combined	67	8	1	0.06335
7	1	rs5277	186,648,197	PTGS2	C/G	Sensitive	Screening	50	5	1	0.01139
							Replication	18	2	0	0.70514
							Combined	68	7	1	0.01819
8	9	rs2290889	93,639,849	SYK	G/A	Sensitive	Screening	50	5	1	0.01183
							Replication	19	1	0	NA
							Combined	69	6	1	0.02838
9	3	rs762803844	71,247,577	FOXP1	G/T	Sensitive	Screening	45	11	0	0.01185
							Replication	20	0	0	NA
							Combined	65	11	0	0.04620
10	5	rs16903989	38,504,303	LIFR	A/T	Sensitive	Screening	27	23	6	0.01225
							Replication	14	5	1	0.09051
							Combined	41	28	7	0.00983

The top 10 variants that revealed the smallest P-values in the screening study. Chr, chromosome; SNPs, single nucleotide polymorphisms; SNP ID, rs ID from the NCBI database of genetic variation (dbSNP). '-', this variant is not identified in dbSNP; Ref., reference; NA, not available.

Table XI. Single nucleotide variants potentially associated with sensitivity to vinblastine.

No.	Chr	SNP ID	Position	Gene	Allele Ref./Variant	Sensitivity	Study set	Variant allele frequency			P-value
								<10%	10-90%	>90%	
1	5	rs351855	176,520,243	FGFR4	G/A	Resistant	Screening	22	22	12	0.00337
							Replication	8	6	6	0.08904
							Combined	30	28	18	0.00225
2	18	-	22,642,741	ZNF521	A/G	Resistant	Screening	33	23	0	0.00613
							Replication	11	9	0	0.42416
							Combined	44	32	0	0.08059
3	18	rs79073678	56,414,592	MALT1	T/C	Sensitive	Screening	42	6	8	0.00676
							Replication	15	3	2	0.89077
							Combined	57	9	10	0.05146
4	3	-	37,067,095	MLH1	A/T	Sensitive	Screening	49	7	0	0.00960
							Replication	9	11	0	0.34137
							Combined	58	18	0	0.15800
5	1	rs117505788	6,535,149	PLEKHG5	A/G	Resistant	Screening	52	3	1	0.01140
							Replication	18	2	0	0.84983
							Combined	70	5	1	0.20339
6	9	rs16909898	98,231,008	PTCH1	A/G	Resistant	Screening	46	8	2	0.01377
							Replication	18	1	1	0.34380
							Combined	64	9	3	0.01214
7	9	rs1805155	98,238,379	PTCH1	A/G	Resistant	Screening	46	8	2	0.01377
							Replication	18	1	1	0.34380
							Combined	64	9	3	0.01214
8	9	rs28448271	98,239,730	PTCH1	G/A	Resistant	Screening	46	8	2	0.01377
							Replication	18	1	1	0.34380
							Combined	64	9	3	0.01214
9	11	rs77233576	44,130,665	EXT2	A/C	Resistant	Screening	50	5	1	0.01647
							Replication	14	5	1	0.62003
							Combined	64	10	2	0.59593
10	3	rs59684491	37,067,097	MLH1	A/T	Sensitive	Screening	49	6	1	0.01677
							Replication	13	6	1	0.96834
							Combined	62	12	2	0.08250

The top 10 variants that revealed the smallest P-values in the screening study. Chr, chromosome; SNPs, single nucleotide polymorphisms; SNP ID, rs ID from the NCBI database of genetic variation (dbSNP). '-', this variant is not identified in dbSNP; Ref., reference; NA, not available.

Table XII. Summary of results for screening and replication study of 35 single nucleotide variants associated with sensitivity to cytotoxic anticancer drugs.

No.	Drug	Chr	SNP ID	Position	Gene	Allele Ref./variant	Feature	Polyphen2 (Score)	SIFT (Score)	Sensitivity	Study set	<10%	10-90%	>90%	P-value	r[c]	P-value[d]
1	CPM	2	rs79555258	148,680,526	ACVR2A	T/C	Intron 9			Resistant	Screening	55	3	1	0.00312	-0.02	0.85
											Replication	18	0	2	0.02313		
											Combined	73	3	3	0.00109		
2	ACNU	1	rs3218625	186,643,541	PTGS2	C/T	Exon 10 (G587R)	Benign (0.012)	Tolerated (0.43)	Sensitive	Screening	55	2	0	0.04147	-0.30	0.15
											Replication	17	3	0	0.00807		
											Combined	72	5	0	0.00117		
3[a]	ACNU	8	rs201432181	37,699,794	GPR124[a]	A/T	Exon 19 (D1313V)	Possibly damaging (0.664)	Tolerated (0.12)	Sensitive	Screening	55	2	0	0.02030	0.14	0.47
											Replication	18	2	0	0.02319		
											Combined	73	4	0	0.00126		
	MMC	8	rs201432181	37,699,794	GPR124[a]	A/T	Exon 19 (D1313V)	Possibly damaging (0.664)	Tolerated (0.12)	Sensitive	Screening	57	2	0	0.02117	-0.28	0.15
											Replication	18	2	0	0.18538		
											Combined	75	4	0	0.00404		
	VLB	8	rs201432181	37,699,794	GPR124[a]	A/T	Exon 19 (D1313V)	Possibly damaging (0.664)	Tolerated (0.12)	Sensitive	Screening	54	2	0	0.03044	-0.16	0.42
											Replication	18	2	0	0.84983		
											Combined	72	4	0	0.01706		
	ADR	8	rs201432181	37,699,794	GPR124[a]	A/T	Exon 19 (D1313V)	Possibly damaging (0.664)	Tolerated (0.12)	Sensitive	Screening	55	2	0	0.03933	0.31	0.11
											Replication	18	2	0	0.61416		
											Combined	73	4	0	0.02917		
4	ADR	7	rs113962761	50,450,446	IKZF1	C/T	Intron 5			Resistant	Screening	47	10	0	0.00365	-0.07	0.54
											Replication	19	1	0	NA		
											Combined	66	11	0	0.00147		
5	CPM	2	rs2020910	48,030,692	MSH6	T/A	Exon 5 (T1102T)			Sensitive	Screening	39	18	2	0.04828	0.20	0.09
											Replication	12	6	2	0.03822		
											Combined	51	24	4	0.00243		
6	CPM	12	rs3217786	4,383,158	CCND2	T/C	Exon 1 (3'UTR)			Resistant	Screening	24	3	32	0.00378	0.02	0.84
											Replication	5	0	15	0.12606		
											Combined	29	3	47	0.00247		
7	ADR	7	rs1050171	55,249,063	EGFR	G/A	Exon 20 (Q787Q)			Resistant	Screening	37	16	4	0.04670	0.22	0.06
											Replication	16	4	0	0.01812		
											Combined	53	20	4	0.00288		
8	5FU	18	-	22,642,739	ZNF521	A/G	Intron 7			Sensitive	Screening	43	13	0	0.01218	-0.39	0.002
											Replication	19	1	0	NA		
											Combined	62	14	0	0.00564		

Number of samples in VAF group: <10%, 10-90%, >90%

Prediction of functional effect: Polyphen2 (Score), SIFT (Score)

Expression: r[c], P-value[d]

Table XII. Continued.

No.	Drug	Chr	SNP ID	Position	Gene	Allele Ref/variant	Feature	Polyphen2 (Score)	SIFT (Score)	Sensitivity	Study set	Number of samples in VAF group <10%	10-90%	>90%	P-value	Expression r[c]	P-value[d]
9	ADR	2	rs4589708	29,498,210	*ALK*	A/G	Intron 10			Sensitive	Screening	4	8	45	0.04147	0.37	0.47
											Replication	1	2	17	0.11220		
											Combined	5	10	62	0.00652		
10	MTX	14	rs3730344	105,241,576	*AKT1*	G/A	Intron 5			Sensitive	Screening	47	3	0	0.01466	-0.03	0.86
											Replication	17	1	0	NA		
											Combined	64	4	0	0.00666		
11	5FU	2	rs1863703	219,544,388	*STK36*	A/G	Exon 8 (K295R)	Benign (0.056)	Tolerated (0.35)	Sensitive	Screening	48	8	0	0.02328	0.01	0.91
											Replication	17	1	2	0.06387		
											Combined	65	9	2	0.00724		
12	5FU	2	rs16859180	219,553,468	*STK36*	C/T	Exon 12 (R477W)	Probably damaging (1.000)	Damaging (0.00)	Sensitive	Screening	48	8	0	0.02328	0.01	0.91
											Replication	17	1	2	0.06387		
											Combined	65	9	2	0.00724		
13	5FU	2	rs12993599	219,563,602	*STK36*	G/A	Exon 26 (R1112Q)	Benign (0.071)	Tolerated (1.00)	Sensitive	Screening	48	8	0	0.02328	0.01	0.91
											Replication	17	1	2	0.06387		
											Combined	65	9	2	0.00724		
14	ACNU	5	rs6962	256,509	*SDHA*	G/A	Exon 15 (V657I)	Benign (0.021)	Tolerated (0.62)	Resistant	Screening	51	6	0	0.01003	0.08	0.47
											Replication	19	1	0	NA		
											Combined	70	7	0	0.00849		
15	5FU	1	rs1699760	144,852,545	*PDE4DIP*	C/T	Intron 43			Resistant	Screening	45	11	0	0.01420	-0.16	0.17
											Replication	10	10	0	0.24114		
											Combined	55	21	0	0.00879		
16[b]	VCR	5	rs16903989	38,504,303	*LIFR*[b]	A/T	Intron 9			Sensitive	Screening	27	23	6	0.01225	0.42	0.0003
											Replication	14	5	1	0.09051		
											Combined	41	28	7	0.00983		
	CPM	5	rs16903989	38,504,303	*LIFR*[b]	A/T	Intron 9			Sensitive	Screening	29	24	6	0.04242	0.36	0.002
											Replication	14	5	1	0.09852		
											Combined	43	29	7	0.02571		
17	5FU	1	rs71664012	144,881,666	*PDE4DIP*	C/A	Intron 24			Resistant	Screening	16	40	0	0.02674	-0.16	0.17
											Replication	5	15	0	0.23847		
											Combined	21	55	0	0.01311		
18	MMC	8	rs17847568	30,973,938	*WRN*	C/T	Exon 20 (T781I)	Possibly damaging (0.807)	Damaging (0.02)	Resistant	Screening	57	0	2	0.04210	0.05	0.82
											Replication	19	1	0	NA		
											Combined	76	1	2	0.01375		

Table XII. Continued.

No.	Drug	Chr	SNP ID	Position	Gene	Allele Ref/variant	Feature	Polyphen2 (Score)	SIFT (Score)	Sensitivity	Study set	<10%	10-90%	>90%	P-value	r^c	P-value[d]
19	MMC	7	rs78004519	151,860,023	MLL3	A/G	Exon 43 (S3547P)	Benign (0.033)	Tolerated (0.30)	Resistant	Screening	57	2	0	0.04887	0.13	0.34
											Replication	19	1	0	NA		
											Combined	76	3	0	0.01530		
20	ACNU	8	rs75858201	103,308,010	UBR5	T/C	Exon 29 (K1222K)			Resistant	Screening	54	3	0	0.04531	-0.11	0.37
											Replication	19	1	0	NA		
											Combined	73	4	0	0.01592		
21	MMC	8	rs138106214	90,947,858	NBN	G/A	Intron 15			Resistant	Screening	56	3	0	0.04167	0.13	0.43
											Replication	19	1	0	NA		
											Combined	75	4	0	0.01617		
22	VCR	10	rs148377922	114,901,092	TCF7L2	G/A	Intron 5			Resistant	Screening	48	7	1	0.03119	-0.10	0.40
											Replication	18	2	0	0.20720		
											Combined	66	9	1	0.01649		
23	CPM	8	rs17652171	113,662,583	CSMD3	A/C	Intron 18			Resistant	Screening	52	7	0	0.04256	-0.15	0.21
											Replication	18	2	0	0.37710		
											Combined	70	9	0	0.01711		
24	MMC	18	-	22,642,748	ZNF521	A/C	Intron 7			Resistant	Screening	51	8	0	0.04618	0.07	0.58
											Replication	19	1	0	NA		
											Combined	70	9	0	0.01743		
25	ADR	11	rs10895289	102,199,611	BIRC3	A/T	Intron 1			Sensitive	Screening	51	6	0	0.01991	-0.05	0.86
											Replication	19	1	0	NA		
											Combined	70	7	0	0.01977		
26	MMC	2	rs61749494	60,689,441	BCL11A	T/C	Exon 4 (E202E)			Sensitive	Screening	46	13	0	0.03616	0.07	0.84
											Replication	16	3	1	0.21878		
											Combined	62	16	1	0.01994		
27	VCR	13	rs2491231	28,610,183	FLT3	A/G	Intron 10			Resistant	Screening	14	13	29	0.04949	-0.25	0.12
											Replication	3	4	13	0.19167		
											Combined	17	17	42	0.02057		
28	5FU	14	rs67737119	95,591,070	DICER1	G/A	Intron 8			Resistant	Screening	22	14	20	0.02660	-0.02	0.85
											Replication	9	5	6	0.61376		
											Combined	31	19	26	0.02387		
29	VCR	6	rs8192585	32,188,823	NOTCH4	G/A	Exon 4 (S244L)	Benign (0.002)	Tolerated (0.84)	Sensitive	Screening	51	5	0	0.04130	0.51	0.38
											Replication	18	2	0	0.34416		
											Combined	69	7	0	0.02414		

Table XII. Continued.

No.	Drug	Chr	SNP ID	Position	Gene	Allele Ref./variant	Feature	Polyphen2 (Score)	SIFT (Score)	Sensitivity	Study set	<10%	10-90%	>90%	P-value	r[c]	P-value[d]
30	5FU	1	rs1539243	206,647,787	IKBKE	T/C	Exon 4 (I67I)			Resistant	Screening	2	3	51	0.03643	0.05	0.79
											Replication	0	1	19	NA		
											Combined	2	4	70	0.02733		
31	ADR	17	rs2735611	8,048,283	PER1	G/A	Exon 18 (G749G)			Resistant	Screening	38	17	2	0.03587	-0.13	0.53
											Replication	12	7	1	0.84700		
											Combined	50	24	3	0.02888		
32	DDP	1	rs12037217	85,742,023	BCL10	C/A	Exon 1 (A5S)	Benign (0.000)	Tolerated (0.10)	Resistant	Screening	53	4	0	0.03770	-0.27	0.73
											Replication	18	2	0	0.44969		
											Combined	71	6	0	0.02953		
33	5FU	18	-	22,642,744	ZNF521	A/G	Intron 7			Resistant	Screening	32	24	0	0.03429	-0.39	0.002
											Replication	13	7	0	0.52596		
											Combined	45	31	0	0.03358		
34	CPM	1	rs139822181	144,863,320	PDE4DIP	T/C	Exon 37 (K2028R)	Probably damaging (-0.998)	Damaging (-0.02)	Sensitive	Screening	50	9	0	0.04988	0.11	0.37
											Replication	19	1	1	NA		
											Combined	69	10	0	0.03433		
35	ADR	20	rs62206933	31,023,500	ASXL1	C/T	Exon 13 (H995H)			Resistant	Screening	51	6	0	0.04955	-0.04	0.84
											Replication	18	2	0	0.48819		
											Combined	69	8	0	0.03538		

5FU, 5-fluorouracil; ACNU, nimustine; ADR, adriamycin; CPM, cyclophosphamide; DDP, cisplatin; MMC, mitomycin C; MTX, methotrexate; VCR, vincristine; VLB, vinblastine; Chr, chromosome; SNP ID, rs ID from the NCBI database of genetic variation (dbSNP). '-', this variant is not identified in dbSNP; Ref., reference; NA, not available; [a]variant allele was suggested to cause multidrug sensitive (ACNU, MMC, VLB and ADR); [b]variant allele was suggested to cause multidrug sensitive (VCR and CPM); [c]expression correlation coefficient (r) had been calculated to estimate positive (sensitive) or negative (resistant) correlation between the gene expression level and sensitivity to each anticancer drug; [d]expression P-value, P-value of Pearson correlation coefficient.
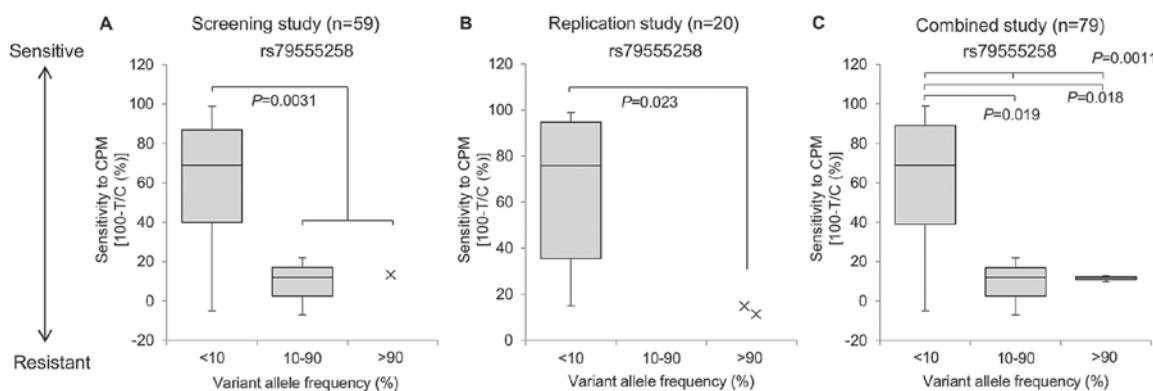
Figure 1. Association between rs79555258 and sensitivity to CPM. The xenografts with higher variant allele frequency in rs79555258 exhibited a lower response to CPM compared with those that presented with a lower variant allele frequency. The (A) screening study, (B) replication study and (C) combined study are presented where the sensitivity to CPM is represented by relative tumor volume of T with respect to C. 'x' represents a single xenograft. Boxes represent the interquartile range (IQR) between first and third quartiles and the line inside represents the median. The whiskers outside the box extend to the highest and lowest value within 1.5 times the IQR. CPM, cyclophosphamide; T, treated mice; C, control.
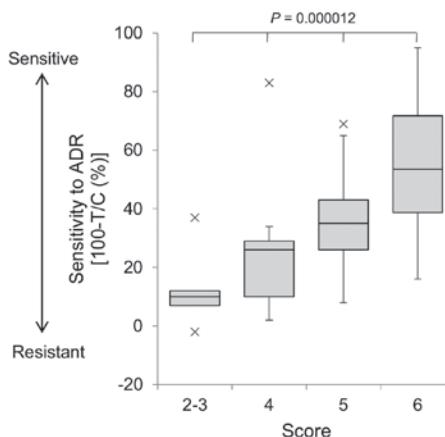
Figure 2. Combined effects of rs4589708, rs113962761 and rs1050171 on sensitivity to ADR. The distribution of ADR sensitivity is presented in the four score groups. The xenografts were classified into four groups based on the sum of the score given to each variant allele frequencies group for the three single nucleotide variants. 'x' represents a single xenograft. Boxes represent the interquartile range (IQR) between first and third quartiles and the line inside represents the median. The whiskers outside the box extend to the highest and lowest value within 1.5 times the IQR. ADR, Adriamycin; T, treated mice; C, control.

rs16903989, which was located in intron 9 of the *LIFR* gene was commonly associated with sensitivity to CPM and VCR. *LIFR* forms a heterodimer with a signal transducer, gp130 and leads to activation of the Janus kinase/signal transducer and activator of transcription and mitogen activated protein kinase cascades (34). *LIFR* has been demonstrated to be downregulated in breast cancer and was identified as a metastasis suppressor (35,36). A single nucleotide polymorphism in *LIFR* (rs3729740) was reported to be a potential predictive marker for sensitivity to a molecular-targeted drug, cetuximab (37). Furthermore, the expression level of *LIFR* was revealed to be associated with sensitivity to VCR in glioblastoma cells (38), and the data of the current study also indicated a positive correlation between the expression level of *LIFR* and sensitivity to VCR. Although the role of *LIFR* in response to anticancer therapy has not yet been clarified, this gene may be associated with a common mechanism

of drug response. The current study also demonstrated that rs201432181 in *GPR124* was typically associated with sensitivity to 4 anticancer drugs (ACNU, ADR, MMC and VLB). rs201432181 is a nonsynonymous substitution (p.D1313 V), and the effect of the substitution on protein function was predicted to be 'possibly damaging' by Polyphen2. *GPR124* is known to regulate vascular endothelial growth factor-induced tumor angiogenesis *in vitro* (39). Therefore, the promotion of tumor angiogenesis by activation of pathway involved with *GPR124* may enhance the delivery of anticancer drugs.

To investigate the tissue specificity of the chemosensitivity-related SNVs identified in the current study, subgroup analysis for breast and gastric cancer xenografts was performed as they included the largest number of tissues (n=12 each) used in the present study. SNVs that were commonly associated with chemosensitivity in the xenografts derived from breast and gastric cancer were identified (rs79555258 for CPM, P=0.031 and 0.086, respectively). By contrast, the study also observed the SNVs associated with chemosensitivity in the xenografts derived from breast cancer, but not in those from gastric cancer.

Of the 409 genes sequenced using CCP in the current study, Excision Repair Cross-Complementation Group 1, Excision Repair Cross-Complementation Group 2, *AKT1* and Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit α have previously been reported to be candidates or promising predictors for sensitivity to cisplatin (40-42). However, no SNVs associated with these genes demonstrated a significant association with cisplatin in the current study, this is potentially because the sample size was too small. Further studies using a large number of xenografts and clinical samples are required to confirm whether they may be a predictive marker for sensitivity to cisplatin clinically.

In conclusion, the present study used 79 human cancer xenografts implanted into nude mice to identify 35 possible genetic variants associated with the sensitivity or resistance to ≥1 anticancer drugs from a total of 9. These findings provide novel insights into personalized selection of chemotherapy for patients with cancer, however; further functional analysis is required to verify the results of the current study and to clarify their biological mechanisms, which have effects on

the clinical outcomes of patients receiving the chemotherapy. Accumulation of data is expected to lead to 'cancer precision medicine' using more effective and less harmful anticancer drugs.

Acknowledgements

References

1. Al-Lazikani B, Banerji U and Workman P: Combinatorial drug therapy for cancer in the post-genomic era. Nat Biotechnol 30: 679-692, 2012.
2. Ou SH, Tong WP, Azada M, Siwak-Tapp C, Dy J and Stiber JA: Heart rate decrease during crizotinib treatment and potential correlation to clinical response. Cancer 119: 1969-1975, 2013.
3. Abubakar MB and Gan SH: Molecular targets in advanced therapeutics of cancers: The role of pharmacogenetics. Oncology 91: 3-12, 2016.
4. Shah DR, Shah RR and Morganroth J: Tyrosine kinase inhibitors: Their on-target toxicities as potential indicators of efficacy. Drug Saf 36: 413-426, 2013.
5. Ranpura V, Pulipati B, Chu D, Zhu X and Wu S: Increased risk of high-grade hypertension with bevacizumab in cancer patients: A meta-analysis. Am J Hypertens 23: 460-468, 2010.
6. Yates LR, Gerstung M, Knappskog S, Desmedt C, Gundem G, Van Loo P, Aas T, Alexandrov LB, Larsimont D, Davies H, *et al*: Subclonal diversification of primary breast cancer revealed by multiregion sequencing. Nat Med 21: 751-759, 2015.
7. Wijdeven RH, Pang B, Assaraf YG and Neefjes J: Old drugs, novel ways out: Drug resistance toward cytotoxic chemotherapeutics. Drug Resist Updat 28: 65-81, 2016.
8. Bedard PL, Hansen AR, Ratain MJ and Siu LL: Tumour heterogeneity in the clinic. Nature 501: 355-364, 2013.
9. Qian CY, Zheng Y, Wang Y, Chen J, Liu JY, Zhou HH, Yin JY and Liu ZQ: Associations of genetic polymorphisms of the transporters organic cation transporter 2 (OCT2), multidrug and toxin extrusion 1 (MATE1), and ATP-binding cassette subfamily C member 2 (ABCC2) with platinum-based chemotherapy response and toxicity in non-small cell lung cancer patients. Chin J Cancer 35: 85, 2016.
10. Fridley BL, Ghosh TM, Wang A, Raghavan R, Dai J, Goode EL and Lamba JK: Genome-wide study of response to platinum, Taxane, and combination therapy in ovarian cancer: In vitro phenotypes, inherited variation, and disease recurrence. Front Genet 7: 37, 2016.
11. Rumiato E, Boldrin E, Malacrida S, Battaglia G, Bocus P, Castoro C, Cagol M, Chiarion-Sileni V, Ruol A, Amadori A and Saggioro D: A germline predictive signature of response to platinum chemotherapy in esophageal cancer. Transl Res 171: 29-37.e1, 2016.
12. Botticelli A, Borro M, Onesti CE, Strigari L, Gentile G, Cerbelli B, Romiti A, Occhipinti M, Sebastiani C, Lionetto L, *et al*: Degradation rate of 5-Fluorouracil in metastatic colorectal cancer: A new predictive outcome biomarker? PLoS One 11: e0163105, 2016.
13. Damerla RR, Chatterjee B, Li Y, Francis RJ, Fatakia SN and Lo CW: Ion Torrent sequencing for conducting genome-wide scans for mutation mapping analysis. Mamm Genome 25: 120-128, 2014.
14. Singh RR, Patel KP, Routbort MJ, Reddy NG, Barkoh BA, Handal B, Kanagal-Shamanna R, Greaves WO, Medeiros LJ, Aldape KD and Luthra R: Clinical validation of a next-generation sequencing screen for mutational hotspots in 46 cancer-related genes. J Mol Diagn 15: 607-622, 2013.
15. Chapman MA, Lawrence MS, Keats JJ, Cibulskis K, Sougnez C, Schinzel AC, Harview CL, Brunet JP, Ahmann GJ, Adli M, *et al*: Initial genome sequencing and analysis of multiple myeloma. Nature 471: 467-472, 2011.
16. Sallman DA, Komrokji R, Vaupel C, Cluzeau T, Geyer SM, McGraw KL, Al Ali NH, Lancet J, McGinniss MJ, Nahas S, *et al*: Impact of TP53 mutation variant allele frequency on phenotype and outcomes in myelodysplastic syndromes. Leukemia 30: 666-673, 2016.
17. Malcovati L, Papaemmanuil E, Bowen DT, Boultwood J, Della Porta MG, Pascutto C, Travaglino E, Groves MJ, Godfrey AL, Ambaglio I, *et al*: Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. Blood 118: 6239-6246, 2011.
18. Verger E, Cassinat B, Chauveau A, Dosquet C, Giraudier S, Schlageter MH, Ianotto JC, Yassin MA, Al-Dewik N, Carillo S, *et al*: Clinical and molecular response to interferon-α therapy in essential thrombocythemia patients with CALR mutations. Blood 126: 2585-2591, 2015.
19. Pratz KW, Sato T, Murphy KM, Stine A, Rajkhowa T and Levis M: FLT3-mutant allelic burden and clinical status are predictive of response to FLT3 inhibitors in AML. Blood 115: 1425-1432, 2010.
20. Kristensen T, Broesby-Olsen S, Vestergaard H, Bindslev-Jensen C and Møller MB; Mastocytosis Centre Odense University Hospital: Serum tryptase correlates with the KIT D816V mutation burden in adults with indolent systemic mastocytosis. Eur J Haematol 91: 106-111, 2013.
21. Du MD, He KY, Qin G, Chen J and Li JY: Adriamycin resistance-associated prohibitin gene inhibits proliferation of human osteosarcoma MG63 cells by interacting with oncogenes and tumor suppressor genes. Oncol Lett 12: 1994-2000, 2016.
22. Kavianpour M, Ahmadzadeh A, Shahrabi S and Saki N: Significance of oncogenes and tumor suppressor genes in AML prognosis. Tumour Biol 37: 10041-10052, 2016.
23. Shin SH, Kim SC, Hong SM, Kim YH, Song KB, Park KM and Lee YJ: Genetic alterations of K-ras, p53, c-erbB-2, and DPC4 in pancreatic ductal adenocarcinoma and their correlation with patient survival. Pancreas 42: 216-222, 2013.
24. Inaba M, Tashiro T, Kobayashi T, Sakurai Y, Maruo K, Ohnishi Y, Ueyama Y and Nomura T: Responsiveness of human gastric tumors implanted in nude mice to clinically equivalent doses of various antitumor agents. Jpn J Cancer Res 79: 517-522, 1988.
25. Zembutsu H, Ohnishi Y, Tsunoda T, Furukawa Y, Katagiri T, Ueyama Y, Tamaoki N, Nomura T, Kitahara O, Yanagawa R, *et al*: Genome-wide cDNA microarray screening to correlate gene expression profiles with sensitivity of 85 human cancer xenografts to anticancer drugs. Cancer Res 62: 518-527, 2002.
26. Ono K, Tanaka T, Tsunoda T, Kitahara O, Kihara C, Okamoto A, Ochiai K, Takagi T and Nakamura Y: Identification by cDNA microarray of genes involved in ovarian carcinogenesis. Cancer Res 60: 5007-5011, 2000.
27. Nomura T, Sakurai Y and Inaba M (eds): The Nude Mouse and Anticancer Drug Evaluation. Central Institute for Experimental Animals, Kawasaki, 1996.
28. Wong ML and Medrano JF: Real-time PCR for mRNA quantitation. Biotechniques 39: 75-85, 2005.
29. Donaldson CJ, Mathews LS and Vale WW: Molecular cloning and binding properties of the human type II activin receptor. Biochem Biophys Res Commun 184: 310-316, 1992.
30. Jung B, Smith EJ, Doctolero RT, Gervaz P, Alonso JC, Miyai K, Keku T, Sandler RS and Carethers JM: Influence of target gene mutations on survival, stage and histology in sporadic microsatellite unstable colon cancers. Int J Cancer 118: 2509-2513, 2006.
31. Loomans HA and Andl CD: Intertwining of activin A and TGFβ signaling: Dual roles in cancer progression and cancer cell invasion. Cancers (Basel) 7: 70-91, 2014.
32. Carlson BC, Hofer MD, Ballek N, Yang XJ, Meeks JJ and Gonzalez CM: Protein markers of malignant potential in penile and vulvar lichen sclerosus. J Urol 190: 399-406, 2013.
33. Massagué J: TGFβ signalling in context. Nat Rev Mol Cell Biol 13: 616-630, 2012.
34. Kishimoto T, Akira S, Narazaki M and Taga T: Interleukin-6 family of cytokines and gp130. Blood 86: 1243-1254, 1995.
35. Chen D, Sun Y, Wei Y, Zhang P, Rezaeian AH, Teruya-Feldstein J, Gupta S, Liang H, Lin HK, Hung MC and Ma L: LIFR is a breast cancer metastasis suppressor upstream of the Hippo-YAP pathway and a prognostic marker. Nat Med 18: 1511-1517, 2012.

36. de la Iglesia N, Konopka G, Puram SV, Chan JA, Bachoo RM, You MJ, Levy DE, Depinho RA and Bonni A: Identification of a PTEN-regulated STAT3 brain tumor suppressor pathway. Genes Dev 22: 449-462, 2008.

37. Kim JC, Kim SY, Cho DH, Ha YJ, Choi EY, Kim CW, Roh SA, Kim TW, Ju H and Kim YS: Novel chemosensitive single-nucleotide polymorphism markers to targeted regimens in metastatic colorectal cancer. Clin Cancer Res 17: 1200-1209, 2011.

38. Balik V, Mirossay P, Bohus P, Sulla I, Mirossay L and Sarissky M: Flow cytometry analysis of neural differentiation markers expression in human glioblastomas may predict their response to chemotherapy. Cell Mol Neurobiol 29: 845-858, 2009.

39. Wang Y, Cho SG, Wu X, Siwko S and Liu M: G-protein coupled receptor 124 (GPR124) in endothelial cells regulates vascular endothelial growth factor (VEGF)-induced tumor angiogenesis. Curr Mol Med 14: 543-554, 2014.

40. Wei HB, Hu J, Shang LH, Zhang YY, Lu FF, Wei M and Yu Y: A meta-analytic review of ERCC1/MDR1 polymorphism and chemosensitivity to platinum in patients with advanced non-small cell lung cancer. Chin Med J (Engl) 125: 2902-2907, 2012.

41. Van Allen EM, Mouw KW, Kim P, Iyer G, Wagle N, Al-Ahmadie H, Zhu C, Ostrovnaya I, Kryukov GV, O'Connor KW, et al: Somatic ERCC2 mutations correlate with cisplatin sensitivity in muscle-invasive urothelial carcinoma. Cancer Discov 4: 1140-1153, 2014.

42. Feldman DR, Iyer G, Van Alstine L, Patil S, Al-Ahmadie H, Reuter VE, Bosl GJ, Chaganti RS and Solit DB: Presence of somatic mutations within PIK3CA, AKT, RAS, and FGFR3 but not BRAF in cisplatin-resistant germ cell tumors. Clin Cancer Res 20: 3712-3720, 2014.