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Mutation analysis of 24 known cancer genes in the NCI-60 cell line set

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Abstract

The panel of 60 human cancer cell lines (the NCI-60) assembled by the National Cancer Institute for anticancer drug discovery is a widely used resource. The NCI-60 has been characterized pharmacologically and at the molecular level more extensively than any other set of cell lines. However, no systematic mutation analysis of genes causally implicated in oncogenesis has been reported. This study reports the sequence analysis of 24 known cancer genes in the NCI-60 and an assessment of 4 of the 24 genes for homozygous deletions. One hundred thirty-seven oncogenic mutations were identified in 14 (*APC, BRAF, CDKN2, CTNNB1, HRAS, KRAS, NRAS, SMAD4, PIK3CA, PTEN, RB1, STK11, TP53,* and *VHL*) of the 24 genes. All lines have at least one mutation among the cancer genes examined, with most lines (73%) having more than one. Identification of those cancer genes mutated in the NCI-60, in combination with pharmacologic and molecular profiles of the cells, will allow for more informed interpretation of anticancer agent screening and will enhance the use of the NCI-60 cell lines for molecularly targeted screens.

Introduction

The NCI-60 cell lines were assembled by the National Cancer Institute as an *in vitro* anticancer drug screen (1-3), which went into operation in 1990. The panel comprises 60 human cancer cell lines representing nine tissue of origin types: breast, colon, central nervous system, renal, lung, melanoma, ovarian, prostate, and hematogenous. More than 100,000 compounds have been screened for anticancer activity against the NCI-60 (chemosensitivity profiles of the NCI-60 cell lines[4] and more refined but less extensive

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[4]http://dtp.nci.nih.gov/docs/cancer/cancer_data.html.

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activity data sets[5] can be accessed online). The resulting data have proved rich in information about the mechanisms of action and resistance of those compounds (4-6). The cells have also been profiled more extensively at the DNA, RNA, protein, chromosomal, and functional levels than any other set of cells (7). For example, DNA copy number changes have been assessed by array-based comparative genomic hybridization (8, 9) and chromosomal aberrations have been catalogued by spectral karyotyping (10). At the DNA sequence level, five known cancer genes have previously been analyzed: *TP53* (11), *KRAS, NRAS*, and *HRAS* (12), and *PIK3CA* (13). RNA expression has been studied on various array-based platforms, and protein expression has been analyzed by two-dimensional gel electrophoresis and by reverse-phase lysate array (7). The various data are being integrated and analyzed, resulting in several leads with possible therapeutic implications (14, 14a). This article and two others in the current issue (14a, 14b) inaugurate *Molecular Cancer Therapeutics*' "Spotlight on Molecular Profiling" series. The data sets have been incorporated into "CellMiner," a searchable relational database for integrative analysis.[5]

Genes encoding protein kinase domains are the most frequently mutated in human cancer (15) and are tractable candidates for therapeutic intervention. The kinase inhibitor imatinib was developed to target the BCR-ABL tyrosine kinase fusion protein in treatment of chronic myelogenous leukemia (16). Response to two other kinase inhibitors, gefitinib and erlotinib, has been linked to activating mutations in the epidermal growth factor receptor (*EGFR*) gene in patients with lung adenocarcinoma (17). There are also promising results from kinase inhibitors targeting the *FLT3* tyrosine kinase receptor in acute myelogenous leukemia (18), in which the gene is frequently mutationally activated. In each of those examples, the acquired mutation renders the cancer cells carrying it more sensitive to the inhibitor. In addition, we have previously identified frequent mutations of the *BRAF* kinase gene in malignant melanoma and other cancers (19), providing the impetus to pursue development of small-molecule inhibitors (20). With respect to nonkinase genes, restoration of wild-type tumor suppressor function is being investigated, as exemplified by the recent use of small-molecule inhibitors of MDM2, a negative modulator of the transcriptional activity and stability of TP53, to restore function to the TP53 pathway (21). However, restoration of tumor suppressor gene function when the gene is inactivated through mutation remains very challenging. It is therefore becoming increasingly clear that understanding the genetics of cancer is key to the further development of targeted therapeutics. Hence, characterization of the genetic abnormalities found in the NCI-60 panel will improve its potential for use in the discovery of new therapies.

Although cancer cell lines are limited, in some instances, with respect to representation of the histopathologic diversity of any given cancer type and may have acquired further genetic events *in vitro*, they are mainstays in drug development programs. As a component of the large-scale systematic sequencing studies being carried out to identify mutations in human cancer by the Wellcome Trust Sanger Institute Cancer Genome Project, we report here the results of sequencing the NCI-60 cell lines for the coding exons and splice junctions of 24 genes causally implicated in oncogenesis. We also report assessment of 4 of the 24 genes for homozygous deletions.

Materials and Methods

Cell Lines

Fifty-nine of the 60 NCI-60 cell lines were kindly provided by the Developmental Therapeutics Program at the National Cancer Institute (Bethesda, MD; Table 1). MDA-N, an

[5] http://discover.nci.nih.gov.

ERBB2 transfectant of MDA-MB-435, was not available at the time of the study because its use was 'restricted' according to the Developmental Therapeutics Program. The cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum and 5 mmol/L L-glutamine. Genomic DNA was extracted using the Qiagen (Hilden, Germany) genomic DNA purification kit.

PCR and Sequencing

PCR primers were designed to amplify the exons and flanking intronic sequences of the 24 cancer genes (Table 2). PCR products were ~500 bp in length, with multiple overlapping amplimers for larger exons (Supplementary Table S1).[6] In total, the coding sequences of the 24 genes covered ~70 kb with PCR amplimers successfully designed for, and sequencing attempted on, 66 kb of the total. PCR amplification of genomic DNA templates and direct sequencing were done as described previously (22).

Sequence Analysis and Confirmation of Putative Variants

Sequence traces were analyzed using a combination of software (Mutation Surveyor and in-house bespoke software) and manual analysis. All putative disease-causing mutations were confirmed by bidirectional sequencing of a second independently amplified PCR product.

Classification of Sequencing Results

The 24 genes screened are commonly mutated in cancer through small intragenic somatic mutations or somatic homozygous deletions[7] or represent plausible drug targets. There are no matched normal DNA samples for the NCI-60 with which to determine the somatic or germ-line nature of the observed variants. We have classified sequence variants into four strata: likely oncogenic mutations, tentative oncogenic variants, variants of unknown significance, and single-nucleotide polymorphisms (SNP). For designation as likely oncogenic mutations, conservative criteria were applied; only those sequence changes that had previously been shown to be somatic mutations in human cancer and/or those consistent with the position and type of mutations for a given gene were included. This class also included homozygous deletions in tumor suppressor genes. Tentative oncogenic variants were those which, though located similarly to known cancer mutations, are different from those previously reported or are present as heterozygous variants in tumor suppressor genes other than missense mutations in *TP53*. All other sequence changes were deemed variants of unknown significance if they were not clearly previously reported SNPs.

Detection of Homozygous Deletions

Exon deletions in *CDKN2A, PTEN, RB1*, and *SMAD4* (*MADH4*) were identified by multiplex PCR. Briefly, PCR primers were designed to amplify exons 1, 2, and 3 of *CDKN2A* together with exon 1 of *ARF*, all 9 exons of *PTEN*, 27 exons of *RB1*, and exons 1 and 3 to 13 of *MADH4*. Control PCR amplimers were designed for β-actin and random intergenic genomic sequences (Supplementary Table S2).[6] PCR products were resolved on 2% agarose gels. All multiplex PCR experiments were done in duplicate.

SNP Genotyping

Cell lines were genotyped for ~10,000 single SNPs using the Affymetrix (Santa Clara, CA) 10K SNP array as described previously (23). The genotype of each cell line was compared with those of the other NCI-60 lines, and a percentage identity score was calculated for each pair of genotypes.[8]

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[6]Supplementary materials for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).
[7]http://www.sanger.ac.uk/genetics/CGP/Census/.

Results/Discussion

More than 60 genes are causally implicated in cancer through the acquisition of somatic small intragenic mutations (15). Twenty-four of those genes were selected for sequence analysis based on mutation frequency and biological interest. In total, 3.9 Mbp of sequence were screened in the 24 genes. Four of the genes are also known to be inactivated frequently by homozygous deletions (*CDKN2A*, 73%; *RB1*, 13%; *SMAD4*, 48%; and *PTEN*, 35%).[9] Therefore, those four genes were also assessed for homozygous deletions. Taking into account point mutations, small insertions/deletions, and homozygous deletions, 14 of 24 cancer genes were found to have likely oncogenic mutations in at least one cell line (*APC, BRAF, CDKN2, CTNNB1, HRAS, KRAS, NRAS, SMAD4, PIK3CA, PTEN, RB1, STK11, TP53,* and *VHL*). Without matched normal DNA from the same individuals, it was not possible to ascertain whether the mutations were somatic, although it is likely that the majority are of somatic origin.

A total of 137 oncogenic mutations were found in the 14 genes (Table 3).[10] *TP53*, the gene most commonly mutated in cancer, had likely oncogenic mutations in 64% (38 of 59) of the cell lines (Table 3). Included was the previously reported large homozygous deletion in HL-60 (24) confirmed via genomic PCR (data not shown). *CDKN2A* single-exon or multiple-exon deletions/point mutations were observed in 56% (33 of 59) of the NCI-60 cell lines. Conversely, mutations were detected only once each in the *HRAS* and *CTNNB1* genes. The number of analyzed cancer genes with likely oncogenic mutations ranged from five in the microsatellite-stable colorectal cancer line HT-29 (*APC, BRAF, SMAD4, PIK3CA*, and *TP53*) to one (*TP53*) in several other lines: the ovarian cancer cell lines OVCAR-3 and OVCAR-4, the lung adenocarcinoma line NCI-H522, and the glioma lines SN12C and SNB-75.

Previously published data on mutations in *KRAS, NRAS, HRAS* (12), and *PIK3CA* (13) for the NCI-60 cell lines are consistent with those in this study. However, with respect to the previously published *TP53* sequence analysis by O'Connor (11), we obtained different results for 9 of the 59 cell lines. Some are annotation differences in the *TP53* data: HS578T has a p.V157F mutation here but p.D157E reported, RPMI-8226 is p.E285K here but has a previous annotation of p.E285L, and SK-MEL-28 is p.L145R here rather than p.C145V (7). In addition, in our analysis, MOLT-4 has a heterozygous *TP53* nonsense mutation (p.R306X) in genomic DNA but no detectable mutation at the cDNA level in the previous study. It is plausible that the mutant *TP53* transcript in MOLT-4 undergoes nonsense-mediated decay and therefore is not detectable in cDNA.

An additional 19 tentative oncogenic variants were identified, including missense variants in the receptor tyrosine kinase genes *EGFR, ERBB2*, and *FLT3*. In addition, a putative splicing mutation in *PDGFRA* was identified in the chronic myelogenous leukemia line K562. The remainder of this class consisted of heterozygous frameshift mutations in tumor suppressor genes found primarily in microsatellite-unstable lines.[11] Of particular interest among these were two different heterozygous frameshift mutations in *BRCA2* in the HCT-15 colorectal cancer cell line. *BRCA2* has not been previously reported to be a target for mutation in microsatellite-unstable cancers. Also included in this category are three heterozygous *TP53* truncating variants and a heterozygous truncating *APC* variant in the KM12 colorectal line. It is likely that a substantial proportion of these heterozygous truncating tumor suppressor

[8] http://www.sanger.ac.uk/genetics/CGP/Genotyping/.
[9] http://www.sanger.ac.uk/genetics/CGP/cosmic/.
[10] http://www.sanger.ac.uk/genetics/CGP/CellLines/.
[11] http://www.sanger.ac.uk/genetics/CGP/MSI/table1.shtml.

gene variants are actually disease causing and that the second allele of the tumor suppressor gene has been inactivated in accordance with the two-hit genetic model. It is possible, for example, that alterations in the second allele have not been detected, given the classes of genetic change that we have not directly addressed (e.g., large rearrangements and promoter methylation) and the fact that it was not possible to sequence every exon in every cell line. All additional data on variants of unknown significance and single SNPs are available in Supplementary Table S3[6] and online.[12]

Based on the resequencing results and genotyping data we generated using Affymetrix 10K SNP Mapping Arrays, there are three pairs among the 59 cell lines analyzed that seem to be derived from the same individuals.[13] Thus, a total of 56 independent cell lines is analyzed in this report. The synonymous pairs are the following: (*a*) "breast" cancer line NCI/ADR-RES and the ovarian cancer OVCAR-8 (9), which have identical *TP53* and *ERBB2* variants and 99% genotype similarity; (*b*) the melanoma line M14 and the "breast" cancer line MDA-MB-435, which have identical *BRAF, CDKN2A*, and *TP53* variants and 97% genotype similarity, with this combination of mutations strongly indicating that MDA-MB-435 is a melanoma (25); and (*c*) two glioma lines SNB-19 and U251, which have identical *TP53, CDKN2A*, and *PTEN* variants and 96% genotype similarity. There is evidence that, though identical at the mutation and genotypic level, those two lines have diverged karyotypically (10).

With respect to the use of the NCI-60 for informing on commonly mutated cancer genes as drug targets, >50% of the NCI-60 are *TP53* mutant. Whereas restoring p53 pathway function in TP53 wild-type cancer cells continues to be a focus of intensive drug development efforts (26), restoring *TP53* function in cells with mutant *TP53* remains challenging. The NCI-60 lines also reflect the mutation patterns seen in the *KRAS* and *NRAS* genes in primary tumors. To date, direct inhibition of activated RAS and hence its downstream effectors has not been effective in cancer therapy. Downstream of RAS there are several BRAF mutations in the NCI-60. Recently, the *BRAF* mutant lines of the NCI-60 have been found to be sensitive to kinase inhibitors of the downstream *BRAF* effector/signaling target mitogen-activated protein/extracellular signal-regulated kinase kinase (14). The mutations in *PIK3CA* and *PTEN* suggest that the panel may be of value for analysis of compounds that target the phosphatidylinositol 3-kinase pathway. *PIK3CA*, a lipid kinase, is a clear target for therapeutic development (13, 27).

Other likely oncogenic mutations of interest included a homozygous *STK11* (*LKB1*) 5-bp deletion in the DU145 prostate cancer cell. Although previous work has implicated *STK11* inactivation in non–small cell lung cancer (28), to our knowledge this is the first report of a mutation in *STK11* in prostate cancer. It will be of interest to extend that observation to a set of primary prostate cancers to determine the prevalence of STK11 inactivation in this common tumor type.

The receptor tyrosine kinases are perhaps the most successfully exploited set of molecular targets in cancer to date. Several family members (*EGFR, ERBB2, FLT3, KIT, MET*, and *PDGFRA*) were included in the set of 24 genes assessed. No mutations identical to those most frequently reported (17) were seen. However, several interesting variants were identified. In *EGFR*, two amino acid substitutions, p.P753S in the SK-MEL-28 melanoma and p.T751I in the RPMI-8226 myeloma line, were identified within the region of the kinase domain frequently affected by in-frame deletions. Both residues are subject to missense substitution as part of more complex deletion/substitution mutations in lung

[12] http://www.sanger.ac.uk/CGP.
[13] http://www.sanger.ac.uk/genetics/CGP/Genotyping/nci60.shtml.

adenocarcinoma.[14] Further investigation of those lines for sensitivity to EGFR inhibitors and the potential role of *EGFR* mutations in a subset of melanoma and myeloma are warranted. An *ERBB2* p.G776V variant was detected in the ovarian cancer line OVCAR-8 (and NCI/ADR-RES). Gly^{776} and the adjacent Val^{777} are somatically mutated in gastric, lung, and colon cancers (29, 30). Recently, an *ERBB2* Gly^{776} mutant non—small cell lung cancer cell line and transformed mouse cells were shown to exhibit *in vitro* sensitivity to a small-molecule ERBB2 kinase inhibitor (31), and there has been a report of clinical response to trastuzumab in a patient with *ERBB2* mutant lung cancer refractory to other treatments (32). Finally, a p.A627T variant in *FLT3* was detected in the CCRF-CEM acute lymphoblastic leukemia cell line. Ala^{627} is just adjacent to the G-loop ATP-binding motif within the kinase domain and is very highly conserved. Internal tandem duplications and point mutations of *FLT3* are frequent in acute myelogenous leukemia.[9]

Sequencing over 1 Mb/case of coding sequence in a series of primary tumors suggests that there are tens of amino acid—changing somatic mutations in most tumors spread across thousands of genes (22). Therefore, every tumor is likely to have a unique somatic mutation pattern in addition to any germ-line variation. Hence, the NCI-60 panel can only contain a small subset of the gene/mutation combinations found in primary tumors. We are continuing our systematic analysis of known cancer genes for mutations in the NCI-60 panel. The work presented here defining the mutation profiles of 24 known cancer genes in the NCI-60 will inform drug development programs and contribute to the growing amount of molecular data on the NCI-60. The data can be analyzed and combined to identify active compounds for further investigation and potential development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

NCI-60 cell lines

Cell line	Tumor type
786-0	renal cell carcinoma
A498	renal cell carcinoma
A549	lung carcinoma
ACHN	renal cell carcinoma
BT-549	breast carcinoma
CAKI-1	renal cell carcinoma
CCRF-CEM	acute lymphoblastic leukaemia
COLO205	colorectal carcinoma
DU-145	prostate carcinoma
EKVX	lung adenocarcinoma
HCC-2998	colorectal carcinoma
HCT-116	colorectal carcinoma
HCT-15	colorectal carcinoma
HL-60	acute myeloid leukaemia
HOP62	lung adenocarcinoma
HOP-92	lung large cell carcinoma
HS578T	breast carcinoma
HT29	colorectal carcinoma
IGROV1	ovarian carcinoma
K-562	chronic myeloid leukaemia
KM12	colorectal carcinoma
LOXIMVI	melanoma
M14	melanoma
MALME-3M	melanoma
MCF7	breast carcinoma
MDA-MB-231	breast carcinoma
MDA-MB-435	melanoma (see text)
MOLT-4	acute lymphoblastic leukaemia
NCI/ADR-RES	ovarian carcinoma (see text)
NCI-H226	lung squamous cell carcinoma
NCI-H23	lung adenocarcinoma
NCI-H322M	lung bronchoalveolar carcinoma
NCI-H460	lung large cell carcinoma
NCI-H522	lung adenocarcinoma
OVCAR3	ovarian carcinoma
OVCAR-4	ovarian carcinoma
OVCAR-5	ovarian carcinoma
OVCAR-8	ovarian carcinoma
PC-3	prostate carcinoma

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Cell line	Tumor type
RPMI-8226	myeloma
RXF393	renal cell carcinoma
SF-268	glioma
SF-295	glioma
SF-539	glioma
SK-MEL-2	melanoma
SK-MEL-28	melanoma
SK-MEL-5	melanoma
SK-OV-3	ovarian carcinoma
SN12C	renal cell carcinoma
SNB19	glioma (see text)
SNB-75	glioma
SR	non Hodgkin lymphoma
SW620	colorectal carcinoma
T47D	breast carcinoma
TK-10	renal cell carcinoma
U251	glioma
UACC-257	melanoma
UACC-62	melanoma
UO-31	renal cell carcinoma

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Table 2

Cancer genes analyzed

Gene symbol	Gene name	National Center for Biotechnology Information gene ID	Likely oncogenic mutations*
APC	Adenomatous polyposis coli	324	8
BRAF	v-raf murine sarcoma viral oncogene homologue B1	673	10
BRCA1	Familial breast/ovarian cancer gene 1	672	0
BRCA2	Familial breast/ovarian cancer gene 2	675	0
CDKN2A	Cyclin-dependent kinase inhibitor 2A, p16	1029	33
CTNNB1	Catenin (cadherin associated protein) β1	1499	1
ERBB2	v-erb-b2 erythroblastic leukemia viral oncogene homologue 2	2064	0
HRAS	v-Ha-ras Harvey rat sarcoma viral oncogene homologue	3265	1
EGFR	Epidermal growth factor receptor	1956	0
FLT3	fms-related tyrosine kinase 3	2322	0
KIT	v-*KIT* Hardy-Zuckerman 4 feline sarcoma viral oncogene homologue	3815	0
KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homologue	3845	11
MAP2K4	Mitogen-activated protein kinase kinase 4	6416	0
MET	met proto-oncogene	4233	0
NRAS	Neuroblastoma RAS viral (v-ras) oncogene homologue	4893	3
PDGFRA	Platelet-derived growth factor receptor, α polypeptide	5156	0
PIK3CA	Phosphoinositide-3-kinase, catalytic, α polypeptide	5290	7
PTEN	Phosphatase and tensin homologue	5728	11
RB1	Retinoblastoma 1	5925	3
RET	ret proto-oncogene	5979	0
SMAD4	SMAD, mothers against DPP homologue 4 (*MADH4*)	4089	2
STK11	Serine/threonine kinase 11/*LKB1* (Peutz-Jehgers syndrome)	6794	4
TP53	Tumor protein p53 (Li-Fraumeni syndrome)	7157	41
VHL	von Hippel-Lindau tumor suppressor	7428	2

*Mutations seen in common ancestor lines counted once.

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Table 3

Mutations/variants identified in 24 cancer genes in the NCI-60

Cell line	Variants identified
786-0	*CDKN2A* Hom c.1_150 del 150, p.? LOM; *PTEN* Hom c.445C>T, p.Q149X LOM; *TP53* Het c.832C>G, P278A c.A560-2A>G, p.? LOM; *VHL* Hom c.311delG p.G105fsX55 LOM
A498	*CDKN2A* Hom c.1_471 del 471, p.? LOM; *VHL* Hom c.426_429delTGAC p.G144fsX14 LOM
A549	*CDKN2A* Hom c.1_471 del 471, p.? LOM; *KRAS* Hom c.34G>A, p.G12S LOM; *STK11* Hom c.109C>T, p.Q37X LOM
ACHN	*CDKN2A* Hom c.1_471 del 471, p.? LOM
BT-549	*RB1* Hom c.265_607 del 343, p.? LOM; *TP53* Hom c.747G>C, p.R249S LOM
CAKI-1	*CDKN2A* Hom c.1_471 del 471, p.? LOM
CCRF-CEM	*CDKN2A* Hom c.1_471 del 471, p.? LOM; *KRAS* Het c.35G>A, p.G12D LOM; *PTEN* Hom c. del 80-492, p.? LOM; *TP53* Het c.743G>A, p.R248Q c.524G>A, p.R175H LOM; *FLT3* Het c.1879G>A p.A627T TOV
COLO-205	*APC* Hom c.4666_4667insA p.T1556fsX3 LOM; *BRAF* Het c.1799T>A, p.V600E LOM; *SMAD4* Hom del exon1-6 LOM; *TP53* Hom c.308_333>TA, p.Y103fsX37 LOM
DU145	*CDKN2A* Hom c.250G>T p.D84Y LOM; *RB1* Hom c.2143A>T, p.K715X LOM; *STK11* Hom c.532_536delAAGCC p.K178fsX86 LOM; *TP53* Het c.820G>T, p.V274F LOM
EKVX	*TP53* Hom c.609_610GG>TT, p.E204X LOM
HCC2998	*APC* Het c.1994T>A, p.L665X c.4348C>T, R1450X LOM; *RB1* Het c.409G>T, p.E137X TOV; *TP53* Het c.637C>T, p.R213X TOV
HCT-116	*CDKN2A* Het c.68_69insG p.R24fsX20 c.220delG p.E74fsX15 (p14) LOM; *CTNNB1* c.133_135 del TCT, p.S45 del LOM; *KRAS* Het c.38G>A, p.G13D LOM; *PIK3CA* Het c.3140A>G, p.H1047R LOM; *BRCA2* Het c.8021_8022insA p.I2675fsX6 TOV
HCT-15	*APC* Het c.6496C>T,p.R2166X Hom c.4248delC p.I1417fsX2 LOM; *KRAS* Het c.38G>A, p.G13D LOM; *PIK3CA* Het c. 1633G>A p. E545K LOM; *TP53* Het c.C1101-2A>C, p.? C722T, S241F LOM; *BRCA2* Het c.3599_3600delGT p.C1200fsX1 c.5351delA p.N1784fsX7 TOV
HL-60	*CDKN2A* Hom c.238C>T p.R80X LOM; *NRAS* Het c.182A>T, p.Q61L LOM; *TP53* Hom deletion LOM
HOP62	*CDKN2A* Hom c.1_471 del 471, p.? LOM; *KRAS* Het c.34G>T, p.G12C LOM; *TP53* Hom c.G673-2A>G, p.? LOM
HOP-92	*CDKN2A* Hom c.1_471 del 471, p.? LOM; *TP53* Hom c.524G>T, p.R175L LOM
Hs-578-T	*CDKN2A* Hom c.1_471 del 471, p.? LOM; *HRAS* Het c.35G>A p.G12D LOM; *TP53* Hom c.469G>T, p.V157F LOM
HT-29	*APC* Het c.2557G>T p.E853X c.4666_4667insA p.T1556fsX3 LOM; *BRAF* Het c.1799T>A, p.V600E LOM; *SMAD4* Hom c.931C>T, p.Q311X LOM; *PIK3CA* Het c.1345C>A p.P449T LOM; *TP53* Hom c.818G>A, p.R273H LOM
IGROV-1	*TP53* Het c.377A>G, p.Y126C LOM; *BRCA1* Het c.1961delA p.K654fsX47 TOV; *SMAD4* Het c.692delG p.G231fsX10 TOV; *PTEN* Het c.955_958delACTT p.T319fsX1 TOV
K-562	*CDKN2A* Hom c.1_471 del 471, p.? LOM; *TP53* Hom c.406_407insC p.Q136fsX13 LOM;

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Cell line	Variants identified
	PDGFRA Het Exon 10 +1 G>A p.? TOV
KM12	*PTEN* Het c.385G>T, p.G129X c.800 del A, p.K267fsX9 LOM; *APC* Het c.5454_5455 ins A, p.N1819fsX7 TOV; *TP53* Het c.215delG, p.R72fsX51 TOV; *BRCA2* Het c.5351delA p.N1784fsX7 TOV
LOXIMVI	*BRAF* Het c.1799T>A, p.V600E LOM; *CDKN2A* Hom c.1_471 del 471, p.? LOM
M14/MDA-MB-435	*BRAF* Het c.1799T>A, p.V600E LOM; *CDKN2A* Het c.150+2 T>C p.? c. 456-+24 AGgtgaggactgatgatctgagaattt >C p.? LOM; *TP53* Het c.797G>A, p.G266E LOM
MALME-3M	*BRAF* Het c.1799T>A, p.V600E LOM; *CDKN2A* Hom c.1_471 del 471, p.? LOM
MCF7	*CDKN2A* Hom c.1_471 del 471, p.? LOM; *PIK3CA* Het c.1633G>A p.E545K LOM
MDA-MB-231	*BRAF* Het c.1391G>T, p.G464V LOM; *CDKN2A* Hom c.1_471 del 471, p.? LOM; *KRAS* Het c.38G>A p.G13D LOM; *TP53* Hom c.839G>A, p.R280K LOM
MOLT-4	*CDKN2A* Hom c.1_471 del 471, p.? LOM; *NRAS* Het c.34G>T p.G12C LOM; *PTEN* Hom c.800delA p.K267fsX9 LOM; *STK11* Het c.640C>T p.Q214X TOV; *TP53* Het c.916C>T, p.R306X TOV
NCI-H226	*CDKN2A* Hom c.1_150 del 150, p.? LOM
NCI-H23	*KRAS* Het c.34G>T, p.G12C LOM; *STK11* Hom c.996G>A, p.W332X LOM; *TP53* Hom c.738G>C, p.M246I LOM
NCI-H322M	*TP53* Hom c.743G>T, p.R248L LOM
NCI-H460	*CDKN2A* Hom del 1_457 del 457, p.? LOM; *KRAS* Hom c.183A>T, p.Q61H LOM; *PIK3CA* Het c.1633G>A p.E545K LOM; *STK11* Hom c.109C>T p. Q37X LOM
NCI-H522	*TP53* Hom 572delC, p.P191fsX56 LOM
OVCAR-3	*TP53* Hom c.743G>A, p.R248Q LOM
OVCAR-4	*TP53* Hom c.388C>G, p.L130V LOM
OVCAR-5	*CDKN2A* Hom c.1_471 del 471, p.? LOM; *KRAS* Hom c.35G>T, p.G12V LOM
OVCAR-8/NCI/ADR-RES	*TP53* Hom c.376-1G>A, p.? LOM; *ERBB2* Het c.2327G>T p.G776V TOV
PC-3	*PTEN* Hom c.165-1026 del 862, p.? LOM; *TP53* Hom c.414delC p.K139fsX31 LOM
RPMI-8226	*KRAS* Het c.35G>C, p.G12A LOM; *TP53* Hom c.853G>A, p.E285K LOM; *EGFR* Het c.2252C>T p.T751I TOV
RXF393	*CDKN2A* Hom c.1_471 del 471, p.? LOM; *PTEN* Hom c.1_164 del 164, p.? LOM; *TP53* Hom c.524G>A, p.R175H LOM
SF-268	*CDKN2A* Hom c.1_471 del 471, p.? LOM; *TP53* Hom c.818G>A, p.R273H LOM
SF-295	*CDKN2A* Hom c.1_471 del 471, p.? LOM; *PTEN* Hom c.697C>T, p.R233X LOM; *TP53* Hom c.743G>A, p.R248Q LOM
SF539	*RB1* Hom c.346_349delACTT p.T116fsX8 LOM; *TP53* Hom c.1024delC p.R342fsX3 LOM; *PTEN* Hom c.1-1026 del 1026, p.? LOM
SK-MEL-2	*NRAS* Hom c.182A>G, p.Q61R LOM; *TP53* Het c.733G>A, p.G245S LOM
SK-MEL-28	*BRAF* Hom c.1799T>A, p.V600E LOM; *TP53* Hom c.435_436G>GT, p.L145R LOM; *EGFR* Hom c.2257C>T p.P753S TOV

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Cell line	Variants identified
SK-MEL-5	*BRAF* Het c.1799T>A, p.V600E LOM; *CDKN2A* Hom c.1_471 del 471, p.? LOM
SK-OV-3	*CDKN2A* Hom del 1_457 del 457, p.? LOM; *PIK3CA* Het c.3140A>G, p.H1047R LOM; *TP53* Hom c. del267C p.S90fsX33 LOM; *APC* Het c.4666delA p.T1556fsX9 TOV
SN12C	*TP53* Hom c.1006G>T, p.E336X LOM
SNB-75	*TP53* Hom c.772G>A, p.E258K LOM
SR	*CDKN2A* Hom c.1_471 del 471, p.? LOM
SW620	*KRAS* Hom c.35G>T, p.G12V LOM; *TP53* Hom c.818G>A, p.R273H C925T, p.P309S LOM; *APC* Hom c.4012C>T, p.Q1338X LOM
T47D	*PIK3CA* Het c.3140A>G, p.H1047R LOM; *TP53* Hom c.580C>T, p.L194F LOM
TK10	*TP53* Het c.791T>G, p.L264R LOM
U251/SNB-19	*CDKN2A* Hom c.1_471 del 471, p.? LOM; *PTEN* Hom c.723_724insTT p.E242fsX15 LOM; *TP53* Hom c.818G>A, p.R273H LOM
UACC-257	*BRAF* Het c.1799T>A, p.V600E LOM
UACC-62	*BRAF* Hom c.1799T>A, p.V600E LOM; *CDKN2A* Hom c.1_471 del 471, p.? LOM; *PTEN* Hom c.741_742insA p.P248fsX5 LOM
UO-31	*CDKN2A* Hom c.1_471 del 471, p.? LOM

Abbreviations: Hom, homozygous; Het, heterozygous; del, deletion; ins, insertion; LOM, likely oncogenic mutation; TOV, tentative oncogenic variant.