의학석사 학위논문

Characterization of Seven Human Hepatocellular Carcinoma Cell Lines

인체 간암 세포주 7종의 특성 분석

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서울대학교 대학원

의과학과 의과학 전공

노 우 리

Abstract

Hepatocellular carcinoma (HCC) continues to secure its spot among top ranks in cancer-related deaths worldwide. Known for its difficulty in management, studies to find effective treatments for HCC are on-going. To assist further *in vitro* study, seven HCC cell lines, SNU-1517, SNU-2655, SNU-2658, SNU-2663, SNU-3058, SNU-3059, and SNU-3160, derived from Korean patients were characterized in this paper. Growth rate varied between HCC cell lines where the slowest growing cell line had doubling time of 74.59 hours and the fastest growing cell line had its doubling time of 42.93 hours. DNA finger printing analysis was carried out for authentication of cell lines. Targeted sequencing was performed for detection of mutations of genes within comprehensive cancer panel. Frame shift deletions, insertions, in frame deletions and insertions, missense, nonsense mutations, start codon deletion and splice site mutations of various genes were found and classified based on their pathogenicity reports. Also, some of these mutations were validated by Sanger sequencing. Also this study confirms that only three (SNU-1517, SNU-2655 and SNU-3058) out of 7 HCC cell lines were shown to have integrated HBV DNA in their genome and were identified via Sanger sequencing. In addition, cell viability assay was carried out to test the sensitivity to three anti-cancer drugs widely used for HCC, 5-fluorouracil (5-FU), cisplatin, and

sorafenib. Among these three chemotherapeutic agents, sorafenib is the most prominent drug known to be used in HCC regimen. Therefore, proteins involved in this pathway were tested for change in gene expression. Phospho-ERK (p-ERK) expression was examined to confirm that sorafenib successfully inhibited Ras-Raf-MEK-ERK signaling pathway. For SNU-1517, SNU-2663, and SNU-3160 cell lines, sorafenib clearly made difference in p-ERK level. However, on the other HCC cell lines, effect of sorafenib was difficult to determine since SNU-2655, 2658, and 3058 had very low basal p-ERK level to verify sorafenib inhibition, and SNU-3059 barely showed any change in expression level after sorafenib treatment. In addition to sorafenib, four phytochemicals, baicalein, curcumin, genistein and resveratrol were tested to evaluate both their individual and synergetic anti-cancer effect when partnered with sorafenib. All phytochemicals were shown to have effect on all HCC cell lines at certain concentrations, although their synergetic effect with sorafenib was ambiguous and demands further study.

Key words: hepatocellular carcinoma, liver cancer cell lines, sorafenib, hepatitis B virus, phytochemicals, characterization

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

Hepatocellular carcinoma (HCC) is one of the most notorious types of cancer known for its high mortality rate. In United States, liver and intrahepatic bile duct cancers were listed fifth for cancer related death estimates for 2016 [1]. A statistical study shows that it is the main cause for death of middle aged Korean male population in their 50's [2]. A predicted death estimates caused by liver originated cancer in South Korea for 2017 is 8,128 for male, and 2,929 for female population [3]. Frequency of HCC incidence is concentrated in Southeast Asia and sub-Saharan Africa. Causes for HCC vary between geographical regions from liver fluke to HBV infection. HBV infection is the predominant cause in Southeast Asia. Alcohol intake and HCV infection was the main cause of HCC development in other regions such as Southern Europe [4]. Statistically, approximately 5% of world population is known to be infected by chronic hepatitis B virus. Among those infected population, about 25% develop chronic hepatitis, cirrhosis, and HCC [5]. Some common therapy to HCC includes surgical resection, liver transplantation, radiofrequency ablation, and sorafenib treatment [6]. Although there are many other chemotherapy regimen involving other drugs such as 5-FU and cisplatin [7], sorafenib is currently

the only prominent anti-cancer drug used in clinics for HCC treatment. Although the precise map of sorafenib mechanism still needs to be constructed, it is known to be a multi-kinase inhibitor targeting RAF kinases of Ras-Raf-MEK-ERK signaling pathway [8]. Sorafenib is also known to hinder angiogenesis, proliferation and induce apoptosis of tumor cells [9]. Various researches are ongoing to develop more effective therapy for HCC including combination drug study involving phytochemicals. Phytochemicals are natural compounds that has been a topic of interest for many years and some are reported to have anti-cancer effect. Curcumin is one of the most studied natural compounds isolated from turmeric. Its effect against hepatotoxic aflatoxin has been reported [10, 11]. Resveratrol is another well-known phytochemical found in wine, grapes and certain kinds of berries and it is noted for anti-invasive effect and decreased HCC proliferation [12]. Potential anti-cancer effect of other phytochemical agents such as baicalein and genistein are also being studied where baicalein had proven to disrupt HCC proliferation [13] and genistein was reported to have protective effect against liver fibrosis through TGF- β/Smad pathway inhibition [14]. Although opinions of anti-cancer effect of phytochemicals are conflicting, the consistent reports which demonstrates their effects

against various cancers show promising future of phytochemicals in cancer therapy. According to Cancer Cell Line Encyclopedia (CCLE) and Korean Cell Line Bank (KCLB), there are 31 established HCC cell lines that are currently being used in researches around the world, and 12 of them are known to be derived from Korean patients. In this study, 7 newly established HCC cell lines, SNU-1517, SNU-2655, SNU-2658, SNU-2663, SNU-3058, SNU-3059, and SNU-3160 are characterized to inspect their traits, growth properties, DNA finger printing, targeted sequencing, hepatitis B expression and their genotyping were done. Furthermore, the cell viability tests were carried out on the treatments of three anti-cancer drugs, 5-FU, cisplatin, and sorafenib, to test the cell line's sensitivity to this anti-cancer drug. Responses to phytochemical agents were also tested, both individually and in combination with sorafenib for their synergetic effect.

Material and methods

Cell culture

Seven human HCC cell lines (SNU-1517, SNU-2655, SNU-2658, SNU-2663, SNU-3058, SNU-3059, and SNU-3160) were established from tumor tissues acquired from Korean HCC patients. SNU-1517, SNU-2655, SNU-2658 and SNU-2663 were established by Korean Cell Line Bank (KCLB, Seoul, Korea), and SNU-3058, SNU-3059 and SNU-3160 were deposited from Liver Research Institute (Seoul, Korea). Established cell lines were cultured in the incubator conditioned at 37 degree Celsius with 5% CO_2 in RPMI-1640 media (Thermo fisher, Massachusetts, United States) with 10% Fetal Bovine Serum (Thermo fisher). Cells were passaged each time of trypsin (Thermo fisher) treatment.

Growth properties and morphology *in vitro*

For growth properties, 2.0x10^3cells were seeded on each well of ten 96 well plates. One of the ten plates were treated with EZ-cytox (DAEIL Lab, Seoul, South Korea), the water soluble tetrazolium salt (WST) solution that gets reduced by succinate-tetrazolium reductase and produce formazan dye,

and incubated at 37℃ for 2 hrs for its optical density (OD) measurement 2 hours after cell seeding. The rest of plates were treated with EZ-cytox treated every day for 13 days. Growth curve and growth properties were drawn and calculated using Graphad Prism software with normalized OD values.

Nucleic acid isolation and cDNA synthesis

Genomic DNA (gDNA) was extracted using DNA Mini Prep Kit (Qiagen, California, USA) following manufacturer's protocol. RNA was isolated using RNeasy mini kit (Qiagen) following manufacturer's protocol and cDNA was synthesized using Reverse Transcription Kit (Qiagen).

DNA fingerprinting

DNA fingerprinting was proceeded with extracted gDNA. Quantified and diluted gDNA solution was added to reaction mixture consisted of Amp FISTR PCR reaction mix, Taq DNA polymerase, and Amp FISTR identifier primer set (Applied Biosystem, California, USA). Then the sequence is amplified by GeneAmp PCR System 9700 (Applied Biosystem) with annealing temperature set to 59℃. 0.05μl of Gene Scan-500 Rox standard

and 9 µl of Hi-Di Formamide (Applied Biosystem) were added to 1 µl of PCR product of each cell line and denatured at 95℃ for 2minutes. This mixture was then analyzed by 3500xL Genetic Analyzer (Applied Biosystem).

Sanger sequencing

PCR product was precipitated by sodium acetate buffer and ethanol mixed solution. Then washed product was set on ice for 10 minutes and centrifuged at 4℃, 14000 rpm. Supernatant was discarded and the product was rinsed this time by 70% ethanol and centrifuged 14000 rpm. Supernatant was discarded then the products were dried using vacuum concentrator (Eppendorf, Hamburg, Germany). 10µl of distilled water was added to dilute precipitated sample. When the product is all diluted in distilled water, cyclic PCR was carried out. Two separate mixtures for forward and reverse sequences were made where they each include 5X sequencing buffer (Applied Biosystem), Big Dye (Applied Biosystem), forward or reverse primer, distilled water, and product from previous step. Cyclic PCR was carried out with denaturation step at 96℃, annealing temperature at 55℃, and elongation at 60℃ for 25 cycles. The cyclic PCR product was then

precipitated with sodium acetate buffer and ethanol mixed solution and set on ice for 10 minutes then it was centrifuged at 4℃ and supernatants were carefully discarded and the final product was dried using the vacuum concentrator. 10μl Hi-Di formamide was added to dilute the dried product. This final product was transferred to 96 well PCR plate and denatured at 95℃ for 2 minutes before taken to 3500xL Genetic Analyzer for sequencing.

HBV PCR and genotyping

For HBV core gene PCR screening, HBV 1μl of gDNA of each cell lines were amplified in 14μl PCR mixture containing 1.5μl of 10X PCR buffer with MgCl2, 0.5 μl of dNTP, 0.25 μl of forward primer (5'-AAGCTGTGCCTTGGGTGGCTT-3'), 0.25μl of reverse primer (5'-CGAGATTGAGATCTTCTGCGAC– 3') [15], and 0.08μl of Taq DNA polymerase (Intron Biotechnology, Kyung-gi, South Korea) was proceeded using GeneAmp PCR System 9700 (Applied Biosystem). Each PCR cycle was set with denaturation step at 94℃ annealing temperature at 61℃, and elongation at 72℃ for 30 cycles. HBV positive SNU cell line SNU-354 was used as positive control. TP53 was used as an internal control gene to solidify the PCR result. PCR products were processed for Sanger sequencing with

method described above in Sanger Sequencing section. Identified forward and reverse nucleotide sequences were aligned to acquire most possible nucleotide sequences, then the final sequences were inputted in HBV Blast Search database to identify their HBV genotypes. HBV surface proteins, different sets of primers were used [16] with same PCR process described above but with annealing temperature of 55℃.

Targeted sequencing

Sequencing process was carried out using Ion Proton sequencer (Thermo Fisher) and comprehensive cancer panel. Sequencing data was analyzed and sorted based on the frequently mutated gene list found in Cancer Cell Line Encyclopedia (CCLE). Clinical significance of sorted mutations were inquired from NCBI (National Center for Biotechnology Information) database, and mutations that were reported to have clinical significance were noted as benign, likely benign, pathogenic, likely pathogenic, potential risk factor, not provided, or conflicting interpretation of pathogenicity. Sorted mutation list for each cell lines were also grouped by its mutation types and the number of each types were represented as pie chart for convenient observation of this data.

Some mutations identified by targeted sequencing, ERBB2 c.(1963-1965)Atc>Gtc (p.I655V), FGFR4 c.(1162-1164)Ggg>Agg (p.G388R), TP53 c.(322-324)ggtfs (p.G108fs), TP53 c.(535-537)cAt>cGt (p.H179R), TP53 c.(634-636)tttfs (p.F212fs), and TP53 c.(841-843)Gac>Tac (p.D281Y) were validated of by PCR followed by Sanger sequencing. Primer sequences used for this process are listed in Table 1. PCR was proceeded with the same method as described above. ERBB2 c.(1963-1965)Atc>Gtc (p.I655V) and TP53 c.(841-843)Gac>Tac (p.D281Y) mutations were carried out as reverse transcription PCR using cDNA, with annealing temperature of 60℃ for both sets of primers. FGFR4 c.(1162-1164)Ggg>Agg (p.G388R), TP53 c.(322-324)ggtfs (p.G108fs), TP53 c.(535-537)cAt>cGt (p.H179R), and TP53 c.(634-636)tttfs (p.F212fs) PCR was run as traditional PCR with gDNA, with annealing temperature of 63℃, 60℃, 62℃, and 61℃ respectively. Sanger sequencing was proceeded as described above in the Sanger sequencing section.

Table 1. PCR primer list

Name	Primer (5' to 3')	Annealing temperature (℃)	Reference
ERBB2 c.1963A>G_F	GCACCCACTCCTGTGTGGAC	60	
ERBB2 c.1963A>G_R	TGCCAAAAGCGCCAGATCCA		
FGFR4 c.1162G>A_F	CGAGGCCAGGTATACGGACA	63	
FGFR4 c.1162G>A_R	CAAAGGCCTCTGCACGTACT		
TP53 c.323delG_F	CTGGTCCTCTGACTGCTCTT	60	
TP53 c.323delG_R	AGGCATTGAAGTCTCATGGA		
TP53 c.536A>G_F	ATGTGTTCACTTGTGCCCTG	62	
TP53 c.536A>G_R	AACCAGCCCTGTCGTCTCTC		
TP53 c.636delT_F	AGGGTCCCCAGGCCTCTGAT	61	
TP53 c.636delT_R	CACCCTTAACCCCTCCTCCC		
TP53 c.841G>T_F	GTGGTGGTGCCCTATGAGCC	60	
TP53 c.841G>T_R	AGGAGCTGGTGTTGTTGGGC		
HBV(C)_F	AAGCTGTGCCTTGGGTGGCTT	61	[15]
HBV_(C)_R	CGAGATTGAGATCTTCTGCGAC		
HBV_(S)_F	TCACCATATTCTTGGGAACAAGA	55	[16]
HBV_(S)_R	CGAACCACTGAACAAATGGC		

Cell proliferation assay to commonly used chemotherapeutic agents

To test sensitivity to 5-FU, cisplatin, and sorafenib of each cell line, cell proliferation assay was carried out. For SNU-1517, SNU-2655, SNU-2658, SNU-3059 and SNU-3160, 4.0x10^3cells/well were seeded on 96 well plate. 8.0x10^3cells/well solution was seeded for SNU-2663 and 1.2x10^4cells were seeded each well for SNU-3058. On the following day, 5-FU, cisplatin, and sorafenib was treated with maximum concentration of 40mM, 5mM, and 20μM respectively and were serially diluted. Drug treated plates were then incubated at 37℃ with 5% CO_2 for 72 hours. After 72 hours of incubation, 10μl of EZ-cytox solutions were added and were incubated for 2 hours to measure cell viability. After incubation, optical density values were measured at 450 nm wavelength using Skanit software. Drug response graph and EC50 value was calculated by Graphpad Prism.

For time dependency test of sorafenib on HCC cell lines, 7.92x10^4 cells/well were seeded on 96 well plates. On the following day, 20μM of sorafenib was treated and each cell lines were treated. OD values were

measured instantly for 0 hour control, then after 3, 6, 9, 12 hours after the sorafenib treatment. Cell proliferation assay was carried out after each time periods using EZ-cytox (DAEIL Lab) and OD values were measured by Multiskan FC Microplate reader (Thermo Fisher), and Skanit for Multiskan FC 3.1 Software.

Similar to the time dependency test, for the concentration dependent sorafenib effect on HCC cell lines, 7.92x10^4 cells/well were seeded on 96 well plates. On the following day, 10μM, 50μM, 100μM of sorafenib was treated and each concentration was treated as triplicates for the accuracy of the experiment. Dimethyl Sulfoxide (DMSO) was also treated as blank control. Sorafenib and DMSO treated cells were then incubated at 37℃ with 5% CO_2 for 6 hours. Optical density was measured with method described for time dependence test. All tests were proceeded as triplicates.

Protein Extraction and Western blotting

To test the change in protein expression after sorafenib treatment of HCC cell lines, 4.0 x 10^5 cells/ml cell solution of each cell lines were seeded on four 60π dish with media. Each dishes for cell lines were treated with DMSO as control, 20μM, 50μM, 100μM of sorafenib then incubated for 6

hours at 37℃ with 5% CO_2. Cell pellets were collected after 6 hours of drug treatment by scraper then rinsed with PBS. Collected pellets were suspended in RIPA lysis and extraction buffer (Atto, Tokyo, Japan) and kept in ice for 15 minutes. The lysates were then centrifuged at 13,000 rpm for 30 minutes at 4℃. Supernatants were collected and concentration was measured by SMART micro BCA protein assay kit (Intron biotechnology). Western blotting was carried out, protein mixture containing 5 μg of extracted protein, SDS buffer (Invitrogen, California, USA), reducing buffer (Invitrogen) and distilled water was boiled at 98 ℃ for 5 minutes then loaded on a Mini-PROTEAN TGX Precast Gels (Bio-Rad) for three and half hours at 50 volt. Then loaded protein was transferred using Trans-Blot Turbo Transfer Packs and Trans-Blot Turbo Transfer System (Bio-Rad, California, USA). Protein transferred membrane was trimmed and shaken at room temperature inside skim milk solution containing 2% skim milk powder (BD Biosciences, New Jersey, USA), 1mM $MgCl_2$, 10% TBS buffer, and 0.2% Tween 20 (VWR Life Science, Pennsylvania, USA) for 1 hour. After blocking, membrane was incubated in primary antibodies which were diluted at ratio of 1:2000 for p-ERK (1/2) (Abcam, Cambridge, UK), 1:1000 for ERK2 (Abcam), 1:200 for K-ras (Abcam), and beta-actin (Santa Cruz Biotechnology, Texas, USA).

Three antibodies except for p-ERK (1/2) were incubated for one hour at room temperature and P-ERK (1/2) was incubated for 2 hours followed by washing process. Then the membrane was incubated in enzyme conjugated secondary antibody (Jackson ImmunoResearch, Pennsylvania, USA) diluted with the ratio of 1:5000 for 1 hour. Membrane was washed then was treated with Sensido ECL Substrate (Recenttec, Taipei, Taiwan) and taken to the darkroom to acquire image on a x-ray film.

Phytochemicals

Four phytochemicals, baicalein, curcumin, genistein, and resveratrol, (Sigma, St. Louis, Missouri, USA) were tested in this study. Same protocol used in sorafenib sensitivity test was carried out to evaluate the sensitivity of HCC cell lines to the phytochemicals.

Combination index

Along with the individual responses to the HCC cell lines, four phytochemicals were also paired up with sorafenib, to test the synergetic effect. For SNU-1517, SNU-2655, SNU-2658, SNU-3059 and SNU-3160, 4.0x10^3 cells/well were seeded as triplicate on 96 well plate. 8.0x10^3

cells/well were seeded for SNU-2663 and 1.2x10^4 cells/well were seeded for SNU-3058. 24 hours after the cell seeding, sorafenib was treated horizontally so its concentration gradate from right to left, and each phytochemicals were treated vertically on 96 well plates so the concentration gradate from bottom to top. Sorafenib was treated with concentration ranged between 3.3x10^-2μM to 1μM. Curcumin concentration ranged between 2.8x10^-2μM to 2.5 μM. Resveratrol was treated with minimum concentration of 3.6x10^-2 μM to maximum concentration of 2 μM. For baicalein, minimum concentration of 3.6x10^-3 μM to 0.2 μM was treated and genistein was treated with concentration between 7.8x10^-5 μM to 2.5x10^-3 μM for all cell lines. OD values were measured after 72 hours of drug treatment via EZ-Cytox assay and combination index and graphs were acquired using CompuSyn software.

Results

Morphology *in vitro* and growth properties of HCC cell lines.

To study the morphology of HCC cell lines, magnified cell images were acquired using Axiovert 100 microscope at 100x magnification (Figure 1). All 7 HCC cell lines, SNU-1517, SNU-2655, SNU-2658, SNU-2663, SNU-3058, SNU-3059, and SNU-3160 grew adherent to the culture flasks with SNU-1517, SNU-2658, and SNU-3059 cell lines showed spindle morphology and the other cell lines showed polygonal morphology.

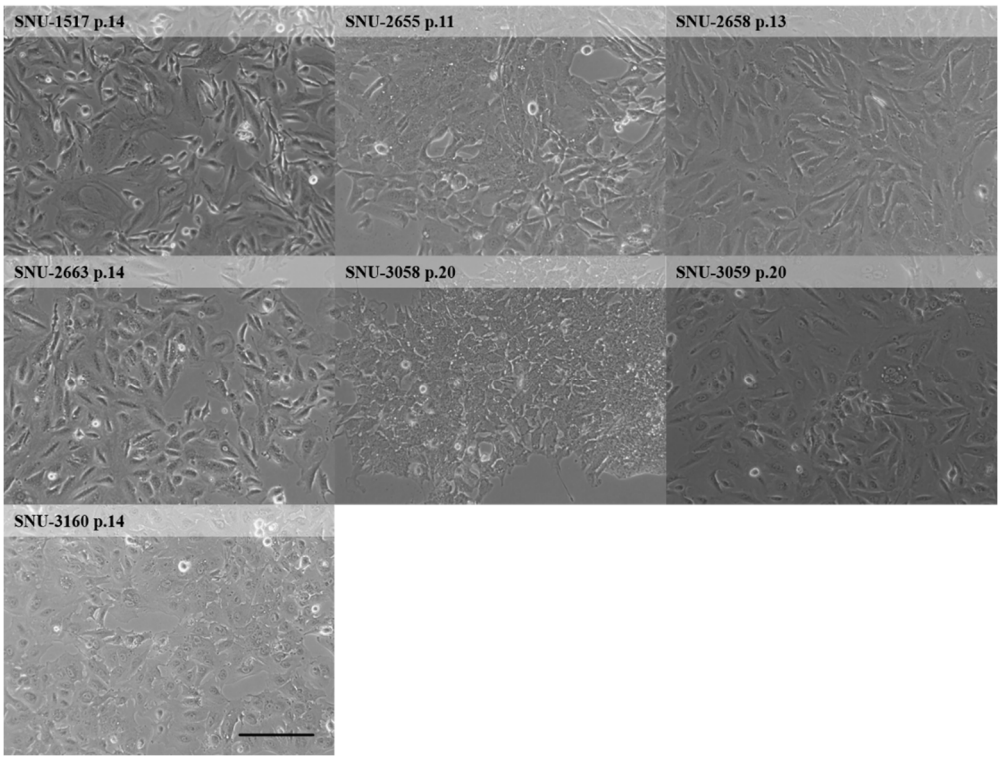

Figure 1. ***in vitro*** **morphology of hepatocellular carcinoma cell lines.**

Cell images were acquired at 100x magnification. Scale bar added indicates 100μm in length. P indicates passage of each cell lines.

For identification of growth properties, each cell lines were seeded on 96 well plates and measured for OD values each day for 13 days. Only the data from day 0 to day 9 were inputted for growth rate calculation since after 9^{th} day, all the cell lines seems to encounter their plateau phase and their OD values disrupted the data of 0 to 9 day period. The growth curve was drawn (Figure 2) and doubling times were calculated by Graphpad software using equation $Y=Start \cdot e^{K \cdot x}$. Y represents the normalized OD value, Start is the starting OD value, K is the growth rate and x is the hours of incubation. Among 7 HCC cell lines, SNU-3058 had highest growth rate of K=0.01614, therefore had steep growth curve and SNU-3160 had lowest growth rate with K value of 0.009293. K values for the rest of cell lines were 0.01166 for SNU-1517, 0.01588 for SNU-2655, 0.01343 for SNU-2658, 0.01334 for SNU-2663, 0.01614 SNU-3058, 0.01096 for SNU-3059. Doubling times for SNU-1517, SNU-2655, SNU-2658, SNU-2663, SNU-3058, SNU-3059, and SNU-3160 were calculated to be 59.45, 43.64, 51.61, 51.95, 42.93, 63.26, and 74.59 hours respectively (Table 2).

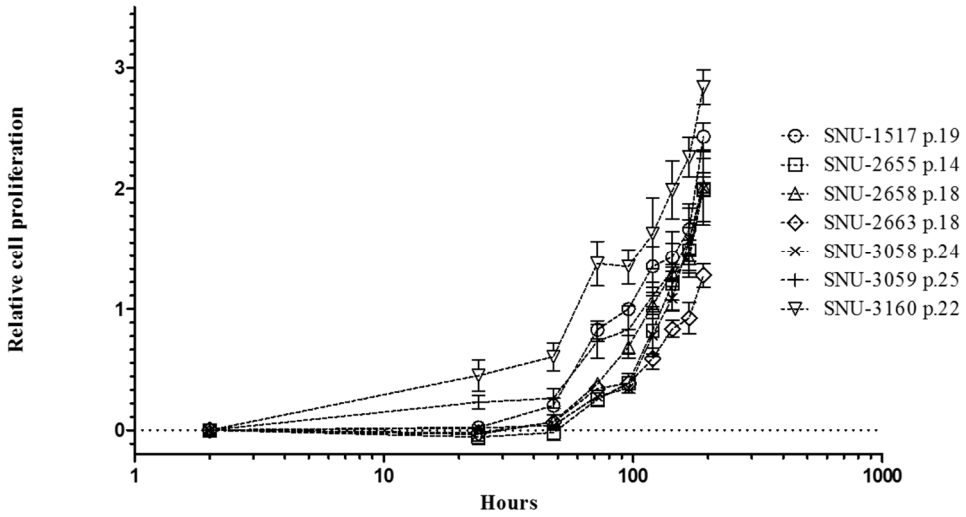

Figure 2. Growth curve of HCC cell lines. X axis shows hours of incubation in log scale, and Y axis shows normalized optical density value. According to this data, among 7 HCC cell lines, SNU-3058 had highest growth rate (K=0.01614), and SNU-3160 was shown to lowest growth rate (K=0.009293).

Table 2. Differentiation level, growth pattern, and doubling time of 7 HCC cell lines.

Cell line name and passage	Differentiation	Cell morphology	Growth pattern	Doubling Time
SNU-1517 p.19		Spindle	Adherent	59.45
SNU-2655 p.14		Polygonal	Adherent	43.64
SNU-2658 p.18		Spindle	Adherent	51.61
SNU-2663 p.18		Polygonal	Adherent	51.95
SNU-3058 p.24	WHO grade G2 (moderately differentiated)	Polygonal	Adherent	42.93
SNU-3059 p.25	WHO grade G2 (moderately differentiated)	Spindle	Adherent	63.26
SNU-3160 p.22	WHO grade G2 (moderately differentiated)	Polygonal	Adherent	74.59

DNA fingerprinting of 7 HCC cell lines.

Total of 16 loci including D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, Vwa, TPOX, D18S51, D5S818, FGA, and Amelogenin for gender identification. Result of DNA fingerprinting showed no cross contamination between each cell lines (Table 3).

Table 3. DNA fingerprinting analysis using 16 STR loci for 7 HCC cell lines

Loci	SNU-1517	SNU-2655	SNU-2658	SNU-2663	SNU-3058	SNU-3059	SNU-3160
D8S1179	14,15	13,14	14	11	13,15	10,15	14,15
D21S11	29,30	30	29,30	29,32.2	31	29	31
D7S820	10	8,10	10,11	10	9,10	8,11	10,12
CSF1PO	9	10,12	9,15	10,12	12	9,12	10,11
D3S1358	15,16	15,16	15,17	15,16	16,17	17	14,17
TH01	9	7	6,8	9	6,7	6	7
D13S317	11	11,14	8	11,12	8	9	8
D16S539	9,12	12	9	9,13	12	12	10,12
D2S1338	18	18,24	19,22	20,25	20	23,24	23
D19S433	14	13,14	13.2,16.2	13,13.2	13,14.2	13	12,13
Vwa	16,18	14,16	14	16,18	14,18	18	15,17
TPOX	8,11	8,11	8,11	9,12	8,11	8,11	9
D18S51	12,17	14,16	15,19	15	13,14	15	13,16
Amelogenin	X,Y	X	X,Y	X	X,Y	X,Y	X
D5S818	13	10,12	10,11	9,10	10,13	11	9,11
FGA	23	19	23	19,23	25	21,23	22,24

HBV PCR and genotyping

Among 7 SNU cell lines tested for HBV DNA integration only 3 cell lines, SNU-1517, SNU-2655 and SNU-3058, were shown to have integrated HBV DNA. HBV core gene PCR bands appear at size 563bp and surface gene PCR showed its bands at size 1,063bp (Figure 3). This result is interesting since all 12 SNU HCC cell lines previously established from Korean patients were HBV positive. Nucleotide sequences for HBV core gene PCR products SNU-2655 and SNU-3058 were identified via Sanger sequencing. Found sequences were then put into HBV sequence database called HBV Blast Search (http://bioafrica.net/blast/hbvblast.html) for genotyping. The result for both SNU-2655 and SNU-3058 cell lines yielded reference sequence name of M12906.1 which indicates HBV subtype C by 96% and 97% match in nucleotide sequences respectively (Figure 4A, B).

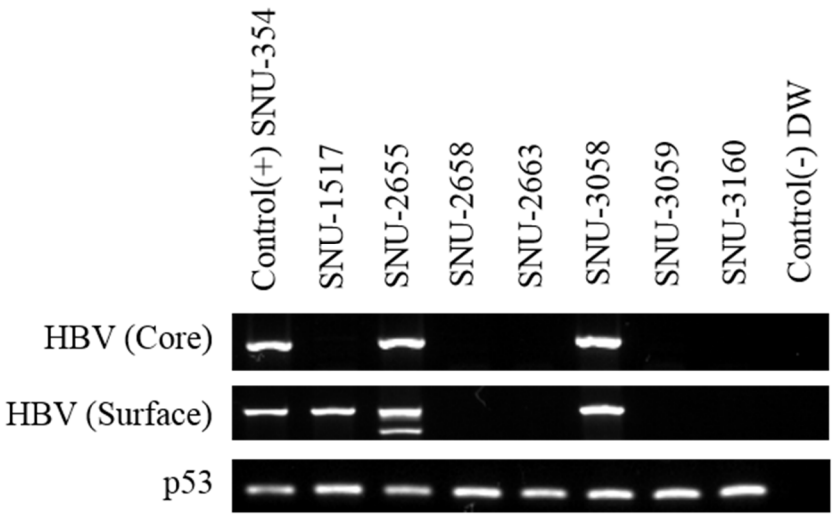

Figure 3. HBV PCR result for HCC cell lines. HBV core and surface gene PCR was carried out with annealing temperature at 61℃ and 55℃ respectively with 30 repeated cycles each. Out of 7 HCC cell lines tested, only SNU-2655 and SNU-3058 were shown to contain HBV core gene DNA. Three cell lines, SNU-1517, SNU-2655, and SNU-3058 showed integrated HBV surface gene DNA. SNU-354 was used as positive control, and p53 was used as an internal control.

(A)

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Query: 1    tgacccgtataaagaatttggagcttctgtggagttactctctttttttgccttctgacttt 60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1909 tgacccgtataaagaatttggagcttctgtggagttactctctttttttgccttctgacttt 1968

Query: 61   ctttccttcaattcgaggatctcctcgacaccgcctctgctctgtatcgggaggccttag 120
            ||||||||| ||||||| ||||||||||||||||||||||||||||||||||||||||||
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Query: 121  agtctccggaacattgttcacctcaccatacagcactcaggcaagctatcctgtgttggg 180
            |||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct: 2028 agtctccggaacattgttcacctcaccatacagcactcaggcaagctattctgtgttggg 2087

Query: 181  gtgagttgatgaatctggccacctgggtgggaagtaacttggaagacccagcatccaggg 240
            |||||||||||||| ||||||||||||||||||||| ||||||||||||||||||||||
Sbjct: 2088 gtgagttgatgaatttggccacctgggtgggaagtaatttggaagacccagcatccaggg 2147

Query: 241  aattagttagtcagctatgtcaatgttaatatgggcctaaaantcagacaaatattgtgg 300
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Query: 361  ggagtgtggattcgcactcctacccctacagaccaccaaatgcccctatcttatccaac 420
            |||||||||||||||||||| || ||||||||||||||||||||||||||||| ||||
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Query: 421  acttccggaaacttgtgttgttagacaacgaggcaggtcccctagaagaagaactccctc 480
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Query: 481  gcctcgcagacgaag 495
            |||||||||||||||
Sbjct: 2386 gcctcgcagacgaag 2400
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(B)

```
Query: 1    ttttttgccttctgacttctttccttctattcgagatctcctcggacaccgcctctgctc 60
            |||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct: 1951 ttttttgccttctgacttctttccttctattcgagatctcctcg-acaccgcctctgctc 2009

Query: 61   tgtatcgggaggccttagagtctccggaacattgttcacctcaccatacagcactcaggc 120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 2010 tgtatcgggaggccttagagtctccggaacattgttcacctcaccatacagcactcaggc 2069

Query: 121  aagatattctgtgttggggtgagttgatgaatctggccacctgggtgggaagtaatttgg 180
            ||| ||||||||||||||||||||||||||||||| ||||||||||||||||||||||||
Sbjct: 2070 aagctattctgtgttggggtgagttgatgaatttggccacctgggtgggaagtaatttgg 2129

Query: 181  aagacccagcatccagggaattantagtcagctatgtcaatgttaatatgggcctaanaa 240
            |||||||||||||||||||| |||||||||||||||||||||||||||||||||| ||
Sbjct: 2130 aagacccagcatccagggaattagtagtcagctatgtcaatgttaatatgggcctaaaaa 2189

Query: 241  tcagacnactattgtggtttcacatttcctgtcttacttttggaagnagaaactgtttct 300
            |||||| |||||||||||||| |||||||||||||||||||| |||||||| ||||
Sbjct: 2190 tcagacaactattgtggtttcatatttcctgtcttacttttggaag-agaaactg-ttct 2247

Query: 301  tgagtatttggtgtcttttggagtgtggattcgcactcctcccgcttacagaccaccaaa 360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 2248 tgagtatttggtgtcttttggagtgtggattcgcactcctcccgcttacagaccaccaaa 2307

Query: 361  tgcccctatcttatcaacactttccggaaactactgttgttagacgacgaggcaggtccc 420
            |||||||||||||||||||| |||||||||||||||||||||||||||||||||||||
Sbjct: 2308 tgcccctatcttatcaacac-ttccggaaactactgttgttagacgacgaggcaggtccc 2366

Query: 421  ctagaagaagaactc 435
            |||||||||||||||
Sbjct: 2367 ctagaagaagaactc 2381
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Figure 4. HBV sequences for HBV positive HCC cell lines. Nucleotide sequences for two HBV positive HCC cell lines (A) SNU-2655 and (B) SNU-3058. Query sequence is the sequence found by Sanger sequencing and subject sequence is the reference nucleotide sequence for C.M12906.1 which indicates HBV genotype C. Letter N indicates the nucleotide that cannot be identified in both forward and reverse sequences. Using HBV Blast Search, both SNU-2655 and SNU-3058 were identified to be HBV subtype C by 96% and 97% respectively.

Targeted sequencing and data analysis

Targeted sequencing using comprehensive cancer panel was performed to identify mutations of cancer related genes. Result was analyzed using online database CCLE and NCBI. Mutations found in each cell lines were sorted based on the list of frequently mutated genes and liver cancer specific mutations provided by CCLE (Table 4~10). Among sorted list of mutations, clinical significance was searched on NCBI ClinVar database. Majority of mutations on the list were either identified to be benign or had no reports on their clinical significance. However, some mutations found in HCC cell lines were reported to be pathogenic. Missense mutation of DPYD (rs1801265) found in SNU-1517, SNU-2655, SNU-2658, SNU-2663, SNU-3058, and SNU-3059, start codon deletion mutation of AR (rs377416804|rs104894742) in SNU-2658, and missense mutation of FGFR4 (rs351855) found in SNU-2663, SNU-3058 were reported as pathogenic mutations. ERCC5 missense mutations (rs587778291|rs9514067) found in SNU-1517, SNU-2655, SNU-2658, SNU-2663, SNU-3058, and SNU-3059, missense mutation of CTNNB1 in SNU-2663 were noted as likely pathogenic mutations. Other pathogenic status found in CCP data include benign, likely benign, risk factor or has conflicting interpretations of pathogenicity.

Number of cell lines mutated by each genes and their mutation types were summarized in Table 11. Some notable mutations are CTNNB1 which is involved in regulation and transcription, cell-cell adhesion, was mutated in one of the cell lines (SNU-2663), FANCA that works as post replication repair and cell cycle checkpoint was mutated in all seven cell lines. FANCD2 was found to be mutated in one cell line, SNU-2663. FGFR4, a tyrosine kinase receptor that is involved in cell proliferation, differentiation, and survival was mutated in 6 cell lines, SNU-1517, SNU-2655, SNU-2658, SNU-2663, SNU-3058, and SNU-3059. Kit was mutated in SNU-2655, SNU-2663, SNU-3059, and SNU-3160 and RET, a proto-oncogene, was found to be mutated in SNU-2658, SNU-2663, SNU-3058 and SNU-3160. TP53, a famous tumor suppressor gene was found to be mutated in SNU-1517, SNU-2655, SNU-2658, SNU-2663, SNU-3058 and SNU-3059.

Pie charts were acquired based on number of different types of mutations on each cell lines (Figure 5). SNU-2658 and SNU-3058 had most diverse list of mutation types where SNU-2658 consists of 1.1% frame shift deletion, 1.1% nonsense mutation, 1.1% splice site mutation, 1.1% start codon deletion and 95.7% missense mutations. SNU-3058 mutations consists with 1.2% frame shift insertion, 1.2% in frame insertion, 2.5% non-sense mutations 1.2%

splice site mutation and 93.8% of missense mutations. For the rest of cell lines, SNU-1517 mutations were composed of 2% nonsense mutation, 3% splice site mutations, and 95% missense mutation. SNU-2655 had 3.4% splice site mutations, 1.1% frame shift deletion mutation, and 95.4% missense mutations. SNU-2663 consists of 1.1% nonsense mutations, 2.2% splice site mutations, 96.7% missense mutations. SNU-3059 contained 2.3% nonsense mutations, 1.1% frame shift deletion, 1.1% in-frame deletion and 95.5% missense mutations. SNU-3160 had 2.5% frame shift deletion, 2.5% in-frame deletion, and 95.0% missense mutations.

Overall, missense mutation was observed most frequently (95.35%) followed by splice site mutations (1.72%) nonsense (1.38%) frame shift deletion (0.69%) and in-frame deletion (0.34%). Frame shift insertion in-frame insertion start codon deletion each took up 0.17% of total mutations.

Sanger sequencing result of ERBB2 c.(1963-1965)Atc>Gtc (p.I655V), FGFR4 c.(1162-1164)Ggg>Agg (p.G388R), TP53 c.(322-324)ggtfs (p.G108fs), TP53 c.(535-537)cAt>cGt (p.H179R) TP53 c.(634-636)tttfs (p.F212fs), TP53 c.(841-843)Gac>Tac (p.D281Y) corresponded to the targeted sequencing data (Figure 6).

Table 4. Sorted targeted sequencing data result for SNU-1517

Cell line name	CCLE list	Variant_Classification	Variant_Type	dbSNP_RS	Genome_Change	cDNA_Change	Codon_Change	Protein_Change	Clinical significance
SNU-1517	AFF3	Missense_Mutation	SNP	rs117319532	g.chr2:100199239T>C	c.2714A>G	c.(2713-2715)aAc>aGc	p.N905S	
	AFF3	Missense_Mutation	SNP	rs4851223	g.chr2:100343557C>T	c.1073G>A	c.(1072-1074)aGt>aAt	p.S358N	
	AKAP9	Missense_Mutation	SNP	rs1063242	g.chr7:91714911C>T	c.8935C>T	c.(8935-8937)Ccc>Tct	p.P2979S	Benign,Likely benign
	ALK	Missense_Mutation	SNP	rs1881421	g.chr2:29416566G>C	c.4587C>G	c.(4585-4587)gaC>gaG	p.D1529E	Benign
	ALK	Missense_Mutation	SNP	rs1881420	g.chr2:29416481T>C	c.4472A>G	c.(4471-4473)aAg>aGg	p.K1491R	Benign
	ALK	Missense_Mutation	SNP	rs1670283	g.chr2:29416572T>C	c.4381A>G	c.(4381-4383)Atc>Gtc	p.I1461V	Benign
	ATR	Missense_Mutation	SNP	rs2229032	g.chr3:142178144C>T	c.7274G>A	c.(7273-7275)cGa>cAa	p.R2425Q	Benign,Likely benign
	ATR	Missense_Mutation	SNP	rs2227928	g.chr3:142281612A>G	c.6321T>C	c.(631-633)aTg>aCg	p.M2111T	Benign,Likely benign
	AURKA	Missense_Mutation	SNP	rs1047972	g.chr20:54961463T>C	c.169A>G	c.(169-171)Att>Gtt	p.I57V	risk factor
	AURKA	Missense_Mutation	SNP	rs2273535	g.chr20:54961541A>T	c.91T>A	c.(91-93)Ttt>Att	p.F31I	
	BAI3	Missense_Mutation	SNP	rs1932618	g.chr6:69666684A>G	c.1508A>G	c.(1507-1509)aAt>aGt	p.N503S	
	BCR	Missense_Mutation	SNP	rs140504	g.chr22:23627369A>G	c.2387A>G	c.(2386-2388)aAt>aGt	p.N796S	
	BUB1B	Missense_Mutation	SNP	rs1801376	g.chr15:40477831G>A	c.1046G>A	c.(1045-1047)cGa>cAa	p.R349Q	Benign
	CASC5	Missense_Mutation	SNP	rs7177192	g.chr15:40898643G>C	c.128G>C	c.(127-129)aGa>aCa	p.R43T	Benign
	CASC5	Missense_Mutation	SNP	rs2412541	g.chr15:40913840G>T	c.1456G>T	c.(1456-1458)Gct>Tct	p.A486S	Benign
	CASC5	Missense_Mutation	SNP	rs11858113	g.chr15:40914177T>C	c.1792T>C	c.(1792-1794)aTg>aCg	p.M598T	Benign
	CASC5	Missense_Mutation	SNP	rs8040502	g.chr15:40915190A>G	c.2806A>G	c.(2806-2808)Agg>Ggg	p.R936G	Benign
	CDH2	Missense_Mutation	SNP		g.chr18:25572641T>C	c.1229A>G	c.(1228-1230)gAc>gGc	p.D410G	
	CSMD3	Missense_Mutation	SNP	rs2219898	g.chr8:114186003T>C	c.657A>G	c.(655-657)atA>atG	p.I219M	
	DPYD	Missense_Mutation	SNP	rs1801265	g.chr1:98348885G>A	c.85C>T	c.(85-87)Cgc>Tgt	p.R29C	Pathogenic
	DST	Missense_Mutation	SNP	rs80260070	g.chr6:56351972G>C	c.20017C>G	c.(20017-20019)Ctg>Gtg	p.L6673V	
	DST	Missense_Mutation	SNP	rs4715630	g.chr6:56417282C>T	c.15675G>A	c.(15673-15675)atG>atA	p.M5225I	
	DST	Missense_Mutation	SNP	rs4715631	g.chr6:56417545T>C	c.15412A>G	c.(15412-15414)Acc>Gcc	p.T5138A	
	DST	Missense_Mutation	SNP	rs141523606	g.chr6:56504561G>A	c.1997C>T	c.(1996-1998)aCa>aTa	p.T666I	
	EGFR	Missense_Mutation	SNP	rs2227983	g.chr7:55229255G>A	c.1427G>A	c.(1426-1428)aGg>aAg	p.R476K	Benign,Likely benign, not provided
	EML4	Missense_Mutation	SNP	rs6736913	g.chr2:42510018A>G	c.847A>G	c.(847-849)Aaa>Gaa	p.K283E	
	EML4	Missense_Mutation	SNP	rs10202624	g.chr2:42515388A>G	c.1144A>G	c.(1144-1146)Att>Gtt	p.I382V	
	EML4	Missense_Mutation	SNP	rs28651764	g.chr2:42515437A>G	c.1193A>G	c.(1192-1194)aAg>aGg	p.K398R	
	EPHA7	Splice_Site	SNP	rs2278106	g.chr6:94120219G>A	c.832C>T	c.(832-834)Ccc>Tcc	p.P278S	
	EPHA7	Missense_Mutation	SNP	rs2278107	g.chr6:94120659T>C	c.412A>G	c.(412-414)Ata>Gta	p.I138V	
	ERBB4	Missense_Mutation	SNP	rs2278107	g.chr2:212615417T>C	c.569A>G	c.(568-570)cAt>cGt	p.H190R	
	ERCC5	Missense_Mutation	SNP	rs2227869	g.chr13:103515085G>C	c.1586G>C	c.(1585-1587)tGt>tCt	p.C529S	Benign,Likely benign, not provided
	ERCC5	Missense_Mutation	SNP	rs9514066	g.chr13:103527849G>C	c.3157G>C	c.(3157-3159)Gga>Cga	p.G1053R	not provided
	ERCC5	Missense_Mutation	SNP	rs587778291ss9514067	g.chr13:103527930G>C	c.3238G>C	c.(3238-3240)Gga>Cga	p.G1080R	Likely pathogenic
	FANCA	Missense_Mutation	SNP	rs9282681	g.chr16:89805914T>C	c.3982A>G	c.(3982-3984)Acc>Gcc	p.T1328A	Benign,Likely benign, not provided
	FANCA	Missense_Mutation	SNP	rs7195066	g.chr16:89836323C>T	c.2426G>A	c.(2425-2427)gGt>gAt	p.G809D	Benign
	FANCA	Missense_Mutation	SNP	rs17232910	g.chr16:89839766G>C	c.1927C>G	c.(1927-1929)Ccc>Gcc	p.P643A	Benign,Likely benign, not provided
	FANCA	Missense_Mutation	SNP	rs2239359	g.chr16:89849480C>T	c.1501G>A	c.(1501-1503)Ggc>Agc	p.G501S	Benign
	FANCA	Missense_Mutation	SNP	rs11646374	g.chr16:89857935G>A	c.1235C>T	c.(1234-1236)gCg>gTg	p.A412V	Benign,Likely benign, not provided
	FANCA	Missense_Mutation	SNP	rs7190823	g.chr16:89866043T>C	c.796A>G	c.(796-798)Acg>Gcg	p.T266A	Benign

Table 4. ***(Continued 1)***

Cell line name	CCLE list	Variant_Classification	Variant_Type	dbSNP_RS	Genome_Change	cDNA_Change	Codon_Change	Protein_Change	Clinical significance
	FANCA	Missense_Mutation	SNP		g.chr16:89881015C>A	c.196G>T	c.(196-198)Ggt>Tgt	p.G66C	
	FGFR3	Missense_Mutation	SNP	rs577990843	g.chr4:1803138C>G	c.490C>G	c.(490-492)Ctg>Gtg	p.L164V	
	FGFR4	Missense_Mutation	SNP	rs1966265	g.chr5:176516631G>A	c.28G>A	c.(28-30)Gtc>Atc	p.V10I	
	FGFR4	Missense_Mutation	SNP	rs376618	g.chr5:176511797C>T	c.407C>T	c.(406-408)cCc>cTc	p.P136L	
	FN1	Missense_Mutation	SNP	rs1250209	g.chr2:216235089C>T	c.6781G>A	c.(6781-6783)Gtc>Atc	p.V2261I	
	FN1	Missense_Mutation	SNP	rs2577301	g.chr2:216272900T>G	c.2449A>C	c.(2449-2451)Acg>Ccg	p.T817P	
	FN1	Missense_Mutation	SNP	rs1250259;rs201366026	g.chr2:216300482T>A	c.44A>T	c.(43-45)cAg>cTg	p.Q15L	
	HIF1A	Missense_Mutation	SNP	rs11549467	g.chr14:62207575G>A	c.1765G>A	c.(1765-1767)Gca>Aca	p.A589T	
	KDR	Missense_Mutation	SNP	rs1870377	g.chr4:55972974T>A	c.1416A>T	c.(1414-1416)caA>caT	p.Q472H	not provided
	KDR	Missense_Mutation	SNP	rs2305948	g.chr4:55979558C>T	c.889G>A	c.(889-891)Gta>Ata	p.V297I	not provided
	LRP1B	Missense_Mutation	SNP	rs12990449	g.chr2:142567910T>C	c.143A>G	c.(142-144)cAg>cGg	p.Q48R	
	MET	Missense_Mutation	SNP		g.chr7:116412023A>G	c.3062A>G	c.(3061-3063)tAc>tGc	p.Y1021C	
	MYH11	Missense_Mutation	SNP	rs16967494;rs587781052	g.chr16:15820863C>T	c.3700G>A	c.(3700-3702)Gca>Aca	p.A1234T	Benign;Likely benign
	MYH9	Missense_Mutation	SNP	rs2269529	g.chr22:36684354T>C	c.4876A>G	c.(4876-4878)Atc>Gtc	p.I1626V	Benign
	NIN	Missense_Mutation	SNP	rs61755995	g.chr14:51219349G>A	c.4837C>T	c.(4837-4839)Cgt>Tgt	p.R1613C	Likely benign
	NIN	Missense_Mutation	SNP	rs2073347	g.chr14:51223789C>T	c.3959G>A	c.(3958-3960)gGg>gAg	p.G1320E	Benign
	NIN	Missense_Mutation	SNP	rs12882191	g.chr14:51224374T>G	c.3374A>C	c.(3373-3375)cAg>cCg	p.Q1125P	Benign
	NIN	Missense_Mutation	SNP	rs2236316	g.chr14:51224417G>C	c.3331C>G	c.(3331-3333)Cca>Gca	p.P1111A	Likely benign
	NLRP1	Missense_Mutation	SNP	rs11651270	g.chr17:5425077T>C	c.3550A>G	c.(3550-3552)Atg>Gtg	p.M1184V	Benign
	NOTCH1	Missense_Mutation	SNP	rs571739078	g.chr9:139396503C>T	c.5422G>A	c.(5422-5424)Gac>Aac	p.D1808N	
	NOTCH1	Missense_Mutation	SNP		g.chr9:139399844C>T	c.4504G>A	c.(4504-4506)Gac>Aac	p.D1502N	
	NSD1	Missense_Mutation	SNP	rs28932178	g.chr5:176637576T>C	c.2176T>C	c.(2176-2178)Tct>Cct	p.S726P	Benign
	NSD1	Missense_Mutation	SNP	rs193290006	g.chr5:176638587A>G	c.3187A>G	c.(3187-3189)Act>Gct	p.T1063A	Benign
	NUP214	Missense_Mutation	SNP	rs103612	g.chr9:134020092C>T	c.1720C>T	c.(1720-1722)Ccc>Tcc	p.P574S	
	PDE4DIP	Missense_Mutation	SNP	rs148370554	g.chr1:144852477G>A	c.7277C>T	c.(7276-7278)cCa>cTa	p.P2426L	
	PDE4DIP	Missense_Mutation	SNP	rs78371650	g.chr1:144854581T>C	c.7144A>G	c.(7144-7146)Act>Gct	p.T2382A	
	PDE4DIP	Missense_Mutation	SNP	rs3863691	g.chr1:144854598C>T	c.7127G>A	c.(7126-7128)cGa>cAa	p.R2376Q	
	PDE4DIP	Missense_Mutation	SNP	rs1613780	g.chr1:144865850G>T	c.5983C>A	c.(5983-5985)gaC>gaA	p.D1995E	
	PDE4DIP	Missense_Mutation	SNP	rs1698605	g.chr1:144871738C>A	c.5479G>T	c.(5479-5481)Gct>Tct	p.A1827S	
	PDE4DIP	Missense_Mutation	SNP	rs1778159	g.chr1:144871755A>T	c.5462T>A	c.(5461-5463)gTg>gAg	p.V1821E	
	PDE4DIP	Missense_Mutation	SNP	rs1778158	g.chr1:144871782A>G	c.5435T>C	c.(5434-5436)tTc>tCc	p.L1812P	
	PDE4DIP	Missense_Mutation	SNP	rs1778155	g.chr1:144874815T>C	c.5201A>G	c.(5200-5202)cAt>cGt	p.H1734R	
	PDE4DIP	Missense_Mutation	SNP	rs2798901	g.chr1:144879264A>G	c.4594T>C	c.(4594-4596)Tgg>Cgg	p.W1532R	
	PDE4DIP	Missense_Mutation	SNP	rs1698647	g.chr1:144882823C>T	c.3607G>A	c.(3607-3609)Gca>Aca	p.A1203T	
	PDE4DIP	Missense_Mutation	SNP	rs1698624	g.chr1:144886197A>T	c.3448T>A	c.(3448-3450)Ttc>Atc	p.F1150I	
	PDE4DIP	Missense_Mutation	SNP	rs1629011	g.chr1:144912233C>T	c.2453G>A	c.(2452-2454)cGc>cAc	p.R818H	
	PDE4DIP	Nonsense_Mutation	SNP	rs1698683	g.chr1:144916676C>T	c.2090G>A	c.(2089-2091)tGg>tAg	p.W697*	
	PDE4DIP	Missense_Mutation	SNP	rs1061308	g.chr1:144918957T>A	c.1640A>T	c.(1639-1641)gAa>gTa	p.E547V	
	PDE4DIP	Missense_Mutation	SNP	rs2455994	g.chr1:144922523C>T	c.1295G>A	c.(1294-1296)cGt>cAt	p.R432H	
	PDE4DIP	Missense_Mutation	SNP	rs1359300	g.chr1:144922583G>A	c.1235C>T	c.(1234-1236)tCg>tTg	p.S412L	

Table 4. ***(Continued 2)***

Cell line name	CCLE list	Variant_Classification	Variant_Type	dbSNP_RS	Genome_Change	cDNA_Change	Codon_Change	Protein_Change	Clinical significance
	PDE4DIP	Missense_Mutation	SNP	rs1664022	g.chr1:144994658C>A	c.485G>T	c.(484-486)cGc>cTc	p.R162L	
	PDE4DIP	Missense_Mutation	SNP	rs77741369	g.chr1:145015877G>T	c.424C>A	c.(424-426)Ctt>Att	p.L142I	
	PDE4DIP	Nonsense_Mutation	SNP	rs2762779	g.chr1:145075683C>T	c.180G>A	c.(178-180)tgG>tgA	p.W60*	
	PKHD1	Missense_Mutation	SNP	rs9381994	g.chr6:51483961T>C	c.12143A>G	c.(12142-12144)cAa>cGa	p.Q4048R	Benign
	PKHD1	Missense_Mutation	SNP	rs4715227	g.chr6:51491884T>C	c.11696A>G	c.(11695-11697)cAg>cGg	p.Q3899R	Benign
	PKHD1	Missense_Mutation	SNP	rs2435322	g.chr6:51875250A>C	c.5608T>G	c.(5608-5610)Ttg>Gtg	p.L1870V	Benign
	PKHD1	Missense_Mutation	SNP	rs9296669	g.chr6:51890823G>A	c.3785C>T	c.(3784-3786)gCt>gTt	p.A1262V	Benign
	PKHD1	Splice_Site	SNP	rs9370096	g.chr6:51914956G>A	c.2278C>T	c.(2278-2280)Cgc>Tgc	p.R760C	Benign
	PTPRT	Missense_Mutation	SNP		g.chr20:41076898G>A	c.1522C>T	c.(1522-1524)Cct>Tct	p.P508S	
	SMARCA4	Splice_Site	SNP		g.chr19:11096082G>T		c.e4+1		
	STK11	Missense_Mutation	SNP	rs59912467	g.chr19:1223125C>G	c.1062C>G	c.(1060-1062)ttC>ttG	p.F354L	Conflicting interpretations of pathogenicity, not provided
	TCF3	Missense_Mutation	SNP	rs2074888	g.chr19:1615796G>A	c.1475C>T	c.(1474-1476)gCg>gTg	p.A492V	
	TCF3	Missense_Mutation	SNP		g.chr19:1619802A>T	c.1144T>A	c.(1144-1146)Tac>Aac	p.Y382N	
	TET1	Missense_Mutation	SNP	rs10823229	g.chr10:70332580A>G	c.485A>G	c.(484-486)gAc>gGc	p.D162G	
	TET2	Missense_Mutation	SNP	rs2454206	g.chr4:106196951A>G	c.5284A>G	c.(5284-5286)Ata>Gta	p.I1762V	not provided
	TNK2	Missense_Mutation	SNP	rs3747673	g.chr3:195611844G>A	c.295C>T	c.(295-297)Cgg>Tgg	p.R99W	
	TP53	Missense_Mutation	SNP	rs587781858	g.chr17:7572989C>T	c.1120G>A	c.(1120-1122)Ggt>Agt	p.G374S	Conflicting interpretations of pathogenicity
	TP53	Missense_Mutation	SNP		g.chr17:7577097C>A	c.841G>T	c.(841-843)Gac>Tac	p.D281Y	
	TRIM33	Missense_Mutation	SNP	rs6537825	g.chr1:114948281A>G	c.2519T>C	c.(2518-2520)aTT>aCt	p.I840T	
	WRN	Missense_Mutation	SNP	rs1801195	g.chr8:30999280G>T	c.3222G>T	c.(3220-3222)ttG>ttT	p.L1074F	Benign/Likely benign, not provided
	XPC	Missense_Mutation	SNP	rs2228000	g.chr3:14199887G>A	c.1496C>T	c.(1495-1497)gCg>gTg	p.A499V	Benign

Table 5. Sorted targeted sequencing data result for SNU-2655

Cell line name	CCLE list	Variant_Classification	Variant_Type	dbSNP_RS	Genome_Change	cDNA_Change	Codon_Change	Protein_Change	Clinical significance
SNU-2655	ADAMTS20	Missense_Mutation	SNP	rs79065113	g.chr12:43846199G>T	c.1957C>A	c.(1957-1959)Cgt>Agt	p.R653S	
	AFF3	Missense_Mutation	SNP	rs4851223	g.chr2:100343557C>T	c.1073G>A	c.(1072-1074)aGt>aAt	p.S358N	
	AKAP9	Missense_Mutation	SNP	rs6960867	g.chr7:91712698A>G	c.8375A>G	c.(8374-8376)aAt>aGt	p.N2792S	Benign,Likely benign
	AKAP9	Missense_Mutation	SNP	rs1063242	g.chr7:91714911C>T	c.8935C>T	c.(8935-8937)Ccc>Tct	p.P2979S	Benign,Likely benign
	ALK	Missense_Mutation	SNP	rs1881421	g.chr2:29416366G>C	c.4587C>G	c.(4585-4587)gaC>gaG	p.D1529E	Benign
	ALK	Missense_Mutation	SNP	rs1881420	g.chr2:29416481T>C	c.4472A>G	c.(4471-4473)aAg>aGg	p.K1491R	Benign
	ALK	Missense_Mutation	SNP	rs1670283	g.chr2:29416572T>C	c.4381A>G	c.(4381-4383)Atc>Gtc	p.I1461V	Benign
	ATR	Missense_Mutation	SNP	rs2229032	g.chr3:142178144C>T	c.7274G>A	c.(7273-7275)cGa>cAa	p.R2425Q	Benign,Likely benign
	ATR	Missense_Mutation	SNP	rs2227928	g.chr3:142281612A>G	c.632T>C	c.(631-633)aTg>aCg	p.M211T	Benign,Likely benign
	ATRX	Missense_Mutation	SNP	rs3088074	g.chrX:76937963G>C	c.2785C>G	c.(2785-2787)Cag>Gag	p.Q929E	Benign,Likely benign
	AURKA	Missense_Mutation	SNP	rs1047972	g.chr20:54961463T>C	c.169A>G	c.(169-171)Att>Gtt	p.I57V	
	BAI3	Missense_Mutation	SNP	rs1932618	g.chr6:69666684A>G	c.1508A>G	c.(1507-1509)aAt>aGt	p.N503S	
	BCR	Missense_Mutation	SNP	rs140504	g.chr22:23627369A>G	c.2387A>G	c.(2386-2388)aAt>aGt	p.N796S	
	BLM	Missense_Mutation	SNP	rs7167216	g.chr15:91354521G>A	c.3568G>A	c.(3568-3570)Gta>Ata	p.V1190I	Benign,Likely benign, not provided
	BUB1B	Missense_Mutation	SNP	rs1801376	g.chr15:40477831G>A	c.1046G>A	c.(1045-1047)cGa>cAa	p.R349Q	Benign
	CARD11	Missense_Mutation	SNP	rs149857605	g.chr7:2953041G>A	c.2899C>T	c.(2899-2901)Cgc>Tgc	p.R967C	
	CSF1R	Missense_Mutation	SNP	rs10079250	g.chr5:149450132T>C	c.1085A>G	c.(1084-1086)cAc>cGc	p.H362R	Benign
	CSMD3	Missense_Mutation	SNP	rs2219898	g.chr8:114186003T>C	c.657A>G	c.(655-657)atA>atG	p.I219M	
	DPYD	Missense_Mutation	SNP	rs1801159	g.chr1:97981395T>C	c.1627A>G	c.(1627-1629)Ata>Gta	p.I543V	Benign,Likely benign, not provided
	DPYD	Missense_Mutation	SNP	rs1801265	g.chr1:98348885G>A	c.85C>T	c.(85-87)Cgt>Tgt	p.R29C	Pathogenic
	DST	Missense_Mutation	SNP	rs4715630	g.chr6:56417282C>T	c.15673G>A	c.(15673-15675)atG>atA	p.M5225I	
	DST	Missense_Mutation	SNP	rs4715631	g.chr6:56417545T>C	c.15412A>G	c.(15412-15414)Acc>Gcc	p.T5138A	
	DST	Missense_Mutation	SNP	rs4712138	g.chr6:56463410T>C	c.11159A>G	c.(11158-11160)cAg>cGg	p.Q3720R	
	EGFR	Missense_Mutation	SNP	rs2227983	g.chr7:55229255G>A	c.1427G>A	c.(1426-1428)aGg>aAg	p.R476K	Benign,Likely benign, not provided
	EML4	Missense_Mutation	SNP	rs6736913	g.chr2:42510018A>G	c.847A>G	c.(847-849)Aaa>Gaa	p.K283E	
	EML4	Missense_Mutation	SNP	rs10202624	g.chr2:42515388A>G	c.1144A>G	c.(1144-1146)Att>Gtt	p.I382V	
	EML4	Missense_Mutation	SNP	rs28651764	g.chr2:42515437A>G	c.1193A>G	c.(1192-1194)aAg>aGg	p.K398R	
	EP300	Missense_Mutation	SNP	rs20551	g.chr22:41548008A>G	c.2989A>G	c.(2989-2991)Att>Gtt	p.I997V	Benign
	ERCC5	Missense_Mutation	SNP	rs9514066	g.chr13:103527849G>C	c.3157G>C	c.(3157-3159)Gga>Cga	p.G1053R	not provided
	ERCC5	Missense_Mutation	SNP	rs587778291;rs 9514067	g.chr13:103527930G>C	c.3238G>C	c.(3238-3240)Gga>Cga	p.G1080R	Likely pathogenic
	ESR1	Missense_Mutation	SNP		g.chr6:152419977G>A	c.1664G>A	c.(1663-1665)cGt>cAt	p.R555H	
	FANCA	Missense_Mutation	SNP	rs142377616	g.chr16:89818581G>A	c.3031C>T	c.(3031-3033)Cgc>Tgc	p.R1011C	Likely benign
	FANCA	Missense_Mutation	SNP	rs2239359	g.chr16:89849480C>T	c.1501G>A	c.(1501-1503)Ggc>Agc	p.G501S	Benign
	FANCA	Missense_Mutation	SNP	rs7190823	g.chr16:89866043T>C	c.796A>G	c.(796-798)Acg>Gcg	p.T266A	Benign
	FGFR4	Missense_Mutation	SNP	rs1966265	g.chr5:176516631G>A	c.28G>A	c.(28-30)Gtc>Atc	p.V10I	
	FGFR4	Missense_Mutation	SNP	rs376618	g.chr5:176517797C>T	c.407C>T	c.(406-408)cCc>cTc	p.P136L	
	FGFR4	Missense_Mutation	SNP	rs55675160	g.chr5:176518037A>G	c.535A>G	c.(535-537)Acg>Gcg	p.T179A	
	FN1	Missense_Mutation	SNP	rs1250209	g.chr2:216235089C>T	c.6781G>A	c.(6781-6783)Gtc>Atc	p.V2261I	
	FN1	Missense_Mutation	SNP	rs13306364	g.chr2:216264021T>G	c.3307A>C	c.(3307-3309)Att>Ctt	p.I1103L	
	FN1	Missense_Mutation	SNP	rs2577301	g.chr2:216272900T>G	c.2449A>C	c.(2449-2451)Acg>Ccg	p.T817P	

Table 5. ***(Continued 1)***

Cell line name	CCLE list	Variant_Classification	Variant_Type	dbSNP_RS	Genome_Change	cDNA_Change	Codon_Change	Protein_Change	Clinical significance
	FN1	Missense_Mutation	SNP	rs1250259|rs2013666026	g.chr2:216300482T>A	c.44A>T	c.(43-45)cAg>cTg	p.Q15L	
	IKBKB	Splice_Site	SNP	rs2272736	g.chr8:42177163G>A	c.1577G>A	c.(1576-1578)cGg>cAg	p.R526Q	
	KDM6A	Missense_Mutation	SNP	rs2230018	g.chrX:44929077C>A	c.2177C>A	c.(2176-2178)aCg>aAg	p.T726K	Benign
	KDR	Missense_Mutation	SNP	rs1870377	g.chr4:55972974T>A	c.1416A>T	c.(1414-1416)caA>caT	p.Q472H	not provided
	KIT	Missense_Mutation	SNP	rs201872586	g.chr4:55561861C>T	c.251C>T	c.(250-252)aCg>aTg	p.T84M	Likely benign
	LAMP1	Missense_Mutation	SNP	rs9577229	g.chr13:113973832C>T	c.611C>T	c.(610-612)gCg>gTg	p.A204V	
	LAMP1	Missense_Mutation	SNP	rs9577230	g.chr13:113975768T>C	c.926T>C	c.(925-927)aTt>aCt	p.I309T	
	MTOR	Missense_Mutation	SNP	rs28730685	g.chr1:11272529G>A	c.3401C>T	c.(3400-3402)gCa>gTa	p.A1134V	
	MYH11	Missense_Mutation	SNP	rs16967494|rs587781052	g.chr16:15820863C>T	c.3700G>A	c.(3700-3702)Gca>Aca	p.A1234T	Benign/Likely benign
	MYH9	Missense_Mutation	SNP	rs2269529	g.chr22:36684354T>C	c.4876A>G	c.(4876-4878)Atc>Gtc	p.I1626V	Benign
	NCOA2	Missense_Mutation	SNP	rs2228591	g.chr8:71039118C>G	c.3846G>C	c.(3844-3846)atG>atC	p.M1282I	
	NLRP1	Missense_Mutation	SNP	rs11651270	g.chr17:5425077T>C	c.3550A>G	c.(3550-3552)Atg>Gtg	p.M1184V	Benign
	NOTCH2	Missense_Mutation	SNP	rs75423398	g.chr1:120471712C>T	c.3779G>A	c.(3778-3780)cGt>cAt	p.R1260H	not provided
	NTRK3	Missense_Mutation	SNP		g.chr15:88679759C>A	c.410G>T	c.(409-411)gGa>gTa	p.G137V	
	NUMA1	Missense_Mutation	SNP	rs3750913	g.chr11:71726168G>C	c.2381C>G	c.(2380-2382)gCt>gGt	p.A794G	
	NUP214	Missense_Mutation	SNP	rs103612	g.chr9:134020092C>T	c.1720C>T	c.(1720-1722)Ccc>Tcc	p.P574S	
	PDE4DIP	Missense_Mutation	SNP	rs148370554	g.chr1:144852477G>A	c.7277C>T	c.(7276-7278)cCa>cTa	p.P2426L	
	PDE4DIP	Missense_Mutation	SNP	rs783711650	g.chr1:144854581T>C	c.7144A>G	c.(7144-7146)Act>Gct	p.T2382A	
	PDE4DIP	Missense_Mutation	SNP	rs3863691	g.chr1:144854598C>T	c.7127G>A	c.(7126-7128)cGa>cAa	p.R2376Q	
	PDE4DIP	Missense_Mutation	SNP	rs1613780	g.chr1:144865850G>T	c.5985C>A	c.(5983-5985)gaC>gaA	p.D1995E	
	PDE4DIP	Missense_Mutation	SNP	rs1698605	g.chr1:144871738C>A	c.5479G>T	c.(5479-5481)Gct>Tct	p.A1827S	
	PDE4DIP	Missense_Mutation	SNP	rs1778159	g.chr1:144871755A>T	c.5462T>A	c.(5461-5463)gTg>gAg	p.V1821E	
	PDE4DIP	Missense_Mutation	SNP	rs1778158	g.chr1:144871782A>G	c.5435T>C	c.(5434-5436)cTc>cCc	p.L1812P	
	PDE4DIP	Missense_Mutation	SNP	rs1778155	g.chr1:144874815T>C	c.5201A>G	c.(5200-5202)cAt>cGt	p.H1734R	
	PDE4DIP	Missense_Mutation	SNP	rs1778120	g.chr1:144879090T>C	c.4768A>G	c.(4768-4770)Aaa>Gaa	p.K1590E	
	PDE4DIP	Missense_Mutation	SNP	rs2798901	g.chr1:144879264A>G	c.4594T>C	c.(4594-4596)Tgg>Cgg	p.W1532R	
	PDE4DIP	Missense_Mutation	SNP	rs12568796	g.chr1:144880832T>C	c.4204A>G	c.(4204-4206)Aag>Gag	p.K1402E	
	PDE4DIP	Missense_Mutation	SNP	rs1698647	g.chr1:144882823C>T	c.3607G>A	c.(3607-3609)Gca>Aca	p.A1203T	
	PDE4DIP	Missense_Mutation	SNP	rs1698624	g.chr1:144886197A>T	c.3448T>A	c.(3448-3450)Ttc>Atc	p.F1150I	
	PDE4DIP	Missense_Mutation	SNP	rs1629011	g.chr1:144912233C>T	c.2453G>A	c.(2452-2454)cGc>cAc	p.R818H	
	PDE4DIP	Missense_Mutation	SNP	rs1061308	g.chr1:144918957T>A	c.1640A>T	c.(1639-1641)gAa>gTa	p.E547V	
	PDE4DIP	Missense_Mutation	SNP	rs1359300	g.chr1:144922583G>A	c.1235C>T	c.(1234-1236)tCg>tTg	p.S412L	
	PDE4DIP	Missense_Mutation	SNP	rs1664022	g.chr1:144994658C>A	c.483G>T	c.(484-486)cGc>cTc	p.R162L	
	PDE4DIP	Nonsense_Mutation	SNP	rs2762779	g.chr1:145075683C>T	c.180G>A	c.(178-180)tgG>tgA	p.W60*	
	PKHD1	Missense_Mutation	SNP	rs9381994	g.chr6:51483961T>C	c.12143A>G	c.(12142-12144)cAa>cGa	p.Q4048R	Benign
	PKHD1	Missense_Mutation	SNP	rs4715227	g.chr6:51491884T>C	c.11696A>G	c.(11695-11697)cAg>cGg	p.Q3899R	Benign
	PKHD1	Missense_Mutation	SNP	rs2435322	g.chr6:51875250A>C	c.5608T>G	c.(5608-5610)Ttg>Gtg	p.L1870V	Benign
	PKHD1	Missense_Mutation	SNP	rs9296669	g.chr6:51890823G>A	c.3785C>T	c.(3784-3786)gCt>gTt	p.A1262V	Benign
	PKHD1	Splice_Site	SNP	rs9370096	g.chr6:51914956G>A	c.2278C>T	c.(2278-2280)Cgc>Tgc	p.R760C	Benign
	TCF3	Missense_Mutation	SNP	rs386805766|rs1052692	g.chr19:1619350C>T	c.1291G>A	c.(1291-1293)Ggc>Agc	p.G431S	

Table 5. ***(Continued 2)***

Cell line name	CCLE list	Variant_Classification	Variant_Type	dbSNP_RS	Genome_Change	cDNA_Change	Codon_Change	Protein_Change	Clinical significance
	TET2	Missense_Mutation	SNP	rs111678678	g.chr4:106158215C>T	c.3116C>T	c.(3115-3117)tCg>tTg	p.S1039L	not provided
	TP53	Frame_Shift_Del	DEL		g.chr17:7578213delA	c.636delT	c.(634-636)tttfs	p.F212fs	
	TPR	Splice_Site	SNP	rs61744267	g.chr1:186321242C>T	c.2335G>A	c.(2335-2337)Gta>Ata	p.V779I	
	TRIM33	Missense_Mutation	SNP	rs6537825	g.chr1:114948281A>G	c.2519T>C	c.(2518-2520)aTt>aCt	p.I840T	
	TRIP11	Missense_Mutation	SNP	rs117748213	g.chr14:92470181G>A	c.4139C>T	c.(4138-4140)aCc>aTc	p.T1380I	Uncertain significance
	TRIP11	Missense_Mutation	SNP	rs186074112	g.chr14:92505923T>A	c.107A>T	c.(106-108)gAt>gTt	p.D36V	Uncertain significance
	UBR5	Missense_Mutation	SNP		g.chr8:103284869C>G	c.6843G>C	c.(6841-6843)gaG>gaC	p.E2281D	

Table 6. Sorted targeted sequencing data result for SNU-2658

Cell line name	CCLE list	Variant_Classification	Variant_Type	dbSNP_RS	Genome_Change	cDNA_Change	Codon_Change	Protein_Change	Clinical significance
SNU-2658	AFF3	Missense_Mutation	SNP	rs117319532	g.chr2:100199239T>C	c.2714A>G	c.(2713-2715)aAc>cGc	p.N905S	
	AFF3	Missense_Mutation	SNP	rs4851223	g.chr2:100343557C>T	c.1073G>A	c.(1072-1074)gGt>gAt	p.S358N	
	AKAP9	Missense_Mutation	SNP	rs6960867	g.chr7:91712698A>G	c.8375A>G	c.(8374-8376)aAt>aGt	p.N2792S	Benign,Likely benign
	AKAP9	Missense_Mutation	SNP	rs1063242	g.chr7:91714911C>T	c.8935C>T	c.(8935-8937)Ccc>Tcc	p.P2979S	Benign,Likely benign
	ALK	Missense_Mutation	SNP	rs1881421	g.chr2:29416366G>C	c.4587C>G	c.(4585-4587)gaC>gaG	p.D1529E	Benign
	ALK	Missense_Mutation	SNP	rs1881420	g.chr2:29416481T>C	c.4472A>G	c.(4471-4473)aAg>aGg	p.K1491R	Benign
	ALK	Missense_Mutation	SNP	rs1670283	g.chr2:29416572T>C	c.4381A>G	c.(4381-4383)Atc>Gtc	p.I1461V	Benign
	AR	Start_Codon_Del	DEL	rs377416804|rs10 4894742	g.chrX:66764986_66765008 delAGGAATGGAAGTGCAG TTAGGGCT				Pathogenic
	ARNT	Missense_Mutation	SNP	rs2275237	g.chr1:150785811G>A	c.2111C>T	c.(2110-2112)cCt>cTt	p.P704L	
	ATM	Missense_Mutation	SNP		g.chr11:108225546A>T	c.8795A>T	c.(8794-8796)gAg>gTg	p.E2932V	
	ATR	Missense_Mutation	SNP	rs2227928	g.chr3:142281612A>G	c.632T>C	c.(631-633)aTg>aCg	p.M211T	Benign,Likely benign
	ATRX	Missense_Mutation	SNP	rs3088074	g.chrX:76937963G>C	c.2785C>G	c.(2785-2787)Cag>Gag	p.Q929E	Benign
	AURKA	Missense_Mutation	SNP	rs1047972	g.chr20:54961463T>C	c.169A>G	c.(169-171)Att>Gtt	p.I57V	
	AURKA	Missense_Mutation	SNP	rs2273535	g.chr20:54961541A>T	c.91T>A	c.(91-93)Ttc>Att	p.F31I	risk factor
	BAI3	Missense_Mutation	SNP	rs1932618	g.chr6:69666684A>G	c.1508A>G	c.(1507-1509)aAt>aGt	p.N503S	
	BCR	Missense_Mutation	SNP	rs140504	g.chr22:23627369A>G	c.2387A>G	c.(2386-2388)aAt>aGt	p.N796S	
	CASC5	Missense_Mutation	SNP	rs7177192	g.chr15:40898643G>C	c.128G>C	c.(127-129)aGa>aCa	p.R43T	Benign
	CASC5	Missense_Mutation	SNP	rs2412541	g.chr15:40913840G>T	c.1456G>T	c.(1456-1458)Gct>Tct	p.A486S	Benign
	CASC5	Missense_Mutation	SNP	rs11858113	g.chr15:40914177T>C	c.1793T>C	c.(1792-1794)aTg>aCg	p.M598T	Benign
	CASC5	Missense_Mutation	SNP	rs8040502	g.chr15:40915190A>G	c.2806A>G	c.(2806-2808)Agg>Ggg	p.R936G	Benign
	CDH2	Missense_Mutation	SNP	rs17445840	g.chr18:25593694C>T	c.259G>A	c.(259-261)Gca>Aca	p.A87T	
	CSF1R	Missense_Mutation	SNP	rs10079250	g.chr5:149450132T>C	c.1085A>G	c.(1084-1086)cAc>cGc	p.H362R	Benign
	CSMD3	Missense_Mutation	SNP		g.chr8:113533825A>G	c.6533T>C	c.(6532-6534)aTt>aCt	p.I2178T	
	CSMD3	Missense_Mutation	SNP		g.chr8:114186034A>G	c.626T>C	c.(625-627)aTc>aCc	p.I209T	
	DPYD	Missense_Mutation	SNP	rs1801265	g.chr1:98348885G>A	c.85C>T	c.(85-87)Cgc>Tgc	p.R29C	Pathogenic
	DST	Missense_Mutation	SNP	rs4715630	g.chr6:56417282C>T	c.15675G>A	c.(15673-15675)atG>atA	p.M5225I	
	DST	Missense_Mutation	SNP	rs4715631	g.chr6:56417545T>C	c.15412A>G	c.(15412-15414)Acc>Gcc	p.T5138A	
	EGFR	Missense_Mutation	SNP	rs2227983	g.chr7:55229255G>A	c.1427G>A	c.(1426-1428)aGg>aAg	p.R476K	Benign,Likely benign, not provided
	EML4	Missense_Mutation	SNP	rs6736913	g.chr2:42510018A>G	c.847A>G	c.(847-849)Aaa>Gaa	p.K283E	
	EML4	Missense_Mutation	SNP	rs10202624	g.chr2:42515388A>G	c.1144A>G	c.(1144-1146)Att>Gtt	p.I382V	
	EML4	Missense_Mutation	SNP	rs28651764	g.chr2:42515437A>G	c.1193A>G	c.(1192-1194)aAg>aGg	p.K398R	
	ERBB2	Missense_Mutation	SNP	rs11136201	g.chr17:37879588A>G	c.1965A>G	c.(1963-1965)Atc>Gtc	p.I655V	Benign
	ERCC5	Missense_Mutation	SNP	rs9514066	g.chr13:103527849G>C	c.3157G>C	c.(3157-3159)Gga>Cga	p.G1053R	not provided
	ERCC5	Missense_Mutation	SNP	rs587778291|rs95 14067	g.chr13:103527910G>C	c.3238G>C	c.(3238-3240)Gga>Cga	p.G1080R	Likely pathogenic
	ERCC5	Missense_Mutation	SNP	rs17655	g.chr13:103528002G>C	c.3310G>C	c.(3310-3312)Gat>Cat	p.D1104H	Benign,Likely benign, not provided
	FANCA	Missense_Mutation	SNP	rs7195066	g.chr16:89836323C>T	c.2426G>A	c.(2425-2427)gGt>gAt	p.G809D	Benign
	FANCA	Missense_Mutation	SNP	rs2239359	g.chr16:89849480C>T	c.1501G>A	c.(1501-1503)Ggc>Agc	p.G501S	Benign
	FANCA	Missense_Mutation	SNP	rs7190823	g.chr16:89866043T>C	c.796A>G	c.(796-798)Acg>Gcg	p.T266A	Benign
	FGFR4	Missense_Mutation	SNP	rs1966265	g.chr5:176516631G>A	c.28G>A	c.(28-30)Gtc>Atc	p.V10I	
	FGFR4	Missense_Mutation	SNP	rs376618	g.chr5:176517797C>T	c.407C>T	c.(406-408)cCc>cTc	p.P136L	

Table 6. ***(Continued 1)***

Cell line name	CCLE list	Variant_Classification	Variant_Type	dbSNP_RS	Genome_Change	cDNA_Change	Codon_Change	Protein_Change	Clinical significance
	FGFR4	Missense_Mutation	SNP	rs351855	g.chr5:176520243G>A	c.1162G>A	c.(1162-1164)Ggg>Agg	p.G388R	Pathogenic
	FN1	Missense_Mutation	SNP	rs1250209	g.chr2:216235089C>T	c.6781G>A	c.(6781-6783)Gtc>Atc	p.V2261I	
	FN1	Missense_Mutation	SNP		g.chr2:216259394C>A	c.3653G>T	c.(3652-3654)gGa>gTa	p.G1218V	
	FN1	Missense_Mutation	SNP	rs2577301	g.chr2:216272900T>G	c.2449A>C	c.(2449-2451)Acg>Ccg	p.T817P	
	FN1	Missense_Mutation	SNP	rs1250259|rs201366026	g.chr2:216300482T>A	c.44A>T	c.(43-45)cAg>cTg	p.Q15L	
	KDR	Missense_Mutation	SNP	rs1870377	g.chr4:55972974T>A	c.1416A>T	c.(1414-1416)caA>caT	p.Q472H	not provided
	LIFR	Missense_Mutation	SNP	rs3729740	g.chr5:38496637C>T	c.1732G>A	c.(1732-1734)Gat>Aat	p.D578N	Benign
	LRP1B	Missense_Mutation	SNP	rs12990449	g.chr2:142567910T>C	c.143A>G	c.(142-144)cAg>cGg	p.Q48R	
	MAML2	Missense_Mutation	SNP		g.chr11:95712798A>T	c.2785T>A	c.(2785-2787)Tct>Act	p.S929T	
	MN1	Missense_Mutation	SNP		g.chr22:28193098G>C	c.3434C>G	c.(3433-3435)cCg>cGg	p.P1145R	
	MYH11	Missense_Mutation	SNP	rs16967494|rs787781052	g.chr16:15820863C>T	c.3700G>A	c.(3700-3702)Gca>Aca	p.A1234T	Benign/Likely benign
	MYH9	Missense_Mutation	SNP	rs2269529	g.chr22:36684354T>C	c.4876A>G	c.(4876-4878)Atc>Gtc	p.I1626V	Benign
	NCOA2	Missense_Mutation	SNP	rs2228591	g.chr8:71039118C>G	c.3846G>C	c.(3844-3846)atG>atC	p.M1282I	
	NIN	Missense_Mutation	SNP	rs2073347	g.chr14:51223789C>T	c.3959G>A	c.(3958-3960)gGg>gAg	p.G1320E	Benign
	NIN	Missense_Mutation	SNP	rs12882191	g.chr14:51224374T>G	c.3374A>C	c.(3373-3375)cAg>cCg	p.Q1125P	Benign
	NLRP1	Missense_Mutation	SNP	rs11651270	g.chr17:5425077T>C	c.3550A>G	c.(3550-3552)Atg>Gtg	p.M1184V	Benign
	NOTCH2	Missense_Mutation	SNP	rs75423398	g.chr1:120471712C>T	c.3779G>A	c.(3778-3780)cGt>cAt	p.R1260H	not provided
	NSD1	Missense_Mutation	SNP	rs28932178	g.chr5:176637576T>C	c.2176T>C	c.(2176-2178)Tct>Cct	p.S726P	Benign
	PDE4DIP	Missense_Mutation	SNP	rs148370554	g.chr1:144852477G>A	c.7277C>T	c.(7276-7278)cCa>cTa	p.P2426L	
	PDE4DIP	Missense_Mutation	SNP	rs78371650	g.chr1:144854581T>C	c.7144A>G	c.(7144-7146)Act>Gct	p.T2382A	
	PDE4DIP	Missense_Mutation	SNP	rs3863691	g.chr1:144854598C>T	c.7127G>A	c.(7126-7128)cGa>cAa	p.R2376Q	
	PDE4DIP	Missense_Mutation	SNP	rs2798855	g.chr1:144854608T>G	c.7117A>C	c.(7117-7119)Aaa>Caa	p.K2373Q	
	PDE4DIP	Missense_Mutation	SNP	rs139822181	g.chr1:144863320T>C	c.6338A>G	c.(6337-6339)aAa>aGa	p.K2113R	
	PDE4DIP	Missense_Mutation	SNP	rs1613780	g.chr1:144865850G>T	c.5985C>A	c.(5983-5985)gaC>gaA	p.D1995E	
	PDE4DIP	Missense_Mutation	SNP	rs143848459	g.chr1:144868094T>C	c.5600A>G	c.(5599-5601)aAc>aGc	p.N1867S	
	PDE4DIP	Missense_Mutation	SNP		g.chr1:144871705A>C	c.5512T>G	c.(5512-5514)Ttg>Gtg	p.L1838V	
	PDE4DIP	Missense_Mutation	SNP	rs1698605	g.chr1:144871738C>A	c.5479G>T	c.(5479-5481)Gct>Tct	p.A1827S	
	PDE4DIP	Missense_Mutation	SNP	rs1778159	g.chr1:144871755A>T	c.5462T>A	c.(5461-5463)gTg>gAg	p.V1821E	
	PDE4DIP	Missense_Mutation	SNP	rs1778158	g.chr1:144871782A>G	c.5435T>C	c.(5434-5436)cTc>cCc	p.L1812P	
	PDE4DIP	Missense_Mutation	SNP	rs1778155	g.chr1:144874815T>C	c.5201A>G	c.(5200-5202)cAt>cGt	p.H1734R	
	PDE4DIP	Missense_Mutation	SNP	rs1778120	g.chr1:144879090T>C	c.4768A>G	c.(4768-4770)Aaa>Gaa	p.K1590E	
	PDE4DIP	Missense_Mutation	SNP	rs2798901	g.chr1:144879264A>G	c.4594T>C	c.(4594-4596)Tgg>Cgg	p.W1532R	
	PDE4DIP	Missense_Mutation	SNP	rs113467089	g.chr1:144879485G>C	c.4373C>G	c.(4372-4374)aCa>aGa	p.T1458R	
	PDE4DIP	Missense_Mutation	SNP	rs12568796	g.chr1:144880832T>C	c.4204A>G	c.(4204-4206)Aag>Gag	p.K1402E	
	PDE4DIP	Missense_Mutation	SNP	rs1698647	g.chr1:144882823C>T	c.3607G>A	c.(3607-3609)Gca>Aca	p.A1203T	
	PDE4DIP	Missense_Mutation	SNP	rs1698624	g.chr1:144886197A>T	c.3448T>A	c.(3448-3450)Ttc>Atc	p.F1150I	
	PDE4DIP	Missense_Mutation	SNP	rs16290111	g.chr1:144912233C>T	c.2453G>A	c.(2452-2454)cGc>cAc	p.R818H	
	PDE4DIP	Missense_Mutation	SNP	rs1061308	g.chr1:144918957T>A	c.1640A>T	c.(1639-1641)gAa>gTa	p.E547V	
	PDE4DIP	Missense_Mutation	SNP	rs1359300	g.chr1:144922583G>A	c.1235C>T	c.(1234-1236)tCg>tTg	p.S412L	
	PDE4DIP	Missense_Mutation	SNP	rs1664022	g.chr1:144994658C>A	c.485G>T	c.(484-486)cGc>cTc	p.R162L	

Table 6. ***(Continued 2)***

Cell line name	CCLE list	Variant_Classification	Variant_Type	dbSNP_RS	Genome_Change	cDNA_Change	Codon_Change	Protein_Change	Clinical significance
	PDE4DIP	Nonsense_Mutation	SNP	rs2762779	g.chr1:145075683C>T	c.180G>A	c.(178-180)tgG>tgA	p.W60*	
	PIK3C2B	Missense_Mutation	SNP		g.chr1:204410669T>C	c.3179A>G	c.(3178-3180)aAt>aGt	p.N1060S	
	PKHD1	Missense_Mutation	SNP	rs9381994	g.chr6:51483961T>C	c.12143A>G	c.(12142-12144)cAa>cGa	p.Q4048R	Benign
	PKHD1	Missense_Mutation	SNP	rs4715227	g.chr6:51491884T>C	c.11696A>G	c.(11695-11697)cAg>cGg	p.Q3899R	Benign
	PKHD1	Missense_Mutation	SNP	rs2435322	g.chr6:51875250A>C	c.5608T>G	c.(5608-5610)Ttg>Gtg	p.L1870V	Benign
	PKHD1	Missense_Mutation	SNP	rs9296669	g.chr6:51890823G>A	c.3785C>T	c.(3784-3786)gCt>gTt	p.A1262V	Benign
	PKHD1	Splice_Site	SNP	rs9370096	g.chr6:51914956G>A	c.2278C>T	c.(2278-2280)Cgc>Tgc	p.R760C	Benign
	RET	Missense_Mutation	SNP	rs9282834	g.chr10:43606856G>A	c.1465G>A	c.(1465-1467)Gac>Aac	p.D489N	Benign/Likely benign, not provided
	TET1	Missense_Mutation	SNP	rs10823229	g.chr10:70332580A>G	c.485A>G	c.(484-486)gAc>gGc	p.D162G	
	TP53	Frame_Shift_Del	DEL	rs587783063	g.chr17:7579364delC	c.323delG	c.(322-324)ggtfs	p.G108fs	Uncertain significance
	TRIM33	Missense_Mutation	SNP	rs6537825	g.chr1:114948281A>G	c.2519T>C	c.(2518-2520)aTT>aCt	p.I840T	
	WRN	Missense_Mutation	SNP	rs1801195	g.chr8:30999280G>T	c.3222G>T	c.(3220-3222)ttG>ttT	p.L1074F	Benign/Likely benign, not provided

Table 7. Sorted targeted sequencing data result for SNU-2663

Cell line name	CCLE list	Variant_Classification	Variant_Type	dbSNP_RS	Genome_Change	cDNA_Change	Codon_Change	Protein_Change	Clinical significance
SNU-2663	ADAMTS20	Missense_Mutation	SNP	rs117455456	g.chr12:43777630C>A	c.4603G>T	c.(4603-4605)Ggg>Tgg	p.G1535W	
	AFF3	Missense_Mutation	SNP	rs4851223	g.chr2:100343557C>T	c.1073G>A	c.(1072-1074)aGt>aAt	p.S358N	
	AFF3	Missense_Mutation	SNP		g.chr2:100625366G>A	c.82C>T	c.(82-84)Cgg>Tgg	p.R28W	
	AKAP9	Missense_Mutation	SNP	rs1063242	g.chr7:91714911C>T	c.8935C>T	c.(8935-8937)Cct>Tct	p.P2979S	Benign/Likely benign
	ALK	Missense_Mutation	SNP	rs1881421	g.chr2:29416366G>C	c.4587C>G	c.(4585-4587)gaC>gaG	p.D1529E	Benign
	ALK	Missense_Mutation	SNP	rs1881420	g.chr2:29416481T>C	c.4472A>G	c.(4471-4473)aAg>aGg	p.K1491R	Benign
	ALK	Missense_Mutation	SNP	rs1670283	g.chr2:29416572T>C	c.4381A>G	c.(4381-4383)Atc>Gtc	p.I1461V	Benign
	AURKA	Missense_Mutation	SNP	rs1047972	g.chr20:54961463T>C	c.169A>G	c.(169-171)Att>Gtt	p.I57V	risk factor
	AURKA	Missense_Mutation	SNP	rs2273535	g.chr20:54961541A>T	c.91T>A	c.(91-93)Ttt>Att	p.F31I	
	BAI3	Missense_Mutation	SNP	rs1932618	g.chr6:69666684A>G	c.1508A>G	c.(1507-1509)aAt>aGt	p.N503S	
	BCL9	Missense_Mutation	SNP	rs3820129	g.chr1:147091972C>T	c.2011C>T	c.(2011-2013)Cct>Tct	p.P671S	
	BLM	Missense_Mutation	SNP	rs7167216	g.chr15:91354521G>A	c.3568G>A	c.(3568-3570)Gta>Ata	p.V1190I	Benign/Likely benign, not provided
	BUB1B	Missense_Mutation	SNP	rs1801376	g.chr15:40477831G>A	c.1046G>A	c.(1045-1047)cGa>cAa	p.R349Q	Benign
	CSF1R	Missense_Mutation	SNP	rs10079250	g.chr5:149450132T>C	c.1085A>G	c.(1084-1086)cAc>cGc	p.H362R	Benign
	CSF1R	Missense_Mutation	SNP	rs3829986	g.chr5:149456893C>T	c.835G>A	c.(835-837)Gtg>Atg	p.V279M	Likely benign
	CSMD3	Missense_Mutation	SNP	rs2219898	g.chr8:114186003T>C	c.657A>G	c.(655-657)atA>atG	p.I219M	
	CTNNB1	Missense_Mutation	SNP	rs121913399|rs121913416	g.chr3:41266103G>A	c.100G>A	c.(100-102)Gga>Aga	p.G34R	Likely pathogenic
	DPYD	Missense_Mutation	SNP	rs1801265	g.chr1:98348885G>A	c.85C>T	c.(85-87)Cgt>Tgt	p.R29C	Pathogenic
	DST	Missense_Mutation	SNP	rs4715630	g.chr6:56417282C>T	c.15673G>A	c.(15673-15675)atG>atA	p.M5225I	
	DST	Missense_Mutation	SNP	rs4715631	g.chr6:56417545T>C	c.15412A>G	c.(15412-15414)Acc>Gcc	p.T5138A	
	DST	Missense_Mutation	SNP	rs11736977	g.chr6:56420538C>T	c.14108G>A	c.(14107-14109)cGt>cAt	p.R4703H	
	DST	Missense_Mutation	SNP	rs4712138	g.chr6:56463410T>C	c.11159A>G	c.(11158-11160)cAg>cGg	p.Q3720R	
	EGFR	Missense_Mutation	SNP	rs2227983	g.chr7:55229255G>A	c.1427G>A	c.(1426-1428)aGg>aAg	p.R476K	Benign/Likely benign, not provided
	EML4	Missense_Mutation	SNP	rs6736913	g.chr2:42510018A>G	c.847A>G	c.(847-849)Aaa>Gaa	p.K283E	
	EML4	Missense_Mutation	SNP	rs10202624	g.chr2:42515388A>G	c.1144A>G	c.(1144-1146)Att>Gtt	p.I382V	
	EML4	Missense_Mutation	SNP	rs28651764	g.chr2:42515437A>G	c.1193A>G	c.(1192-1194)aAg>aGg	p.K398R	
	EP300	Missense_Mutation	SNP	rs20551	g.chr22:41548008A>G	c.2989A>G	c.(2989-2991)Att>Gtt	p.I997V	Benign
	EPHA3	Missense_Mutation	SNP		g.chr3:89391021T>A	c.1087T>A	c.(1087-1089)Tgt>Agt	p.C363S	
	EPHA3	Missense_Mutation	SNP		g.chr3:89462383A>T	c.1855A>T	c.(1855-1857)Acc>Tcc	p.T619S	
	EPHA3	Missense_Mutation	SNP	rs35124509	g.chr3:89521693T>C	c.2770T>C	c.(2770-2772)Ttg>Ctg	p.W924R	
	ERBB3	Missense_Mutation	SNP		g.chr12:56477612G>A	c.160G>A	c.(160-162)Gag>Aag	p.E54K	
	ERCC5	Missense_Mutation	SNP	rs9514066	g.chr13:103527849G>C	c.3157G>C	c.(3157-3159)Gga>Cga	p.G1053R	not provided
	ERCC5	Missense_Mutation	SNP	rs587778291|rs9514067	g.chr13:103527930G>C	c.3238G>C	c.(3238-3240)Gga>Cga	p.G1080R	Likely pathogenic
	ESR1	Missense_Mutation	SNP		g.chr6:152332868G>A	c.1174G>A	c.(1174-1176)Gtc>Atc	p.V392I	
	FANCA	Missense_Mutation	SNP	rs7195066	g.chr16:89836323C>T	c.2426G>A	c.(2425-2427)gGt>gAt	p.G809D	Benign
	FANCA	Missense_Mutation	SNP	rs2239359	g.chr16:89849480C>T	c.1501G>A	c.(1501-1503)Ggc>Agc	p.G501S	Benign
	FANCA	Missense_Mutation	SNP	rs7190823	g.chr16:89866043T>C	c.796A>G	c.(796-798)Acg>Gcg	p.T266A	Benign
	FANCD2	Missense_Mutation	SNP	rs1450999733	g.chr3:10108987A>C	c.2480A>C	c.(2479-2481)gAa>gCa	p.E827A	Uncertain significance
	FGFR4	Missense_Mutation	SNP	rs1966265	g.chr5:176516631G>A	c.28G>A	c.(28-30)Gtc>Atc	p.V10I	

Table 7. ***(Continued 1)***

Cell line name	CCLE list	Variant Classification	Variant Type	dbSNP RS	Genome Change	cDNA Change	Codon Change	Protein Change	Clinical significance
	FGFR4	Missense_Mutation	SNP	rs376618	g.chr5:176517797C>T	c.407C>T	c.(406-408)cCc>cTc	p.P136L	
	FGFR4	Missense_Mutation	SNP	rs55675160	g.chr5:176518037A>G	c.535A>G	c.(535-537)Acg>Gcg	p.T179A	
	FGFR4	Missense_Mutation	SNP	rs351855	g.chr5:176520243G>A	c.1162G>A	c.(1162-1164)Ggg>Agg	p.G388R	Pathogenic
	FN1	Missense_Mutation	SNP	rs1250209	g.chr2:216235089C>T	c.6781G>A	c.(6781-6783)Gtc>Atc	p.V2261I	
	FN1	Missense_Mutation	SNP	rs2577301	g.chr2:216272900T>G	c.2449A>C	c.(2449-2451)Acg>Ccg	p.T817P	
	FN1	Missense_Mutation	SNP	rs1250259;rs201366026	g.chr2:216300482T>A	c.44A>T	c.(43-45)cAg>cTg	p.Q15L	
	IL6ST	Missense_Mutation	SNP	rs2228043	g.chr5:55251931G>C	c.1189C>G	c.(1189-1191)Cta>Gta	p.L397V	
	IL6ST	Missense_Mutation	SNP	rs2228044	g.chr5:55264153C>G	c.442G>C	c.(442-444)Ggt>Cgt	p.G148R	
	KIT	Missense_Mutation	SNP		g.chr4:55597580G>T	c.2228G>T	c.(2227-2229)aGa>aTa	p.R743I	
	LRP1B	Missense_Mutation	SNP		g.chr2:141641509A>T	c.4046T>A	c.(4045-4047)aTa>aAa	p.I1349K	
	MYH11	Missense_Mutation	SNP	rs16967494;rs7781052	g.chr16:15820863C>T	c.3700G>A	c.(3700-3702)Gca>Aca	p.A1234T	Benign;Likely benign
	MYH9	Missense_Mutation	SNP	rs2269529	g.chr22:36684354T>C	c.4876A>G	c.(4876-4878)Atc>Gtc	p.I1626V	Benign
	NCOA2	Missense_Mutation	SNP	rs2228591	g.chr8:71039118C>G	c.3846G>C	c.(3844-3846)atG>atC	p.M1282I	
	NIN	Missense_Mutation	SNP	rs144624455	g.chr14:51196324G>A	c.5995C>T	c.(5995-5997)Cgc>Tgc	p.R1999C	
	NIN	Missense_Mutation	SNP	rs2073347	g.chr14:51223789C>T	c.3959G>A	c.(3958-3960)gCg>gAg	p.G1320E	Benign
	NIN	Missense_Mutation	SNP	rs12882191	g.chr14:51224374T>G	c.3374A>C	c.(3373-3375)cAg>cCg	p.Q1125P	Benign
	NIN	Missense_Mutation	SNP	rs2236316	g.chr14:51224417G>C	c.3331C>G	c.(3331-3333)Cca>Gca	p.P1111A	Likely benign
	NLRP1	Missense_Mutation	SNP	rs2137722	g.chr17:5418799G>A	c.4096C>T	c.(4096-4098)Cgc>Tgc	p.R1366C	
	NLRP1	Missense_Mutation	SNP	rs11651270	g.chr17:5425077T>C	c.3550A>G	c.(3550-3552)Atg>Gtg	p.M1184V	Benign
	NSD1	Missense_Mutation	SNP	rs28932178	g.chr5:176637576T>C	c.2176T>C	c.(2176-2178)Tct>Cct	p.S726P	Benign
	NUP214	Missense_Mutation	SNP	rs103612	g.chr9:134020092C>T	c.1720C>T	c.(1720-1722)Ccc>Tcc	p.P574S	
	PDE4DIP	Missense_Mutation	SNP	rs148370554	g.chr1:144852477G>A	c.7277C>T	c.(7276-7278)cCa>cTa	p.P2426L	
	PDE4DIP	Missense_Mutation	SNP	rs78371650	g.chr1:144854581T>C	c.7144A>G	c.(7144-7146)Act>Gct	p.T2382A	
	PDE4DIP	Missense_Mutation	SNP	rs3863691	g.chr1:144854598C>T	c.7127G>A	c.(7126-7128)cGa>cAa	p.R2376Q	
	PDE4DIP	Missense_Mutation	SNP	rs1613780	g.chr1:144868850G>T	c.5985C>A	c.(5983-5985)gaC>gaA	p.D1995E	
	PDE4DIP	Missense_Mutation	SNP	rs1698605	g.chr1:144871738C>A	c.5479G>T	c.(5479-5481)Gct>Tct	p.A1827S	
	PDE4DIP	Missense_Mutation	SNP	rs1778159	g.chr1:144871755A>T	c.5462T>A	c.(5461-5463)gTg>gAg	p.V1821E	
	PDE4DIP	Missense_Mutation	SNP	rs1778158	g.chr1:144871782A>G	c.5435T>C	c.(5434-5436)cTc>cCc	p.L1812P	
	PDE4DIP	Missense_Mutation	SNP	rs1778155	g.chr1:144874815T>C	c.5201A>G	c.(5200-5202)cAt>cGt	p.H1734R	
	PDE4DIP	Missense_Mutation	SNP	rs2798901	g.chr1:144879264A>G	c.4594T>C	c.(4594-4596)Ttg>Ctg	p.W1532R	
	PDE4DIP	Missense_Mutation	SNP	rs1698647	g.chr1:144882823C>T	c.3607G>A	c.(3607-3609)Gca>Aca	p.A1203T	
	PDE4DIP	Missense_Mutation	SNP	rs1698624	g.chr1:144886197A>T	c.3448T>A	c.(3448-3450)Ttc>Atc	p.F1150I	
	PDE4DIP	Missense_Mutation	SNP	rs1629011	g.chr1:144912233C>T	c.2453G>A	c.(2452-2454)cGc>cAc	p.R818H	
	PDE4DIP	Missense_Mutation	SNP	rs1061308	g.chr1:144918957T>A	c.1640A>T	c.(1639-1641)gAa>gTa	p.E547V	
	PDE4DIP	Missense_Mutation	SNP	rs1359300	g.chr1:144922583G>A	c.1235C>T	c.(1234-1236)tCg>tTg	p.S412L	
	PDE4DIP	Missense_Mutation	SNP	rs1664022	g.chr1:144994658C>A	c.485G>T	c.(484-486)cGc>cTc	p.R162L	
	PDE4DIP	Missense_Mutation	SNP	rs11538401	g.chr1:145021150T>C	c.251A>G	c.(250-252)gAt>gGt	p.D84G	
	PKHD1	Nonsense_Mutation	SNP	rs2762779	g.chr1:145075683C>T	c.180G>A	c.(178-180)tgG>tgA	p.W60*	
	PKHD1	Missense_Mutation	SNP	rs9381994	g.chr6:51483961T>C	c.12143A>G	c.(12142-12144)cAa>cGa	p.Q4048R	Benign
	PKHD1	Missense_Mutation	SNP	rs2435322	g.chr6:51875250A>C	c.5608T>G	c.(5608-5610)Ttg>Gtg	p.L1870V	Benign

Table 7. ***(Continued 2)***

Cell line name	CCLE list	Variant_Classification	Variant_Type	dbSNP_RS	Genome_Change	cDNA_Change	Codon_Change	Protein_Change	Clinical significance
	PKHD1	Missense_Mutation	SNP	rs9296669	g.chr6:51890823G>A	c.3785C>T	c.(3784-3786)gCt>gTt	p.A1262V	Benign
	PKHD1	Splice_Site	SNP	rs9370096	g.chr6:51914956G>A	c.2278C>T	c.(2278-2280)Cgc>Tgc	p.R760C	Benign
	RET	Missense_Mutation	SNP	rs1924489011	g.chr10:43596033G>A	c.200G>A	c.(199-201)cGc>cAc	p.R67H	Conflicting interpretation s of pathogenicity, not pr ovided
	RET	Missense_Mutation	SNP	rs1799939	g.chr10:43610119G>A	c.2071G>A	c.(2071-2073)Ggt>Agt	p.G691S	Benign/Likely benign, no t provided
	RET	Missense_Mutation	SNP	rs17158558	g.chr10:43620335C>T	c.2944C>T	c.(2944-2946)Cgc>Tgc	p.R982C	Benign/Likely benign, no t provided, risk factor
	TCF3	Missense_Mutation	SNP	rs386805766[rs1052692	g.chr19:1619350C>T	c.1291G>A	c.(1291-1293)Ggc>Agc	p.G431S	
	TET1	Missense_Mutation	SNP	rs10823229	g.chr10:70332580A>G	c.485A>G	c.(484-486)gAc>gGc	p.D162G	
	TET2	Missense_Mutation	SNP	rs12498609	g.chr4:106155185C>G	c.86C>G	c.(85-87)cCt>cGt	p.P29R	not provided
	TP53	Splice_Site	SNP		g.chr17:7577610T>A	c.e7-2			
	TPR	Missense_Mutation	SNP	rs3753565	g.chr1:186316488C>T	c.2879G>A	c.(2878-2880)aGc>aAc	p.S960N	
	TRIM33	Missense_Mutation	SNP	rs6537825	g.chr1:114948281A>G	c.2519T>C	c.(2518-2520)aTr>aCt	p.I840T	
	TRIP11	Missense_Mutation	SNP	rs186074112	g.chr14:92505923T>A	c.107A>T	c.(106-108)gAt>gTt	p.D36V	Uncertain significance
	WRN	Missense_Mutation	SNP	rs1801195	g.chr8:30999280G>T	c.3222G>T	c.(3220-3222)ttG>ttT	p.L1074F	Benign/Likely benign, no t provided

Table 8. Sorted targeted sequencing data result for SNU-3058

Cell line name	CCLE list	Variant_Classification	Variant_Type	dbSNP_RS	Genome_Change	cDNA_Change	Codon_Change	Protein_Change	Clinical significance
SNU-3058	AFF3	Missense_Mutation	SNP	rs4851223	g.chr2:100345557C>T	c.1073G>A	c.(1072-1074)gGt>aAt	p.S358N	
	AKAP9	Missense_Mutation	SNP	rs1063242	g.chr7:91714911C>T	c.8935C>T	c.(8935-8937)Ccc>Tct	p.P2979S	Benign;Likely benign
	ALK	Missense_Mutation	SNP	rs1881421	g.chr2:29416366G>C	c.4587C>G	c.(4585-4587)gaC>gaG	p.D1529E	Benign
	ALK	Missense_Mutation	SNP	rs1881420	g.chr2:29416481T>C	c.4472A>G	c.(4471-4473)aAg>aGg	p.K1491R	Benign
	ALK	Missense_Mutation	SNP	rs1670283	g.chr2:29416572T>C	c.4381A>G	c.(4381-4383)Atc>Gtc	p.I1461V	Benign
	ARID1A	Frame_Shift_Ins	INS	rs377622327	g.chr1:27100175_27100176insC	c.3971_3972insC	c.(3970-3975)tacccch	p.YP1324fs	
	AURKA	Missense_Mutation	SNP	rs1047972	g.chr20:54961465T>C	c.169A>G	c.(169-171)Att>Gtt	p.I57V	
	AURKA	Missense_Mutation	SNP	rs2273535	g.chr20:54961541A>T	c.91T>A	c.(91-93)Ttt>Att	p.F31I	risk factor
	BAI3	Missense_Mutation	SNP	rs1932618	g.chr6:69666684A>G	c.1508A>G	c.(1507-1509)aAt>aGt	p.N503S	
	BCR	Missense_Mutation	SNP	rs1400504	g.chr22:23627369A>G	c.2387A>G	c.(2386-2388)aAt>aGt	p.N796S	
	CSF1R	Missense_Mutation	SNP	rs3829986	g.chr5:149456893C>T	c.835G>A	c.(835-837)Gtg>Atg	p.V279M	Likely benign
	CSMD3	Missense_Mutation	SNP	rs200867276	g.chr8:113275949G>T	c.9781C>A	c.(9781-9783)Cca>Aca	p.P3261T	
	CSMD3	Missense_Mutation	SNP	rs138226564	g.chr8:113349885C>T	c.6728G>A	c.(6727-6729)gGt>gAt	p.G2243D	
	CSMD3	Missense_Mutation	SNP	rs2219898	g.chr8:114186003T>C	c.657A>G	c.(655-657)atA>atG	p.I219M	
	DPYD	Missense_Mutation	SNP	rs18011159	g.chr1:97981395T>C	c.1627A>G	c.(1627-1629)Ata>Gta	p.I543V	Benign;Likely benign, not provided
	DPYD	Missense_Mutation	SNP	rs18011265	g.chr1:98348885G>A	c.85C>T	c.(85-87)Cgt>Tgt	p.R29C	Pathogenic
	DST	Missense_Mutation	SNP	rs80260070	g.chr6:56351972G>C	c.20017C>G	c.(20017-20019)Ctg>Gtg	p.L6673V	
	DST	Missense_Mutation	SNP	rs4715630	g.chr6:56417282C>T	c.15675G>A	c.(15673-15675)atG>atA	p.M5225I	
	DST	Missense_Mutation	SNP	rs4715631	g.chr6:56417545T>C	c.15412A>G	c.(15412-15414)Acc>Gcc	p.T5138A	
	DST	Missense_Mutation	SNP	rs4712138	g.chr6:56463410T>C	c.11159A>G	c.(11158-11160)cAg>cGg	p.Q3720R	
	EGFR	Missense_Mutation	SNP	rs2227983	g.chr7:55229255G>A	c.1427G>A	c.(1426-1428)gGg>aAg	p.R476K	Benign;Likely benign, not provided
	EML4	Missense_Mutation	SNP	rs6736913	g.chr2:42510018A>G	c.847A>G	c.(847-849)Aaa>Gaa	p.K283E	
	EML4	Missense_Mutation	SNP	rs10202624	g.chr2:42515388A>G	c.1144A>G	c.(1144-1146)Att>Gtt	p.I382V	
	EML4	Missense_Mutation	SNP	rs28651764	g.chr2:42515437A>G	c.1193A>G	c.(1192-1194)aAg>aGg	p.K398R	
	EP300	Missense_Mutation	SNP		g.chr22:41543915C>A	c.2206C>A	c.(2206-2208)Cat>Aat	p.H736N	
	EP300	Missense_Mutation	SNP	rs188035979	g.chr22:41574196A>G	c.6481A>G	c.(6481-6483)Atg>Gtg	p.M2161V	Likely benign
	EPHB6	In_Frame_Ins	INS	rs143667367?rs372769362	g.chr7:142562051_142562052insCCT	c.493_494insCCT	c.(493-495)ccc>cCCTcc	p.176_177insS	
	EPHB6	Missense_Mutation	SNP	rs8177146	g.chr7:142563253T>G	c.970T>G	c.(970-972)Tct>Gct	p.S324A	not provided
	ERCC5	Missense_Mutation	SNP	rs9514066	g.chr13:103527849G>C	c.3157G>C	c.(3157-3159)Gga>Cga	p.G1053R	
	ERCC5	Missense_Mutation	SNP	rs587778291?rs9514067	g.chr13:103527930G>C	c.3238G>C	c.(3238-3240)Gga>Cga	p.G1080R	Likely pathogenic
	ERCC5	Missense_Mutation	SNP	rs17655	g.chr13:103528002G>C	c.3310G>C	c.(3310-3312)Gat>Cat	p.D1104H	Benign;Likely benign, not provided
	FANCA	Missense_Mutation	SNP	rs7195066	g.chr16:89836323C>T	c.2426G>A	c.(2425-2427)gGt>gAt	p.G809D	Benign
	FANCA	Missense_Mutation	SNP	rs2239359	g.chr16:89849480C>T	c.1501G>A	c.(1501-1503)Ggc>Agc	p.G501S	
	FANCA	Missense_Mutation	SNP	rs7190823	g.chr16:89866043T>C	c.796A>G	c.(796-798)Acg>Gcg	p.T266A	
	FGFR4	Missense_Mutation	SNP	rs1966265	g.chr5:176516631G>A	c.28G>A	c.(28-30)Gtc>Atc	p.V10I	
	FGFR4	Missense_Mutation	SNP	rs376618	g.chr5:176517797C>T	c.407C>T	c.(406-408)cCc>cTc	p.P136L	
	FGFR4	Missense_Mutation	SNP	rs351855	g.chr5:176520243G>A	c.1162G>A	c.(1162-1164)Ggg>Agg	p.G388R	Pathogenic
	FN1	Missense_Mutation	SNP	rs1250209	g.chr2:216235089C>T	c.6781G>A	c.(6781-6783)Gtc>Atc	p.V2261I	
	FN1	Missense_Mutation	SNP	rs2577301	g.chr2:216272900T>G	c.2449A>C	c.(2449-2451)Acg>Ccg	p.T817P	
	FN1	Missense_Mutation	SNP	rs1250259?rs3366026	g.chr2:216300482T>A	c.44A>T	c.(43-45)cAg>cTg	p.Q15L	
	JAK1	Missense_Mutation	SNP		g.chr1:65305443G>T	c.2685C>A	c.(2683-2685)gaC>gaA	p.D895E	

Table 8. ***(Continued 1)***

Cell line name	CCLE list	Variant_Classification	Variant_Type	dbSNP_RS	Genome_Change	cDNA_Change	Codon_Change	Protein_Change	Clinical significance
	LIFR	Missense_Mutation	SNP	rs3729740	g.chr5:38498637C>T	c.1732G>A	c.(1732-1734)Gat>Aat	p.D578N	Benign
	LRP1B	Missense_Mutation	SNP	rs12990449	g.chr2:142567910T>C	c.143A>G	c.(142-144)cAg>cGg	p.Q48R	
	MYH9	Missense_Mutation	SNP	rs2269529	g.chr22:36684354T>C	c.4876A>G	c.(4876-4878)Atc>Gtc	p.I1626V	Benign
	NIN	Missense_Mutation	SNP	rs61755995	g.chr14:51219349G>A	c.4837C>T	c.(4837-4839)Cgt>Tgt	p.R1613C	Likely benign
	NLRP1	Missense_Mutation	SNP	rs2137722	g.chr17:5418799G>A	c.4096C>T	c.(4096-4098)Cgc>Tgc	p.R1366C	
	NLRP1	Missense_Mutation	SNP	rs11651270	g.chr17:5425077T>C	c.3550A>G	c.(3550-3552)Atg>Gtg	p.M1184V	Benign
	NOTCH1	Missense_Mutation	SNP	rs374230681	g.chr9:139402516T>C	c.3401A>G	c.(3400-3402)cAg>cGg	p.Q1134R	not provided
	NSD1	Missense_Mutation	SNP	rs28932178	g.chr5:176637576T>C	c.2176T>C	c.(2176-2178)Tct>Cct	p.S726P	Benign
	PDE4DIP	Missense_Mutation	SNP	rs148370554	g.chr1:144852477G>A	c.7277C>T	c.(7276-7278)cCa>cTa	p.P2426L	
	PDE4DIP	Missense_Mutation	SNP	rs78371650	g.chr1:144854581T>C	c.7144A>G	c.(7144-7146)Act>Gct	p.T2382A	
	PDE4DIP	Missense_Mutation	SNP	rs3863691	g.chr1:144854598C>T	c.7127G>A	c.(7126-7128)cGa>cAa	p.R2376Q	
	PDE4DIP	Missense_Mutation	SNP	rs1613780	g.chr1:144865850G>T	c.5985C>A	c.(5983-5985)gaC>gaA	p.D1995E	
	PDE4DIP	Missense_Mutation	SNP	rs1698605	g.chr1:144871738C>A	c.5479G>T	c.(5479-5481)Gct>Tct	p.A1827S	
	PDE4DIP	Missense_Mutation	SNP	rs1778159	g.chr1:144871755A>T	c.5462T>A	c.(5461-5463)gTg>gAg	p.V1821E	
	PDE4DIP	Missense_Mutation	SNP	rs1778158	g.chr1:144871782A>G	c.5435T>C	c.(5434-5436)cTc>cCc	p.L1812P	
	PDE4DIP	Missense_Mutation	SNP	rs1778155	g.chr1:144874815T>C	c.5201A>G	c.(5200-5202)cAt>cGt	p.H1734R	
	PDE4DIP	Missense_Mutation	SNP	rs2798901	g.chr1:144879264A>G	c.4594T>C	c.(4594-4596)Ttg>Ctg	p.W1532R	
	PDE4DIP	Missense_Mutation	SNP	rs1698647	g.chr1:144882823C>T	c.3607G>A	c.(3607-3609)Gca>Aca	p.A1203T	
	PDE4DIP	Missense_Mutation	SNP	rs1698624	g.chr1:144886197A>T	c.3448T>A	c.(3448-3450)Ttc>Atc	p.F1150I	
	PDE4DIP	Missense_Mutation	SNP	rs16290011	g.chr1:144912233C>T	c.2453G>A	c.(2452-2454)cGc>cAc	p.R818H	
	PDE4DIP	Missense_Mutation	SNP	rs1061308	g.chr1:144918957T>A	c.1640A>T	c.(1639-1641)gAa>gTa	p.E547V	
	PDE4DIP	Missense_Mutation	SNP	rs1359300	g.chr1:144922583G>A	c.1235C>T	c.(1234-1236)tCg>tTg	p.S412L	
	PDE4DIP	Missense_Mutation	SNP	rs1664022	g.chr1:144994658C>A	c.485G>T	c.(484-486)cGc>cTc	p.R162L	
	PDE4DIP	Nonsense_Mutation	SNP	rs2762779	g.chr1:145075683C>T	c.180G>A	c.(178-180)tgG>tgA	p.W60*	
	PIK3CG	Missense_Mutation	SNP	rs17847825	g.chr7:106509331C>A	c.1325C>A	c.(1324-1326)tCc>tAc	p.S442Y	
	PKHD1	Missense_Mutation	SNP	rs9381994	g.chr6:51483961T>C	c.12143A>G	c.(12142-12144)cAa>cGa	p.Q4048R	Benign
	PKHD1	Missense_Mutation	SNP	rs4715227	g.chr6:51491884T>C	c.11696A>G	c.(11695-11697)cAg>cGg	p.Q3899R	Benign
	PKHD1	Missense_Mutation	SNP	rs2435322	g.chr6:51875250A>C	c.5608T>G	c.(5608-5610)Ttg>Gtg	p.L1870V	Benign
	PKHD1	Missense_Mutation	SNP	rs92966669	g.chr6:51890823G>A	c.3783C>T	c.(3784-3786)gC>gT	p.A1262V	Benign
	PKHD1	Splice_Site	SNP	rs9370096	g.chr6:51914956G>A	c.2278C>T	c.(2278-2280)Cgc>Tgc	p.R760C	Benign
	PTPRD	Missense_Mutation	SNP	rs10977171	g.chr9:8518052G>C	c.1339C>G	c.(1339-1341)Cag>Gag	p.Q447E	
	RET	Missense_Mutation	SNP	rs1799939	g.chr10:43610119G>A	c.2071G>A	c.(2071-2073)Ggt>Agt	p.G691S	Benign/Likely benign, n ot provided
	TET1	Missense_Mutation	SNP	rs10823229	g.chr10:70332580A>G	c.485A>G	c.(484-486)gAc>gGc	p.D162G	
	TET1	Missense_Mutation	SNP	rs12773594	g.chr10:70332672T>A	c.577T>A	c.(577-579)Tcc>Acc	p.S193T	
	TET1	Missense_Mutation	SNP	rs12221107	g.chr10:70332862C>T	c.767C>T	c.(766-768)gCa>gTa	p.A256V	
	TET1	Missense_Mutation	SNP	rs16925541[rs71483917	g.chr10:70405539A>G	c.3053A>G	c.(3052-3054)aAt>aGt	p.N1018S	
	TNK2	Missense_Mutation	SNP	rs3747673	g.chr3:195611844G>A	c.295C>T	c.(295-297)Cgg>Tgg	p.R99W	
	TP53	Missense_Mutation	SNP		g.chr17:7578394T>C	c.536A>G	c.(535-537)cAt>cGt	p.H179R	
	TRIM33	Missense_Mutation	SNP	rs6537825	g.chr1:114948281A>G	c.2519T>C	c.(2518-2520)aTt>aCt	p.I840T	
	ZNF521	Nonsense_Mutation	SNP		g.chr18:22807056C>A	c.826G>T	c.(826-828)Gag>Tag	p.E276*	

Table 9. Sorted targeted sequencing data result for SNU-3059

Cell line name	CCLE list	Variant_Classification	Variant_Type	dbSNP_RS	Genome_Change	cDNA_Change	Codon_Change	Protein_Change	Clinical significance
SNU-3059	ABL2	Missense_Mutation	SNP	rs370175435	g.chr1:179077317C>A	c.3085G>T	c.(3085-3087)Gca>Tca	p.A1029S	
	ABL2	Missense_Mutation	SNP	rs56565202	g.chr1:179100604C>T	c.233G>A	c.(232-234)xGt>xAt	p.R78H	
	AFF3	Missense_Mutation	SNP	rs4851223	g.chr2:100343557C>T	c.1073G>A	c.(1072-1074)aGt>aAt	p.S358N	
	AKAP9	Missense_Mutation	SNP	rs1063242	g.chr7:91714911C>T	c.8935C>T	c.(8935-8937)Cct>Tct	p.P2979S	Benign/Likely benign
	ALK	Missense_Mutation	SNP	rs1881421	g.chr2:29416366G>C	c.4587C>G	c.(4585-4587)gaC>gaG	p.D1529E	Benign
	ALK	Missense_Mutation	SNP	rs1881420	g.chr2:29416481T>C	c.4472A>G	c.(4471-4473)aAg>aGg	p.K1491R	Benign
	ALK	Missense_Mutation	SNP	rs1670283	g.chr2:29416572T>C	c.4381A>G	c.(4381-4383)Atc>Gtc	p.I1461V	Benign
	AR	In_Frame_Del	DEL		g.chrX:66766365_66766374delGGCGCGGCGGCGGC	c.1375_1386delGGCGCGGCGGC	c.(1375-1386)ggcggcggcggc	p.GGGG467del	
	ARID1A	Nonsense_Mutation	SNP		g.chr1:27106915C>T	c.5875C>T	c.(5875-5877)Cag>Tag	p.Q1959*	
	ARNT	Missense_Mutation	SNP	rs2275237	g.chr1:150785811G>A	c.2111C>T	c.(2110-2112)cCt>cTt	p.P704L	
	AURKA	Missense_Mutation	SNP	rs1047972	g.chr20:54961463T>C	c.169A>G	c.(169-171)Att>Gtt	p.I57V	
	AURKA	Missense_Mutation	SNP	rs2273535	g.chr20:54961541A>T	c.91T>A	c.(91-93)Ttt>Att	p.F31I	risk factor
	BAI3	Missense_Mutation	SNP	rs1932618	g.chr6:69666684A>G	c.1508A>G	c.(1507-1509)aAt>aGt	p.N503S	
	BCR	Missense_Mutation	SNP	rs140504	g.chr22:23627369A>G	c.2387A>G	c.(2386-2388)aAt>aGt	p.N796S	
	CDH2	Missense_Mutation	SNP	rs17445840	g.chr18:25593694C>T	c.259G>A	c.(259-261)Gca>Aca	p.A87T	
	CSF1R	Missense_Mutation	SNP	rs10079250	g.chr5:149450132T>C	c.1085A>G	c.(1084-1086)cAc>cGc	p.H362R	Benign
	CSMD3	Missense_Mutation	SNP	rs2219898	g.chr8:114186003T>C	c.657A>G	c.(655-657)atA>atG	p.I219M	
	DPYD	Missense_Mutation	SNP	rs1801265	g.chr1:98348885G>A	c.85C>T	c.(85-87)Cgt>Tgt	p.R29C	Pathogenic
	DST	Missense_Mutation	SNP	rs4715630	g.chr6:56417282C>T	c.15675G>A	c.(15673-15675)atG>atA	p.M5225I	
	DST	Missense_Mutation	SNP	rs4715631	g.chr6:56417545T>C	c.15412A>G	c.(15412-15414)Acc>Gcc	p.T5138A	
	DST	Missense_Mutation	SNP	rs148547958	g.chr6:56426246A>G	c.13537T>C	c.(13537-13539)Tcc>Ccc	p.S4513P	
	EGFR	Missense_Mutation	SNP	rs2227983	g.chr7:55219255G>A	c.1427G>A	c.(1426-1428)aGg>aAg	p.R476K	Benign/Likely benign, not provided
	EML4	Missense_Mutation	SNP	rs67369913	g.chr2:42510018A>G	c.847A>G	c.(847-849)Aaa>Gaa	p.K283E	
	EML4	Missense_Mutation	SNP	rs10202624	g.chr2:42515388A>G	c.1144A>G	c.(1144-1146)Att>Gtt	p.I382V	
	EML4	Missense_Mutation	SNP	rs286517764	g.chr2:42515437A>G	c.1193A>G	c.(1192-1194)aAg>aGg	p.K398R	
	EPHA7	Missense_Mutation	SNP	rs1443369113	g.chr6:93969124G>T	c.1872C>A	c.(1870-1872)ttC>ttA	p.F624L	
	ERBB2	Missense_Mutation	SNP	rs11136201	g.chr17:37879588A>G	c.1963A>G	c.(1963-1965)Atc>Gtc	p.I655V	Benign
	ERCC5	Missense_Mutation	SNP	rs9514066	g.chr13:103527849G>C	c.3157G>C	c.(3157-3159)Gga>Cga	p.G1053R	not provided
	ERCC5	Missense_Mutation	SNP	rs587778291|rs9514067	g.chr13:103527930G>C	c.3238G>C	c.(3238-3240)Gga>Cga	p.G1080R	Likely pathogenic
	ERCC5	Missense_Mutation	SNP	rs17655	g.chr13:103528002G>C	c.3310G>C	c.(3310-3312)Gat>Cat	p.D1104H	Benign/Likely benign, not provided
	FANCA	Missense_Mutation	SNP	rs2014943 04	g.chr16:89805318G>A	c.4232C>T	c.(4231-4233)cGg>tGg	p.P1411L	Benign/Likely benign, not provided
	FANCA	Missense_Mutation	SNP	rs9282681	g.chr16:89805914T>C	c.3982A>G	c.(3982-3984)Acc>Gcc	p.T1328A	Benign/Likely benign, not provided
	FANCA	Missense_Mutation	SNP	rs7195066	g.chr16:89836323C>T	c.2426G>A	c.(2425-2427)gG t>gA t	p.G809D	Benign
	FANCA	Missense_Mutation	SNP	rs17232910	g.chr16:89839766G>C	c.1927C>G	c.(1927-1929)Ccc>Gcc	p.P643A	Benign/Likely benign, not provided
	FANCA	Missense_Mutation	SNP	rs2239359	g.chr16:89849480C>T	c.1501G>A	c.(1501-1503)Ggc>Agc	p.G501S	Benign
	FANCA	Missense_Mutation	SNP	rs11646374	g.chr16:89857935G>A	c.1235C>T	c.(1234-1236)gCg>gTg	p.A412V	Benign/Likely benign, not provided
	FANCA	Missense_Mutation	SNP	rs7190823	g.chr16:89866043T>C	c.796A>G	c.(796-798)Acg>Gcg	p.T266A	Benign
	FGFR4	Missense_Mutation	SNP	rs1966265	g.chr5:176516631G>A	c.28G>A	c.(28-30)Gtc>Atc	p.V10I	
	FN1	Missense_Mutation	SNP	rs1250209	g.chr2:216235089C>T	c.6781G>A	c.(6781-6783)Gtc>Atc	p.V2261I	
	FN1	Missense_Mutation	SNP		g.chr2:216237077G>C	c.6269C>G	c.(6268-6270)aCc>aGc	p.T2090S	

Table 9. ***(Continued 1)***

Cell line name	CCLE list	Variant_Classification	Variant_Type	dbSNP_RS	Genome_Change	cDNA_Change	Codon_Change	Protein_Change	Clinical significance
	FN1	Missense_Mutation	SNP	rs2577301	g.chr2:216272900T>G	c.2449A>C	c.(2449-2451)Acg>Ccg	p.T817P	
	FN1	Missense_Mutation	SNP	rs1250259/rs201366026	g.chr2:216300482T>A	c.44A>T	c.(43-45)cAg>cTg	p.Q15L	
	KIT	Missense_Mutation	SNP	rs3822214	g.chr4:55593464A>C	c.1621A>C	c.(1621-1623)Atg>Ctg	p.M541L	Benign/Likely benign, not provided
	LIFR	Missense_Mutation	SNP	rs3729740	g.chr5:38496637C>T	c.1732G>A	c.(1732-1734)Gat>Aat	p.D578N	Benign
	LTK	Missense_Mutation	SNP	rs148513655	g.chr15:41797721G>T	c.799C>A	c.(799-801)Cgc>Agc	p.R267S	
	MSH2	Missense_Mutation	SNP	rs56170584	g.chr2:47630344C>A	c.14C>A	c.(13-15)cCg>cAg	p.P5Q	Uncertain significance
	MYB	Missense_Mutation	SNP	rs373144674	g.chr6:135516937G>A	c.1000G>A	c.(1000-1002)Gac>Aac	p.D334N	
	MYH11	Missense_Mutation	SNP	rs16967494/rs387781052	g.chr16:15820863C>T	c.3700G>A	c.(3700-3702)Gca>Aca	p.A1234T	Benign/Likely benign
	MYH9	Missense_Mutation	SNP	rs2269529	g.chr22:36684354T>C	c.4876A>G	c.(4876-4878)Atc>Gtc	p.I1626V	Benign
	NIN	Missense_Mutation	SNP	rs61755995	g.chr14:51219349G>A	c.4837C>T	c.(4837-4839)Cgt>Tgt	p.R1613C	Likely benign
	NIN	Missense_Mutation	SNP	rs2073347	g.chr14:51223789C>T	c.3959G>A	c.(3958-3960)gGg>gAg	p.G1320E	Benign
	NIN	Missense_Mutation	SNP	rs12882191	g.chr14:51224374T>G	c.3374A>C	c.(3373-3375)cAg>cCg	p.Q1125P	Benign
	NOTCH1	Missense_Mutation	SNP	rs61751543	g.chr9:139401233C>T	c.3836G>A	c.(3835-3837)cGt>cAt	p.R1279H	Benign
	NUP214	Missense_Mutation	SNP	rs103612	g.chr9:134020092C>T	c.1720C>T	c.(1720-1722)Ccc>Tcc	p.P574S	
	PDE4DIP	Missense_Mutation	SNP	rs148370554	g.chr1:144852477G>A	c.7277C>T	c.(7276-7278)cCa>cTa	p.P2426L	
	PDE4DIP	Missense_Mutation	SNP	rs78371650	g.chr1:144854581T>C	c.7144A>G	c.(7144-7146)Act>Gct	p.T2382A	
	PDE4DIP	Missense_Mutation	SNP	rs3863691	g.chr1:144854598C>T	c.7127G>A	c.(7126-7128)cGa>cAa	p.R2376Q	
	PDE4DIP	Missense_Mutation	SNP	rs2798851	g.chr1:144859929C>T	c.6410G>A	c.(6409-6411)cGa>cAa	p.R2137Q	
	PDE4DIP	Missense_Mutation	SNP	rs71225704	g.chr1:144863401T>C	c.6257A>G	c.(6256-6258)gAg>gGg	p.E2086G	
	PDE4DIP	Missense_Mutation	SNP	rs1613780	g.chr1:144865850G>T	c.5985C>A	c.(5983-5985)gaC>gaA	p.D1995E	
	PDE4DIP	Missense_Mutation	SNP	rs1698597	g.chr1:144866688G>A	c.5809C>T	c.(5809-5811)Cgg>Tgg	p.R1937W	
	PDE4DIP	Missense_Mutation	SNP	rs1698605	g.chr1:144871738C>A	c.5479G>T	c.(5479-5481)Gct>Tct	p.A1827S	
	PDE4DIP	Missense_Mutation	SNP	rs1778159	g.chr1:144871755A>T	c.5462T>A	c.(5461-5463)gTg>gAg	p.V1821E	
	PDE4DIP	Missense_Mutation	SNP	rs1778158	g.chr1:144871782A>G	c.5435T>C	c.(5434-5436)cTc>cCc	p.L1812P	
	PDE4DIP	Missense_Mutation	SNP	rs1778155	g.chr1:144874815T>C	c.5201A>G	c.(5200-5202)cAt>cGt	p.H1734R	
	PDE4DIP	Missense_Mutation	SNP	rs1778120	g.chr1:144879090T>C	c.4768A>G	c.(4768-4770)Aaa>Gaa	p.K1590E	
	PDE4DIP	Missense_Mutation	SNP	rs2798901	g.chr1:144879264A>G	c.4594T>C	c.(4594-4596)Tgg>Cgg	p.W1532R	
	PDE4DIP	Missense_Mutation	SNP	rs1747960	g.chr1:144880814G>A	c.4222C>T	c.(4222-4224)Ctc>Ttc	p.L1408F	
	PDE4DIP	Missense_Mutation	SNP	rs12568796	g.chr1:144880832T>C	c.4204A>G	c.(4204-4206)Aag>Gag	p.K1402E	
	PDE4DIP	Missense_Mutation	SNP	rs2762877	g.chr1:144881583A>G	c.4024T>C	c.(4024-4026)Tca>Cca	p.S1342P	
	PDE4DIP	Missense_Mutation	SNP	rs1698647	g.chr1:144882823C>T	c.3607G>A	c.(3607-3609)Gca>Aca	p.A1203T	
	PDE4DIP	Missense_Mutation	SNP	rs1698624	g.chr1:144886197A>T	c.3448T>A	c.(3448-3450)Ttc>Atc	p.F1150I	
	PDE4DIP	Missense_Mutation	SNP	rs16290111	g.chr1:144912233C>T	c.2453G>A	c.(2452-2454)cGc>cAc	p.R818H	
	PDE4DIP	Missense_Mutation	SNP	rs1061308	g.chr1:144918957T>A	c.1640A>T	c.(1639-1641)gAa>gTa	p.E547V	
	PDE4DIP	Missense_Mutation	SNP	rs1359300	g.chr1:144922583G>A	c.1235C>T	c.(1234-1236)tCg>tTg	p.S412L	
	PDE4DIP	Missense_Mutation	SNP	rs1664022	g.chr1:144994658C>A	c.485G>T	c.(484-486)cGc>cTc	p.R162L	
	PDE4DIP	Nonsense_Mutation	SNP	rs2762779	g.chr1:145075683C>T	c.180G>A	c.(178-180)tgG>tgA	p.W60*	
	PDGFRA	Missense_Mutation	SNP	rs35597368	g.chr4:55139771T>C	c.1432T>C	c.(1432-1434)Tcc>Ccc	p.S478P	Benign
	PIK3C2B	Missense_Mutation	SNP	rs17847749	g.chr1:204419139G>C	c.2073C>G	c.(2071-2073)tgC>tgG	p.C691W	
	PKHD1	Missense_Mutation	SNP	rs9381994	g.chr6:51483961T>C	c.12143A>G	c.(12142-12144)cAa>cGa	p.Q4048R	Benign

Table 9. ***(Continued 2)***

Cell line name	CCLE list	Variant_Classification	Variant_Type	dbSNP_RS	Genome_Change	cDNA_Change	Codon_Change	Protein_Change	Clinical significance
	PKHD1	Missense_Mutation	SNP	rs4715227	g.chr6:51491884T>C	c.11696A>G	c.(11695-11697)cAg>cGg	p.Q3899R	Benign
	PKHD1	Missense_Mutation	SNP	rs2435322	g.chr6:51875250A>C	c.5608T>G	c.(5608-5610)Ttg>Gtg	p.L1870V	Benign
	TCF3	Missense_Mutation	SNP	rs386805766|rs1052692	g.chr19:1619350C>T	c.1291G>A	c.(1291-1293)Ggc>Agc	p.G431S	
	TET1	Missense_Mutation	SNP	rs108233229	g.chr10:70332580A>G	c.485A>G	c.(484-486)gAc>gGc	p.D162G	
	TP53	Frame_Shift_Del	DEL		g.chr17:7572991delT	c.1118delA	c.(1117-1119)aagfs	p.K373fs	
	TPR	Missense_Mutation	SNP	rs3753565	g.chr1:186316488C>T	c.2879G>A	c.(2878-2880)aGc>aAc	p.S960N	
	TRIM33	Missense_Mutation	SNP	rs6537825	g.chr1:114948281A>G	c.2519T>C	c.(2518-2520)aTt>aCt	p.I840T	
	WRN	Missense_Mutation	SNP	rs1801195	g.chr8:30999280G>T	c.3222G>T	c.(3220-3222)ttG>ttT	p.L1074F	Benign/Likely benign, not provided

Table 10. Sorted targeted sequencing data result for SNU-3160

Cell line name	CCLE list	Variant_Classification	Variant_Type	dbSNP_RS	Genome_Change	cDNA_Change	Codon_Change	Protein_Change	Clinical significance
SNU-3160	ABL2	Missense_Mutation	SNP		g.chr1:179078146A>C	c.2256T>G	c.(2254-2256)tT>tG	p.F752L	
	AFF3	Missense_Mutation	SNP	rs4851223	g.chr2:100343557C>T	c.1073G>A	c.(1072-1074)gGt>aAt	p.S358N	
	ALK	Missense_Mutation	SNP	rs1881420	g.chr2:29416481T>C	c.4472A>G	c.(4471-4473)aAg>aGg	p.K1491R	Benign
	AR	In_Frame_Del	DEL		g.chrX:66765228_667652 42delAGAGACTAGCCC CAG	c.240_254delAG AGACTAGCCC CAG	c.(238-255)caaggagactagccccca ggc>cag	p.ETSPR81del	
	ATR	Missense_Mutation	SNP		g.chr3:142242908T>G	c.4079A>C	c.(4078-4080)gAa>gCa	p.E1360A	
	BAI3	Missense_Mutation	SNP	rs1932618	g.chr6:69666684A>G	c.1508A>G	c.(1507-1509)aAt>aGt	p.N503S	
	BMPR1A	Missense_Mutation	SNP		g.chr10:88679084C>G	c.1024C>G	c.(1024-1026)Ctg>Gtg	p.L342V	
	BUB1B	Missense_Mutation	SNP	rs1801376	g.chr15:40477831G>A	c.1046G>A	c.(1045-1047)cGa>cAa	p.R349Q	Benign
	CDH2	Missense_Mutation	SNP	rs17445840	g.chr18:25593694C>T	c.259G>A	c.(259-261)Gca>Aca	p.A87T	
	CSF1R	Missense_Mutation	SNP		g.chr5:149435697G>A	c.2446C>T	c.(2446-2448)Cgc>Tgc	p.R816C	
	DST	Missense_Mutation	SNP	rs4712138	g.chr6:56463410T>C	c.11159A>G	c.(11158-11160)cAg>cGg	p.Q3720R	
	EML4	Missense_Mutation	SNP	rs6736913	g.chr2:42510018A>G	c.847A>G	c.(847-849)Aaa>Gaa	p.K283E	
	EML4	Missense_Mutation	SNP	rs10202624	g.chr2:42515388A>G	c.1144A>G	c.(1144-1146)Att>Gtt	p.I382V	
	ERCC5	Missense_Mutation	SNP	rs17655	g.chr13:103528002G>C	c.3310G>C	c.(3310-3312)Ggt>Cgt	p.D1104H	Benign,Likely benign, n ot provided
	FANCA	Missense_Mutation	SNP	rs7195066	g.chr16:89836323C>T	c.2426G>A	c.(2425-2427)gGt>gAt	p.G809D	Benign
	FN1	Missense_Mutation	SNP	rs2577301	g.chr2:216272900T>G	c.2449A>C	c.(2449-2451)Acg>Ccg	p.T817P	
	FN1	Missense_Mutation	SNP	rs1250259|rs201 366026	g.chr2:216300482T>A	c.44A>T	c.(43-45)cAg>cTg	p.Q15L	
	GNAS	Missense_Mutation	SNP		g.chr20:57429555A>G	c.1235A>G	c.(1234-1236)gAc>gGc	p.D412G	
	GNAS	Missense_Mutation	SNP	rs77059021	g.chr20:57429568A>C	c.1058A>C	c.(1057-1059)cAc>cCc	p.H353P	
	IGF2R	Missense_Mutation	SNP	rs629849	g.chr6:160494409A>G	c.4855A>G	c.(4855-4857)Agg>Ggg	p.R1619G	
	IGF2R	Missense_Mutation	SNP	rs1805075	g.chr6:160505207A>G	c.6059A>G	c.(6058-6060)aAc>aGc	p.N2020S	
	KIT	Missense_Mutation	SNP	rs3822214	g.chr4:55593464A>C	c.1621A>C	c.(1621-1623)Atg>Ctg	p.M541L	Benign,Likely benign, n ot provided
	LRP1B	Missense_Mutation	SNP	rs12990449	g.chr2:142567910T>C	c.143A>G	c.(142-144)cAg>cGg	p.Q48R	
	MSH6	Missense_Mutation	SNP	rs1042821	g.chr2:48010488G>A	c.116G>A	c.(115-117)gGg>gAg	p.G39E	Uncertain significance
	NIN	Missense_Mutation	SNP	rs2073347	g.chr14:51223789C>T	c.3959G>A	c.(3958-3960)gGg>gAg	p.G1320E	Benign
	NLRP1	Missense_Mutation	SNP	rs11651270	g.chr17:5425077T>C	c.3550A>G	c.(3550-3552)Atg>Gtg	p.M1184V	Benign
	PDE4DIP	Missense_Mutation	SNP	rs16986605	g.chr1:144871738C>A	c.5479G>T	c.(5479-5481)Gct>Tct	p.A1827S	
	PDE4DIP	Missense_Mutation	SNP	rs11778159	g.chr1:144871755A>T	c.5462T>A	c.(5461-5463)gTg>gAg	p.V1821E	
	PDE4DIP	Missense_Mutation	SNP	rs1061308	g.chr1:144918957T>A	c.1640A>T	c.(1639-1641)gAa>gTa	p.E547V	
	PDE4DIP	Frame_Shift_Del	DEL	rs3215779|rs369 106087	g.chr1:144923729delT	c.1140delA	c.(1138-1140)gaafs	p.E380fs	Benign
	PKHD1	Missense_Mutation	SNP	rs9381994	g.chr6:51483961T>C	c.12143A>G	c.(12142-12144)cAa>cGa	p.Q4048R	Benign
	PTPRD	Missense_Mutation	SNP	rs10977171	g.chr9:8518052G>C	c.1339C>G	c.(1339-1341)Cag>Gag	p.Q447E	
	RET	Missense_Mutation	SNP	rs1799939	g.chr10:43610119G>A	c.2071G>A	c.(2071-2073)Ggt>Agt	p.G691S	Benign,Likely benign, n ot provided
	TAL1	Missense_Mutation	SNP	rs743269	g.chr1:47691377C>G	c.184G>C	c.(184-186)Ggc>Cgc	p.G62R	
	TCF3	Missense_Mutation	SNP	rs2074888	g.chr19:1615796G>A	c.1475C>T	c.(1474-1476)gCg>gTg	p.A492V	
	TCF3	Missense_Mutation	SNP	rs386805766|rs1 052692	g.chr19:1619350C>T	c.1291G>A	c.(1291-1293)Ggc>Agc	p.G431S	
	TET2	Missense_Mutation	SNP		g.chr4:106157533A>G	c.2434A>G	c.(2434-2436)Ata>Gta	p.I812V	
	TRIM33	Missense_Mutation	SNP	rs6537825	g.chr1:114948281A>G	c.2519T>C	c.(2518-2520)aTt>aCt	p.I840T	
	WRN	Missense_Mutation	SNP	rs77969734	g.chr8:30949398C>G	c.1882C>G	c.(1882-1884)Cta>Gta	p.L628V	Benign
	WRN	Missense_Mutation	SNP	rs1801195	g.chr8:30999280G>T	c.3222G>T	c.(3220-3222)ttG>ttT	p.L1074F	Benign,Likely benign, n ot provided

Table 11. Summary of targeted sequencing result by mutated genes.

CCLE list	Variant_Classification	#
ABL2	Missense_Mutation	2
ADAMTS20	Missense_Mutation	2
AFF3	Missense_Mutation	7
AKAP9	Missense_Mutation	6
ALK	Missense_Mutation	7
AR	Start_Codon_Del	3
ARID1A	Frame_Shift_Ins	1
ARID1A	Nonsense_Mutation	1
ARNT	Missense_Mutation	2
ATM	Missense_Mutation	1
ATR	Missense_Mutation	4
ATRX	Missense_Mutation	2
AURKA	Missense_Mutation	6
BAI3	Missense_Mutation	7
BCL9	Missense_Mutation	1
BCR	Missense_Mutation	5
BLM	Missense_Mutation	2
BMPR1A	Missense_Mutation	1
BUB1B	Missense_Mutation	4
CARD11	Missense_Mutation	1
CASC5	Missense_Mutation	2
CDH2	Missense_Mutation	4
CSF1R	Missense_Mutation	6
CSMD3	Missense_Mutation	6
CTNNB1	Missense_Mutation	1
DPYD	Missense_Mutation	6
DST	Missense_Mutation	7
EGFR	Missense_Mutation	6
EML4	Missense_Mutation	7
EP300	Missense_Mutation	3
EPHA3	Missense_Mutation	1
EPHA7	Splice_Site	1
EPHA7	Missense_Mutation	2
EPHB6	In_Frame_Ins	1
EPHB6	Missense_Mutation	1
ERBB2	Missense_Mutation	2
ERBB3	Missense_Mutation	1
ERBB4	Missense_Mutation	1
ERCC5	Missense_Mutation	7
ESR1	Missense_Mutation	2
FANCA	Missense_Mutation	7
FANCD2	Missense_Mutation	1
FGFR3	Missense_Mutation	1
FGFR4	Missense_Mutation	6
FN1	Missense_Mutation	7
GNAS	Missense_Mutation	1
HIF1A	Missense_Mutation	1
IGF2R	Missense_Mutation	1
IKBKB	Splice_Site	1
IL6ST	Missense_Mutation	1
JAK1	Missense_Mutation	1
KDM6A	Missense_Mutation	1
KDR	Missense_Mutation	3
KIT	Missense_Mutation	4
LAMP1	Missense_Mutation	1
LIFR	Missense_Mutation	3
LRP1B	Missense_Mutation	5
LTK	Missense_Mutation	1
MAML2	Missense_Mutation	1
MET	Missense_Mutation	1
MN1	Missense_Mutation	1
MSH2	Missense_Mutation	1
MSH6	Missense_Mutation	1
MTOR	Missense_Mutation	1
MYB	Missense_Mutation	1
MYH11	Missense_Mutation	5
MYH9	Missense_Mutation	5
NCOA2	Missense_Mutation	3
NIN	Missense_Mutation	6
NLRP1	Missense_Mutation	6
NOTCH1	Missense_Mutation	3
NOTCH2	Missense_Mutation	2
NSD1	Missense_Mutation	4
NTRK3	Missense_Mutation	1
NUMA1	Missense_Mutation	1
NUP214	Missense_Mutation	4
PDE4DIP	Missense_Mutation	7
PDE4DIP	Nonsense_Mutation	6
PDE4DIP	Frame_Shift_Del	1
PDGFRA	Missense_Mutation	1
PIK3C2B	Missense_Mutation	2
PIK3CG	Missense_Mutation	1
PKHD1	Missense_Mutation	7
PKHD1	Splice_Site	5
PTPRD	Missense_Mutation	2
PTPRT	Missense_Mutation	1
RET	Missense_Mutation	4
SMARCA4	Splice_Site	1
STK11	Missense_Mutation	1
TAL1	Missense_Mutation	1
TCF3	Missense_Mutation	5
TET1	Missense_Mutation	5
TET2	Missense_Mutation	4
TNK2	Missense_Mutation	2
TP53	Missense_Mutation	2
TP53	Frame_Shift_Del	3
TP53	Splice_Site	1
TPR	Splice_Site	1
TPR	Missense_Mutation	2
TRIM33	Missense_Mutation	7
TRIP11	Missense_Mutation	2
UBR5	Missense_Mutation	1
WRN	Missense_Mutation	5
XPC	Missense_Mutation	1
ZNF521	Nonsense_Mutation	1

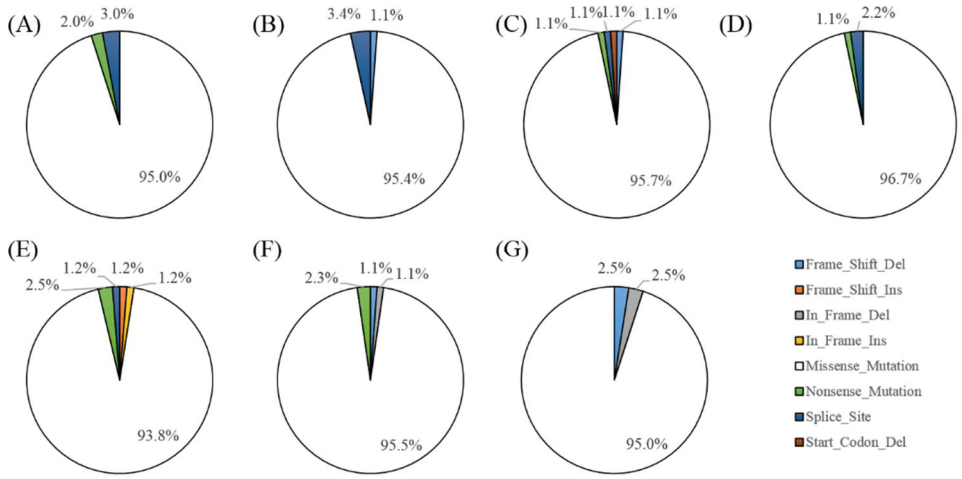

Figure 5. Mutations found by comprehensive cancer panel in each HCC cell lines. All 7 HCC cell lines were mainly mutated by missense mutation, which occupies 95.35% of all mutations. Other types of mutations such as frame shift deletions, insertions, in-frame deletions, insertions, missense mutations, nonsense mutations, splice site mutations and start codon deletions only occupy total of 4.65% all together.

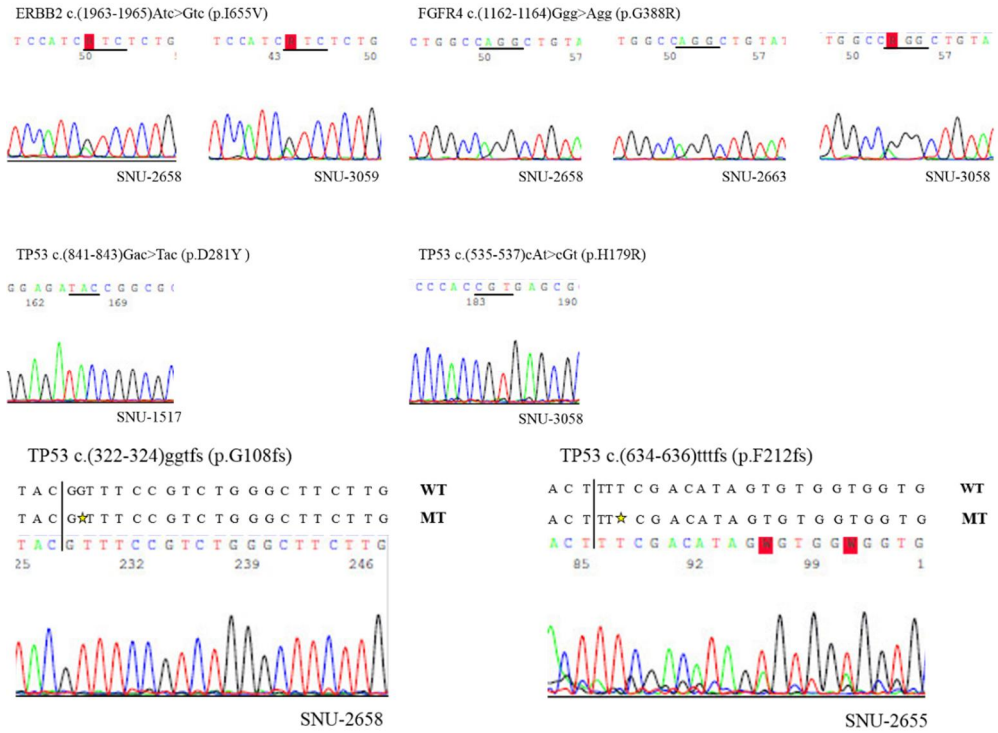

Figure 6. Validation of selected mutations obtained by targeted sequencing. ERBB2 c.(1963-1965)Atc>Gtc (p.I655V), FGFR4 c.(1162-1164)Ggg>Agg (p.G388R), TP53 c.(322-324)ggtfs (p.G108fs), TP53 c.(535-537)cAt>cGt (p.H179R), TP53 c.(634-636)tttfs (p.F212fs), and TP53 c.(841-843)Gac>Tac (p.D281Y) were tested and the result agreed with the targeted sequencing data. For SNU-2655 and SNU-2658 frame shift deletion mutations, WT indicates wild type allele and MT indicates the mutant allele with their deleted nucleotide marked with a star ( ).

Sensitivity to chemotherapeutic agents of HCC cell lines.

Seven HCC cell lines responded to cisplatin and sorafenib without significant divergence in their responses. Average EC50 of each cell lines (Table 12) and sigmoidal graphs (Figure 7, 8) were achieved by Graphpad Prism software. For 5-FU, EC 50 value of 3.698, 9.759, 0.997, 4.540, 5.404, 7174, and 5211.5 were acquired for SNU-1517, SNU-2655, SNU-2658, SNU-2663, SNU-3058, SNU-3059, and SNU-3160 respectively. For cisplatin, EC50 values of 489.250, 338.333, 336.433, 442.800, 218.967, 578.333, and 496.100 were obtained. SNU-1517 had average EC50 value of 2.369, SNU-2655 had 1.759, and SNU-2658 had 1.615, SNU-2663 1.294, 2.176 for SNU-3059, and 1.959 for SNU-3160 for sorafenib. Average EC50 value on sorafenib for SNU-3058 was not calculated as its EC50 values of drug test trials were ambiguous due to cell line conditions.

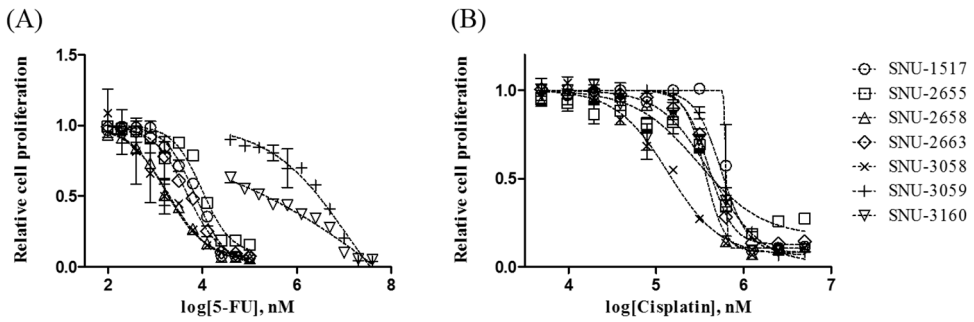

Figure 7. Sigmoidal graph representing 5-FU and cisplatin responses of HCC cell lines. SNU-3059 and SNU-3160 showed relatively resistant responses to 5-FU (A) whereas all 7 cell lines showed similar responses to cisplatin (B).

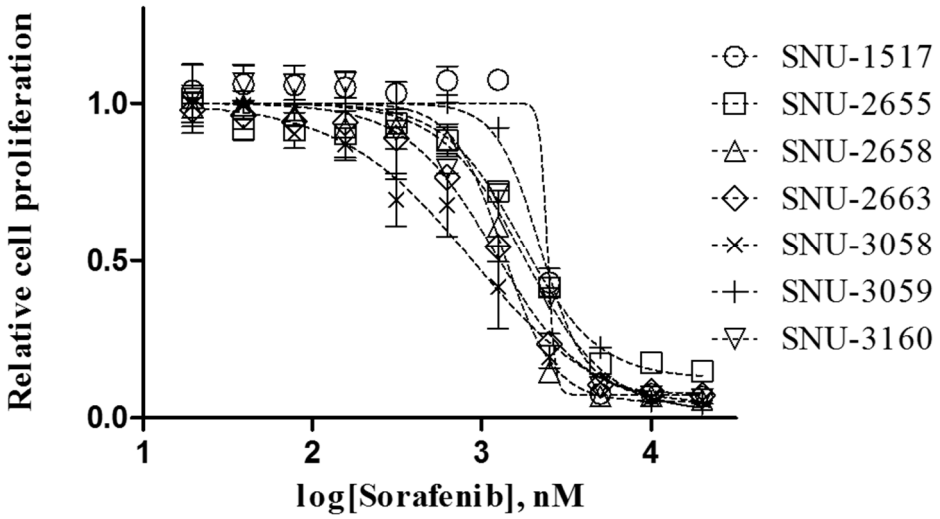

Figure 8. Sigmoidal graph representing sorafenib responses of HCC cell lines. SNU-1517 has the highest EC50 value which indicates resistance to sorafenib treatment, whereas SNU-3059 has the lowest average EC50 value indicating sensitivity to sorafenib. However this result seems to be insignificant since sigmoidal graphs for each cell lines were plotted within the same range of sorafenib concentration.

Table 12. EC50 values to 5-FU, cisplatin, and sorafenib of HCC cell lines

	5-FU	Cisplatin	Sorafenib
SNU-1517	3.698 ± 2.978	489.250 ± 29.769	2.369 ± 0.369
SNU-2655	9.759	338.333 ± 39.177	1.759 ± 0.068
SNU-2658	0.997 ± 0.712	336.433 ± 28.166	1.615 ± 0.340
SNU-2663	4.540 ± 3.45	442.800 ± 94.118	1.294 ± 0.101
SNU-3058	5.404 ± 5.279	218.967 ± 136.196	
SNU-3059	7174 ± 5726.151	578.333 ± 99.972	2.176 ± 0.047
SNU-3160	5211.500 ± 4083.542	496.100 ± 75.156	1.959 ± 0.198

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Western blot analysis of sorafenib treated HCC cell lines.

Time and concentration dependent effect of sorafenib yielded no reliable data. Western blot result yielded varying levels of sorafenib effect on each cell lines. SNU-2655, SNU-2658, and SNU-3058 showed very little basal p-ERK expression. SNU-3059 showed very slight change in protein level in all concentrations therefore the effect of sorafenib was difficult to determine. On the other hand, SNU-1517, SNU-2663, and SNU-3160 clearly expressed decrease in p-ERK level as concentration of sorafenib increase. Sorafenib treatments did not effect on ERK2 and Kras protein level, noting that SNU-2658 only had slight ERK 2 expression even before the sorafenib treatment.

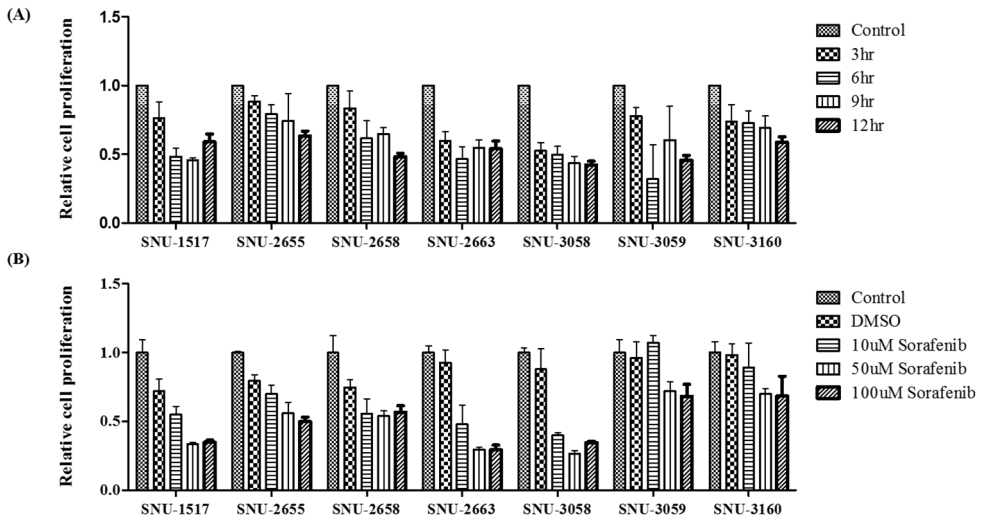

Figure 9. Sorafenib responses of hepatocellular carcinoma cell lines depending on time and concentration.

Cell viability was measured after sorafenib treatment. (A) shows responses of each cell lines after treatment of 20μM sorafenib. Cell viability was measured after 0, 3, 6, 9, 12 hours to see the time dependent differences in response to sorafenib of each HCC cell lines. (B) represents the responses of each cell lines after treatment of different concentrations of sorafenib. Measurements were taken after 3 hours of treatment to observe concentration dependent differences of each cell lines

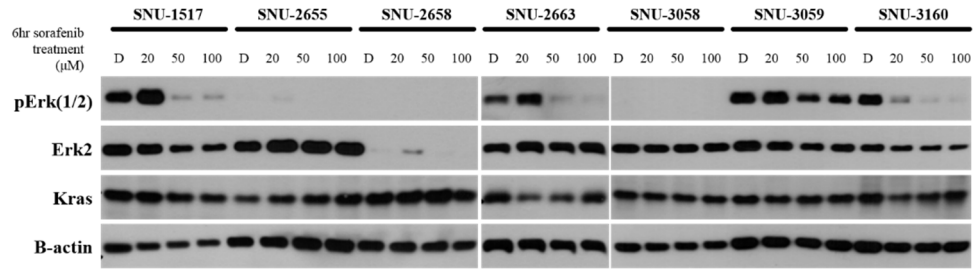

Figure 10. Concentration dependent effect of sorafenib on HCC cell lines. Each sorafenib treated cell lines showed different levels of protein change. SNU-1517, SNU-2663, and SNU-3160 showed decreased level of phospho-ERK as concentration of sorafenib increase. SNU-3059 showed mere change in protein expression while SNU-2655, SNU-2658 and SNU-3058 had very slight expression of p-ERK even before the sorafenib treatment. Sorafenib treatments seems to have no effect on ERK2 and Kras protein level, with exception of SNU-2658, which showed mere basal expression of ERK2.

Individual and combination effect of phytochemicals.

Four phytochemicals, baicalein, curcumin, genistein, and resveratrol were treated on HCC cell lines to test its effect. Sigmoidal graph was plotted for baicalein when treated with maximum concentration of 200μM, curcumin with 1000μM, genistein with 0.2μM, and resveratrol with 200μM (Figure 11). For combination drug treatment of each phytochemicals with sorafenib, no prominent synergetic effects were shown, with their Combination index (CI) value above 0.5.

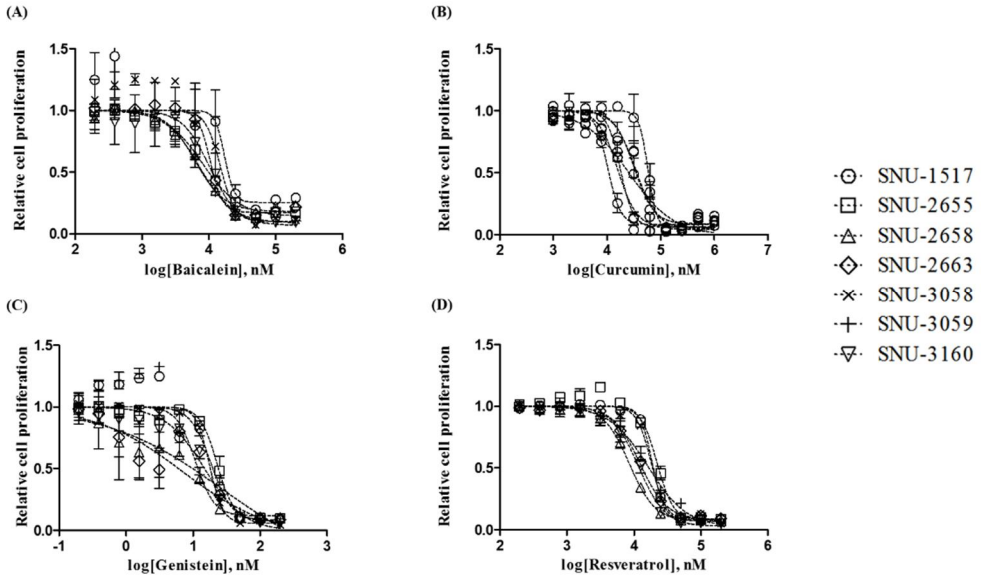

Figure 11. Sensitivity of HCC cell lines to phytochemicals. Cell proliferation assay was carried out after treatment of each phytochemicals and results are as shown. (A) is the result for baicalein, (B) for curcumin, (C) for genistein, and (D) for resveratrol.

Discussion

Hepatocellular carcinoma (HCC) is currently ranked as the third most deadly cancer worldwide [17]. In South Korea, a statistical study of cancer related death in 2014 placed liver cancer as third most deadly cancer [18]. One of the most prevalent cause of HCC in Southeast Asia is liver cirrhosis caused by HBV infection. A large fraction of world population is infected with chronic HBV and many of them develop liver cirrhosis which frequently eventually progresses to HCC. Common approaches to HCC treatments include surgical resection, liver transplantation, radiofrequency ablation, and sorafenib treatment. However, due to heterogeneity of HCC, these treatments do not guarantee patients with the absolute survival [19, 20]. Studies to find alternative therapeutic agents are ongoing, several of them studying possible therapeutic effects of phytochemicals on cancer. According to these studies, phytochemicals seem to have promising effect at interrupting cancer progression while opposing opinions still exists

To overcome complications in HCC therapy, more *in vivo* and *in vitro* studies are much needed. In this work, 7 SNU HCC cell lines derived from Korean patients, SNU-1517, SNU-2655, SNU-2658, SNU-2663, SNU-3058, SNU-3059, and SNU-3160 were characterized on their morphology, growth

rate, drug sensitivity, and HBV expression to provide apparatus for future studies.

Result of growth property experiment showed varying degree in growth rate between HCC cell lines. Doubling times for SNU-1517, SNU-2655, SNU-2658, SNU-2663, SNU-3058, SNU-3059, and SNU-3160 were 59.45, 43.64, 51.61, 51.95, 42.93, 63.26, 74.59 hours correspondingly.

DNA fingerprinting with 16 loci, D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, Vwa, TPOX, D18S51, Amelogenin, D5S818, and FGA were performed to rigidify each cell lines and the result confirmed that all cell lines had no cross contamination.

To verify mutation status of HCC cell lines, targeted sequencing result showed that SNU-2658 and SNU-3058 had the most diverse mutation list where SNU-2658 contained of 1.1% frame shift deletion, 1.1% nonsense mutation, 1.1% splice site mutation, 1.1% start codon deletion and 95.7% missense mutations and SNU-3058 mutations consists with 1.2% frame shift insertion, 1.2% in frame insertion, 2.5% non-sense mutations 1.2% splice site mutation and 93.8% of missense mutations while other five cell lines, SNU-1517 DNA were composed of 2% nonsense mutation, 3% splice site

mutations, and 95% missense mutation. SNU-2655 had 3.4% splice site mutations, 1.1% frame shift deletion mutation, and 95.4% missense mutations. SNU-2663 consists of 1.1% nonsense mutations, 2.2% splice site mutations, 96.7% missense mutations. SNU-3059 had 2.3% nonsense mutations, 1.1% frame shift deletion, 1.1% in-frame deletion and 95.5% missense mutations. SNU-3160 had 2.5% frame shift deletion, 2.5% in-frame deletion, and 95.0% missense mutations.

Overall, missense mutations were observed most frequently (95.35%) followed by splice site mutations (1.72%) nonsense (1.38%) frame shift deletion (0.69%) and in-frame deletion (0.34%). Frame shift insertion in-frame insertion start codon deletion each took up 0.17% of total mutations.

Sanger sequencing of ERBB2 c.(1963-1965)Atc>Gtc (p.I655V), FGFR4 c.(1162-1164)Ggg>Agg (p.G388R), TP53 c.(322-324)ggtfs (p.G108fs), TP53 c.(535-537)cAt>cGt (p.H179R) TP53 c.(634-636)tttfs (p.F212fs), TP53 c.(841-843)Gac>Tac (p.D281Y) was carried out and the result corresponds with the targeted sequencing data (Figure 3).

Result of HBV PCR showed that only 3 cell lines (SNU-1517, SNU-2655 and SNU-3058) out of 7 have integrated HBV DNA in their genome. This outcome is rather unique since majority of HCC cases in Asia-Pacific region

were known to be the chronic carriers of HBV [21]. Considering that all previously established HCC cell lines from Korean patients are HBV positive, these newly established HBV negative HCC cell lines are expected to be useful resources for future studies. In addition, Sanger sequencing was proceeded for genotyping. HBV sequences were also identified by Sanger sequencing and these sequences were inputted in the online database called HBV Blast Search for the genotyping. Both HBV positive cell lines, SNU-2655 and SNU-3058, matched HBV type C nucleotide sequence by 96 and 97% respectively.

Sorafenib, the widely used anti-cancer drug for HCC, which is known to be the multi kinase inhibitor of Ras-Raf-MEK-ERK signaling pathway, was treated on HCC cell lines and its EC50 values were calculated and graphs were plotted for this study. No definite variations to sorafenib response was shown where relatively sensitive cell line, SNU-3059, yielded EC50 value of 0.0471487 and relatively resistant HCC cell line, SNU-1517, yielded 0.3691097. However, when the response to sorafenib was examined at protein level via western blot analysis, change was more evident where cell lines such as SNU-1517 showed clear decrease in pERK level after sorafenib treatment while SNU-3059 showed very little or no change in pERK

expression which provides evidence in divergent responses of HCC cell lines to sorafenib. In order to find the possible drug that can partner up with sorafenib to improve its effect, four phytochemicals, baicalein, curcumin, genistein, and resveratrol were tested for their anti-cancer effect. Curcumin is known to work against hepatotoxic aflatoxin [10, 11]. Resveratrol and baicalein is known to decrease HCC proliferation [12] and genistein is known to inhibit TGF- β/Smad pathway. All four agents successfully decreased cell viability. However, its combination index was calculated by CompySyn software and the result showed no synergetic effect of sorafenib and phytochemicals. However, further study of phytochemicals and their effects on HCC deserve more attention.

Change in protein expression after the sorafenib treatments were also examined, and response level of sorafenib of each HCC cell lines were different. SNU-1517, SNU-2663, and SNU-3160 showed decreased level in p-ERK as concentration of sorafenib increase. SNU-3059 expressed slight change in protein level, while SNU-2655, SNU-2658 and SNU-3058 had little expression of p-ERK originally. Sorafenib treatments seems to have no effect on ERK2 and Kras protein level, with exception of SNU-2658, which

showed very low level of ERK2 expression even without the sorafenib treatment.

In this study, 7 HCC cell lines established from Korean patients were characterized as the ground work for further studies to develop better management method of HCC. Further and more in depth study of HCC would be required for complete control of this disease, as it is one of the difficult types of cancer to treat yielding very low survival rate for the patients.

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국문 초록

간세포암은 세계적으로 가장 사망률이 높은 암 중 하나이다. 치료 방법으로는 수술적 절제, 이식, sorafenib 등을 이용한 항암치료 등이 병행되고 있지만 효율이 낮아 이를 극복하고자 관련 연구들이 활발히 진행되고 있다. 간세포암 관련 연구에 기반을 다지고자 본 논문에서는 인체유래 간암세포주 SNU-1517, SNU-2655, SNU-2658, SNU-2663, SNU-3058, SNU-3059, SNU-3160 의 특성 분석 연구를 진행하였다. 각각의 세포주마다 성장 속도가 다르게 나타났으며 가장 느리게 자라는 세포주는 두 배로 증식하기까지 74.59 시간이 걸렸고 가장 빠르게 자라는 세포주는 42.93 시간이 걸렸다. 각 세포주를 동정하고, 상호 오염 여부 확인을 위하여 16 개의 STR loci 를 이용한 DNA 지문분석실험을 하였다. 409 개의 유전자로

이루어진 comprehensive cancer panel 을 활용한 타깃 시퀀싱을 이용하여 각각의 세포주에 대한 돌연변이 검사를 하였다. 이 검사 결과로 얻은 돌연변이 목록은 돌연변이의 종류 또는 임상적 영향에 따라 분류하였고 이 중 몇 가지 유전자 돌연변이를 골라 생거시퀀싱으로 결과의 정확성을 인증하였다. 총 일곱 개의 세포주의 HBV 감염 여부를 확인 한 결과, 세 개의 세포주 (SNU-1517, SNU-2655, SNU-3058) 에서 B 형 간염 바이러스가 양성이었으며 생거시퀀싱을 통한 유전형질 분석을 진행하였다. 간암 세포주에 5-FU, cisplatin, sorafenib 을 처리하여 항암제 감수성 및 세포 증식 속도를 확인하였으며, 항암제에 대한 반응을 확인하고자 간암치료에 가장 많이 쓰이는 sorafenib 처리 후 농도에 따른 세포 내 단백질 발현양의 차이를 확인했다. 세포주에 따라 반응 정도에 차이가 있었지만 sorafenib 이 SNU-1517, SNU-2663, 그리고 SNU-3160 에서 Ras-Raf-MEK-ERK 신호전달 체계에 영향을 준다는 것을 확인 할 수 있었다. 또한, 식물추출물로서 항암작용이 있다고 알려진 네 가지 피토케미칼, baicalein, curcumin, genistein, resveratrol 이 간암세포주의 증식에 미치는 영향을 피토케미칼

자체와 더불어 sorafenib 의 항암제 민감성을 증진시키기 위한 목적으로 실험하였다. 피토케미칼을 단독으로 혹은 sorafenib 과 함께 처리한 결과, 피토케미칼이 일정농도에서 암세포 증식을 억제하였으나 sorafenib 과의 상승효과는 나타나지 않았다.

본 논문에서 확인한 간암 세포주의 성장 특징, 돌연변이 목록, B 형 바이러스 감염여부와 유전형질, 항암제와 피토케미칼의 세포 증식 감소 효과, 세포 내 단백질 발현 변화, sorafenib 과 각 피토케미칼들에 대한 상승효과 확인 결과가 후속 연구에 활용되어 도움이 될 것이라고 생각된다.

주요어: 간세포암, 간암 세포주, 특성 분석, **sorafenib**, **B** 형 간염, 피토케미칼