이학 석사 학위논문

Establishment and Characterization of 26 Human Pancreatic Cancer Cell Lines: Pathway Approach and Mutation Analysis Related to Drug Reactivity

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Establishment and Characterization of 26 Human Pancreatic Adenocarcinoma Cell Lines: Pathway Approach and Mutation Analysis Related to Drug Reactivity

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ABSTRACT

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Pancreatic cancer is one of the most fatal malignancies, with a low survival rate of 9% for a patient's 5-year average survival rate. Pancreatic cancer is difficult to elucidating mutational characteristics due to the high heterogeneity between tumors. We established 26 different pancreatic cancer cell lines and performed whole exome sequencing, RNA sequencing and western blotting to confirm the mutational properties and protein expression of each cell line and examine the anti-cancer drug sensitivity. KRAS mutations, known to be representative of pancreatic cancer, were found in codon 12 a total of in 22 of the 26 cell lines, and four cell line didn’t harbor KRAS mutations. Pancreatic cancer cell lines with the wild type KRAS displays good response to anti-cancer drugs, except for the SNU-3752 cell line, and protein expression and different genetic factors

contribute to this difference. Other mutations in the KRAS wild-type cell lines were found to be related to signal transduction about composition and degradation of the extracellular matrix. High inter-tumor heterogeneity was observed in KRAS mutant cell lines in terms of anti-cancer drug differences and protein expression. For instance, a strong positive correlation between trametinib sensitivity and p-ERK 1/2 protein expression levels in KRAS mutant cell lines was confirmed. HER2 over-expressing pancreatic cancer cell lines generally showed high resistance to anti-cancer drugs, and exhibited various results according to differences in genetic variations and expression of certain proteins. Based on these results, anti-cancer drug reactivity can be predicted by associating each mutant property and protein expression level.

Keywords: Pancreatic cancer, Drug sensitivity, Mutational characteristics

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1. Introduction

The pancreatic cancer (PA) is the seventh common cause of tumor-related mortality worldwide with a median survival rate of 6 months and the 5-year survival rate of less than 5% (1). Despite low incidences, pancreatic cancer is ranked to the highest mortality in 5-year survival rates (1,2). Advances in sequencing technology have enabled various omics analyses such as whole exome sequencing (WES), RNA sequencing, and reverse-phase protein lysate microarray (RPPA), which has allowed the discovery of mutations that can be considered characteristics of various carcinomas. Pancreatic cancer is known for its difficulty in initial diagnosis, rapid and aggressive local invasions, the potential for metastasis, and poor prognosis (3-5).

Histologically, most pancreatic cancers occur in exocrine pancreas, and 85% of the tumors were originate from the epithelial lining of the pancreatic duct. As a feature of the pancreas composed of both exocrine and endocrine structure, the configuration of the pancreas is complicated. Consequently, even with a surgical resection, the tumor

often proceeds with recurrences or local metastasis, which leads to a poor prognosis of pancreatic cancer patients. Another cause of poor prognosis is the heterogeneity of mutational characteristics in pancreatic cancer. The high rates of KRAS mutations (>80%), inactivation of the tumor suppressor genes TP53, SMAD4, and CDKN2A occur at rates of >30% (6-8). The prevalence of mutations involved in chromatin modification, DNA damage repair, and other mechanisms important for carcinogenesis is known to be less than 10%.

The cause of pancreatic cancer is also unclear. There are a significant number of KRAS mutations present in patients, but this is a consequential interpretation. Mutated KRAS does not affect alone in tumor progression (9,10). Along with mutated KRAS, inflammation in pancreas is essential for progression to carcinoma (10,11). In addition, it has been reported that activation of notch signaling has an effect on tumor development (12). Even though molecular classification of pancreatic cancer is significant to precise diagnosis, and it has not been studied with Korean patients' cohorts. The pancreatic cancer cell line status established worldwide has 21 types in ATCC (https://www.atcc.org/), 10 types in DSMZ (https://www.dsmz.de/), 20

types in RIKEN cell bank (https://cell.brc.riken.jp/en/), and 3 types in ECACC (https://www.phe-culturecollections.org.uk/products/celllines/generalcell/). And the Korea Cell Line Bank (http://cellbank.snu.ac.kr) stores 18 types, including 4 types of overseas cell lines and 14 types established by itself. Therefore, we investigated the mutational characteristics of pancreatic cancer from newly established 26 pancreatic cancer cell lines through whole exome sequencing, RNA sequencing, and drug sensitivity assays. In addition, 26 established pancreatic cancer cell lines were divided into KRAS wild-type, mutated KRAS, and HER2 over-expressing with mutated KRAS according to the status of KRAS and HER2. Each group compared mutational characteristics and anti-cancer drug resistance.

2. Material and methods

2.1. Establishment and maintenance of human pancreatic cancer cell lines

Cell lines from pathologically proven pancreatic carcinomas were established. 22 pancreatic carcinoma samples are obtained from patients in Seoul national university hospital. 5 pancreatic cancer cell lines are derived from organoid culture system. Solid tumors were finely minced with scissors and dispersed into small aggregates by pipetting. Appropriate amounts of fine neoplastic tissue fragments were seeded into 25 cm 2 flasks. Most of the tumor cells were initially cultured in Opti-MEM I (Thermo Fisher Scientific, MA, USA) supplemented with 5% fetal bovine serum. Confined-area trypsinization or scraping method was used to attain a pure tumor cell population when stromal cells like mesothelial cells or fibroblasts grew in the initial culture. Established cell lines were sustained in RPMI 1640 medium with 10% fetal bovine serum and 1% (v/v) penicillin and streptomycin (10,000U/ml). Cultures were maintained in humidified

incubators at 37°C in an atmosphere of 5% CO2 and 95% air. The initial passage was assigned when substantial tumor cell growth was detected, and successive passages were given at sub-confluence after trypsinization. When one culture population contains both floating and adherent cells, floating cells were gathered by centrifuging the medium and dispersed by pipetting. Established cell lines were deposited to the Korean Cell Line Bank (Seoul, Korea)

2.2. Cell growth properties

At 70-80% of confluency, Cells were detached from a T75 flask with 2X trypsin. After centrifugation, the cells were re-suspended with a culture medium. Suspensions of 5×10^4 to 2×10^5 cells were seeded on 24 identical well of 96-well culture plates in 80 μL of complete culture medium and incubated in humidified incubators at 37°C in an atmosphere of 5% CO2 and 95% air. Since the first cell seeding, in every 24 hours of incubation, 10 μL of CellTiter-Glo® (Promega) was added to well of each seeded pancreatic cancer cells in triplicate. After 10 minutes adding CellTiter-Glo®, the optical density was measured at

fluorescence using Luminoskan Ascent™ (Thermo Fisher Scientific, MA, USA). The number of cells was counted at 24-hour intervals for at least 5 days. The morphology of cells grown in T-75-cm^2 culture flasks was observed daily by phase-contrast microscopy. In addition, mycoplasma contamination was tested by the 16S-rRNA gene-based PCR amplification method using the e-Myco Mycoplasma PCR detection kit (Intron Biotechnology, Gyeonggi, Korea).

2.3. Nucleic acid isolation and complementary DNA synthesis

Genomic DNA was extracted from the cell lines using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and RNA was extracted using the TRIzol (Life technologies, CA, USA) and RNeasy Plus Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

2.4. DNA fingerprinting analysis using 15 Short Tandem Repeat (STR) loci and amelogenin marker

The genomic DNA from each cell line was amplified using an

AmpFlSTR identifier polymerase chain reaction (PCR) amplification kit (Applied Biosystems, CA, USA). A single cycle of PCR amplified 15 short tandem repeat markers (CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX and vWA) and an amelogenin gender-determining marker containing highly polymorphic microsatellite markers. Amplified PCR products were analyzed by an ABI 3500XL Genetic analyzer (Applied Biosystems, CA, USA).

2.5. Drug sensitivity test and analyze

Cell suspensions were prepared in the previously described. According to various growth rates, 2-8 x 10^5 cells/mL were seeded on 96-well tissue culture plates in 80 μL of complete culture medium and incubated in humidified incubators at 37°C for 24 hours in an atmosphere of 5% CO2 and 95% air. 18 Anti-cancer agents were serially diluted in DPBS and were then added to each well with a volume of 20 μL. After 72 hours of incubation, 10 μL of CellTiter-Glo® (Promega, WI, USA) was added to each well. After 20 minutes of incubation at 37°C, the optical

density was measured at fluorescence using Luminoskan Ascent™ (Thermo Fisher Scientific, MA, USA). To compare and analyze between established cell lines, calculates area under curve (AUC) using R program version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria) with various packages

2.6. Whole exome sequencing

Whole-exome capture was performed on all samples with the SureSelect Human All Exon V5 Kit (Agilent Technologies, Tokyo, Japan), using the Bravo automated liquid handler. The captured targets were subjected to sequencing using HiSeq 2500 (Illumina, San Diego, CA, USA) with the pair-end 100 bp read option for organoid samples and 200 bp read option for tissue materials. The sequence data were processed through an in-house pipeline. Briefly, paired-end sequences are firstly mapped to the human genome, where the reference sequence is UCSC assembly hg19 (original GRCh37 from NCBI, Feb. 2009) using the mapping program BWA (version 0.7.12), and generated a mapping result file in BAM format using BWA-MEM. Then, Picard-

tools (ver.1.130) were applied in order to remove PCR duplicates. The local realignment process is performed to locally realign reads with BAM files reducing those reads identically match to a position at start into a single one, using MarkDuplicates.jar, which requires reads to be sorted. By using Genome Analysis Toolkit, base quality score recalibration (BQSR) and local realignment around insertion, deletions (indels) were performed. Haplotype Caller of GATK (GATKv3.4.0) was used for variant genotyping for each sample based on the BAM file previously generated (SNP and short indels candidates are detected). Those variants are annotated by SnpEff v4.1g, to vcf file format, filtering with dbSNP for the version of 142 and SNPs from the 1000 genome project. Then, SnpEff was applied to filter additional databases, including ESP6500, ClinVar, dbNSFP 2.9.

2.7. RNA sequencing and fusion gene analysis

Total RNA was isolated from cell lysate using TRIzol (Qiagen, Hilden, Germany) and Qiagen RNeasy kit (Qiagen, Hilden, Germany). Sequencing libraries were prepared using the Illumina TruSeq stranded

total RNA library prep kit. Fifty-one million reads were obtained from the cell lysates. Following base-calling and alignment with the Tuxedo Suite, the rejected reads were analyzed using FusionMap, ChimeraScan, and Defuse with default parameters for RNA and alignment to GRCh37.72.

The fusion gene extracted from FusionMap and Defuse was sorted through fusionGDB, fusion gene annotation data base.

2.8. Protein isolation and western blotting

Cells were harvested with a cell scraper after washing with cold PBS. Whole protein was extracted with EzRIPA buffer (ATTO Co., Tokyo, JAPAN) supplied with 1% protease inhibitor and 1% of phosphatase inhibitor. The protein concentration was calculated by Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, MA, USA), Equal amounts of protein were loaded on 4%-15% Mini-PROTEAN TGX Precast Gels (BIO-RAD, Hercules, CA) and blotted at 50 V for 2 hours. Proteins were transferred to a Trans-Blot Turbo Transfer Pack (BIO-RAD) using the Trans-Blot Turbo Transfer System V1.02 machine

(BIO-RAD) at 2.5 A and 25 V. The membrane was incubated in 1.5 % skim milk containing 0.5 % Tween 20 for an hour at room temperature. Primary antibodies against KRAS (Abcam, Cambridge, UK) (1:500), EGFR (Cell Signaling Technology, Danvers, MA, USA) (1:1000), Phospho-EGFR-Tyr1068 (Cell Signaling Technology, Danvers, MA, USA) (1:1000), HER2 (Cell Signaling Technology, Danvers, MA, USA) (1:1000), ERK (Applied Biological Materials Inc., Richmond, BC, Canada) (1:1000), Phospho-ERK-Thr202/Tyr204 (Cell Signaling Technology, Danvers, MA, USA) (1:1000), PTEN (Cell Signaling Technology, Danvers, MA, USA) (1:1000), mTOR (Cell Signaling Technology, Danvers, MA, USA) (1:1000), Phospho-mTOR-Ser2448 (Cell Signaling Technology, Danvers, MA, USA) (1:1000), Akt (Cell Signaling Technology, Danvers, MA, USA) (1:1000), Phospho-Akt-Thr308 (Cell Signaling Technology, Danvers, MA, USA) (1:500), β-actin (Invitrogen, Carlsbad, CA, USA) (1:1000) were introduced to the membrane and incubated at room temperature for 1 hour. Peroxidase-conjugated mouse or rabbit IgG antibody (Jackson Immunoresearch, West Grove, PA, USA) (1:5000) was added as a secondary antibody and incubated at room temperature for 1 hour. After chemiluminescent working solution, SuperSignal™ West Pico PLUS (Thermo Fisher

Scientific, MA, USA), was decanted to the membrane. The membrane was exposed to Fuji RX film for 1-5 minutes.

2.9. Mutational signature analysis

The Analysis was performed using R program version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria) with various packages including dplyr, stringr, tidyr, MutationalPattern. The R package 'MutationalPattern' was also used to identify significant sub-networks of differentially mutated genes.

2.10. Mutation profiling and enriched pathway analysis

The Analysis was performed using R program version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria) with various packages including dplyr, rvest, stringr, tidyr, clusterProfiler, org.Hs.eg.db, ReactomePA. The R package 'ReactomePA' was also used to identify significant sub-networks of differentially mutated genes.

To identifying significant mutations, we searched for what was reported in ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) for a given rs number. An indexing file was created by matching the gene defined in Cancer Gene Census (https://cancer.sanger.ac.uk/census) (13) and indexing its role in cancer. After that, we predicted the weight of each mutation and selected only those with significant influence.

2.11. Code availability

Computational pipelines for calling significant somatic mutations and procedurally explained instructions are available to the public repository (https://github.com/jh-HOS/panc-by-R.git).

3. Results

3.1. General characteristics of the cell lines

The clinical characteristic of the established cell lines is summarized in Table 1. Twenty-one cell lines were established from tumor tissues, and 5 cell line were derived from organoid cultures (Table 1). Morphology and growth pattern of most cell lines displayed an adherent polygonal shape. SNU-2822 and SNU-2913 cell lines showed fibroblast-like shape, and SNU-3375 had densely aggregated and round morphologies (Figure 1, Table 2). All cell lines were subjected to characteristic analysis after passaged at least 3 times. Growth rates were ranged from 22.6 to 119.5 hours. According to fifteen tetranucleotide repeat loci and Amelogen sex-determining markers, short tandem repeats (STR) fingerprinting, each marker was heterogeneously distributed and all newly established cell lines were not cross-contaminated (Table 3). All cell lines were confirmed to be free of contamination from mycoplasma (Figure 2). All cell lines introduced in this study including its molecular characterization and drug responses will be deposited to Korean Cell

Line Bank (http://cellbank.snu.ac.kr) at initial passages to be distributed to researchers worldwide.

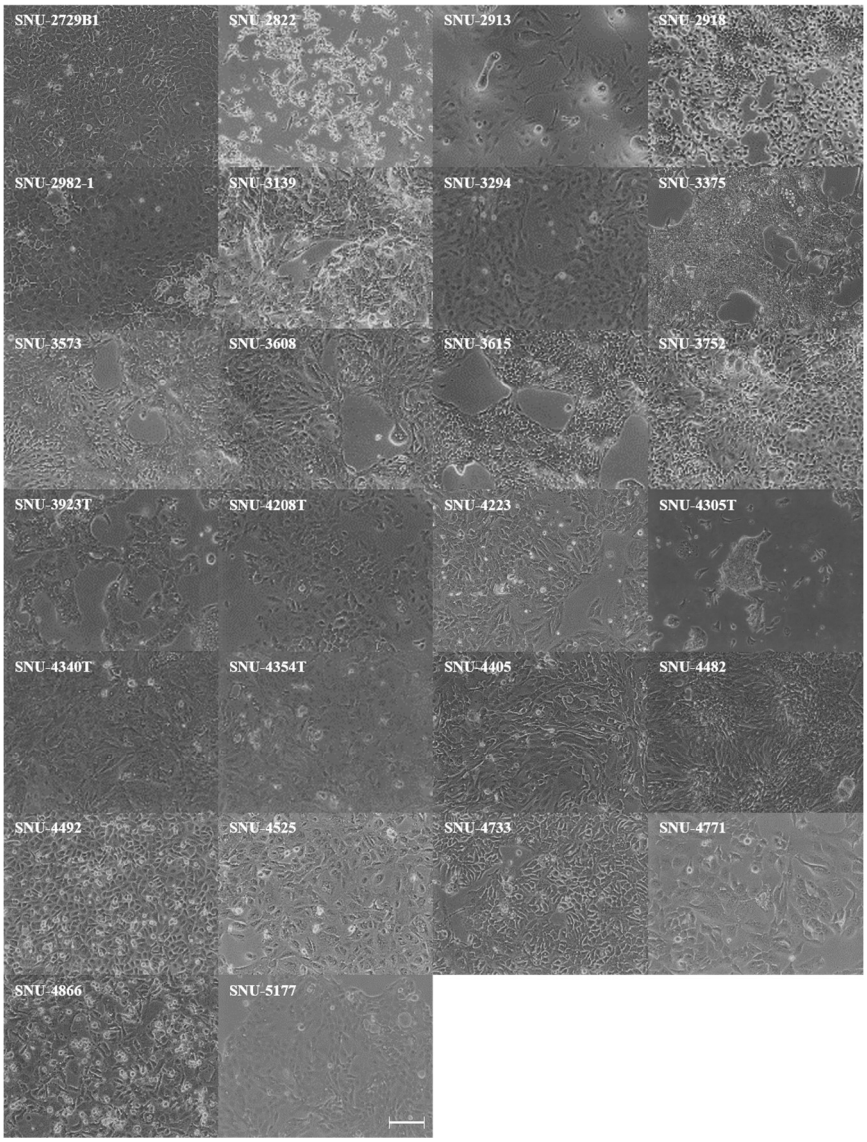

Figure 1. Phase-contrast microscopy of 26 established pancreatic cancer cell lines. Phase-contrast microscopy of 26 pancreatic cancer cell lines. Scale bar in microscope of SNU-5177 represent 50 μm and all microscopy have same magnification.

Table 1. Clinical characteristics of 26 established pancreatic cancer cell lines

No.	Cell line	Sex	Age	Derived Origin
1	SNU-2729B1	M	46	Tissue
2	SNU-2822	M	N.A.	Tissue
3	SNU-2913	M	N.A.	Tissue
4	SNU-2918	F	N.A.	Tissue
5	SNU-2982-1	F	N.A.	Tissue
6	SNU-3139	M	78	Tissue
7	SNU-3294	M	66	Tissue
8	SNU-3375	M	55	Tissue
9	SNU-3573	M	49	Tissue
10	SNU-3608	M	76	Tissue
11	SNU-3615	M	77	Tissue
12	SNU-3752	F	70	Tissue
13	SNU-3923T	M	69	Organoid
14	SNU-4208T	F	N.A.	Organoid
15	SNU-4223	F	47	Tissue
16	SNU-4305T	F	N.A.	Organoid
17	SNU-4340T	F	N.A.	Organoid
18	SNU-4354T	F	N.A.	Organoid
19	SNU-4405	F	61	Tissue
20	SNU-4482	M	65	Tissue
21	SNU-4492	F	80	Tissue
22	SNU-4525	M	56	Tissue
23	SNU-4733	M	61	Tissue
24	SNU-4771	M	73	Tissue
25	SNU-4866	M	67	Tissue
26	SNU-5177	M	N.A.	Tissue

Table 2. *In vivo* characteristics of 26 established pancreatic cancer cell lines

No.	Cell line	Growth Pattern	Doubling Time (hr)	Cell Morphology
1	SNU-2729B1	Adherent	50.4	Polygonal
2	SNU-2822	Adherent	60.7	Fibroblast-like
3	SNU-2913	Adherent	22.6	Fibroblast-like/Polygonal
4	SNU-2918	Adherent	75	Oval
5	SNU-2982-1	Adherent	66	Polygonal
6	SNU-3139	Adherent	77.2	Polygonal
7	SNU-3294	Adherent	85.4	Polygonal
8	SNU-3375	Adherent	52.3	Round
9	SNU-3573	Adherent	67.6	Polygonal
10	SNU-3608	Adherent	59.5	Polygonal
11	SNU-3615	Adherent	68.4	Oval/Round
12	SNU-3752	Adherent	113	Polygonal/Round
13	SNU-3923T	Adherent	80.4	Round
14	SNU-4208T	Adherent	61.9	Round
15	SNU-4223	Adherent	79.3	Polygonal
16	SNU-4305T	Adherent	45.1	Polygonal
17	SNU-4340T	Adherent	85.1	Polygonal
18	SNU-4354T	Adherent	73.2	Polygonal
19	SNU-4405	Adherent	119.5	Polygonal
20	SNU-4482	Adherent	48.6	Polygonal
21	SNU-4492	Adherent	37.9	Oval/Round
22	SNU-4525	Adherent	78.2	Oval
23	SNU-4733	Adherent	63.8	Polygonal
24	SNU-4771	Adherent	48.3	Polygonal
25	SNU-4866	Adherent	85.7	Polygonal
26	SNU-5177	Adherent	92.3	Polygonal

Table 3. DNA fingerprinting using 15 STR loci and Amelogenin marker

No.	Cell line	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539
1	SNU-2729B1	11,12	29,30	8	11,12	15	6,9.3	12	9,10
2	SNU-2822	15,16	30,31	8,11	7,11	15,19	6	10,11	9,13
3	SNU-2913	13	30,32.2	11	10,12	15,17	7,8	8,12	9,11
4	SNU-2918	13,15	31	10,12	11,13	15,17	6,7	8	9
5	SNU-2982-1	10,12	31.2	11	12,13	15	6,9	10,12	9,13
6	SNU-3139	13,15	30	11,12	10,11	15,17	6,9	11	9,11
7	SNU-3294	10,11	29,30	10,12	11,12	16,17	9	9,11	11,13
8	SNU-3375	10,16	32.2	11	12	14,17	6,7	8,10	12,13
9	SNU-3573	13,15	30	10,13	11	16	7	11	9,11
10	SNU-3608	11,13	32.2	9,11	12,13	16	7,9	9,13	9,12
11	SNU-3615	10	30	11,13	15,14	17	7,9	8	11,12
12	SNU-3752	11,15	30,32.2	12	9,13	15,18	6,7	11	11,15
13	SNU-3923T	14	29	8,12	12	15,17	6	8,12	9,11
14	SNU-4208T	10	31.2	11	11	15	7,9	11	10,11
15	SNU-4223	11,14	31.2	9,11	10,11	16	9	10	10
16	SNU-4305T	14,15	29	10	12	15,16	7	8,12	11
17	SNU-4340T	10,15	30,32	10	11	15,16	7,9	9,12	10
18	SNU-4354T	10,11	31.2,32.2	11,12	11,12	15,17	6	9,11	11,14
19	SNU-4405	13,15	29,32.2	10,11	12	15,16	6,9	8,13	9,13
20	SNU-4482	13,14	30,31	7,11	11,12	15,16	7,9	12	9,10
21	SNU-4492	14,16	29	10	11	17	7,9	10	9,11
22	SNU-4525	10,14	29,31	11,12	11,13	17	9	9	9,11
23	SNU-4733	13,14	29	11	12	16	7	11,12	10,11
24	SNU-4771	13,14	29	8,11	10	16,19	6,9	12	9,12
25	SNU-4866	11,16	30,31	8,12	12,13	17,18	7,9	11	11,13
26	SNU-5177	15	29,31	10,11	11,12	16,17	9	8,12	9,13

Continued

No.	Cell line	D2S1338	D19S433	Vwa	TPOX	D18S51	Amelogenin	D5S818	FGA
1	SNU-2729B1	24,25	14	17	8,11	16	X,Y	12	21
2	SNU-2822	18,24	14	14	8	14,15	X,Y	11,12	21,22
3	SNU-2913	17,18	13,16.2	16	8,11	19	X,Y	10,11,12	17,20
4	SNU-2918	20,23	12,13	16,18	8	14	X	11,12	22
5	SNU-2982-1	17,18	13	17	11	18	X	11	19,22
6	SNU-3139	19,20	13,15	17,18	8,11	19,22	X,Y	9,11	24,25
7	SNU-3294	20,21	13,14	16,19	8	13,15	X,Y	11,13	22,26
8	SNU-3375	18,23	14.2	17,18	11	15	X,Y	11,13	23,24
9	SNU-3573	18,19	13,16.2	17	8	14,20	X,Y	11	22,23
10	SNU-3608	18,23	14,14.2	14,16	9,11	13,15	X,Y	10	26
11	SNU-3615	22,23	13,15.2	18	8,9	12	X,Y	9,11	23,24
12	SNU-3752	19,24	14,14.2	17,18	8,9	18	X	9,10	19,28
13	SNU-3923T	20,27	14	18	8	14	X,Y	11	23
14	SNU-4208T	22,25	14,15.2	16	8	20	X	12	23
15	SNU-4223	17,23	12,14	17	8	16	X	11	24
16	SNU-4305T	23	13	17,18	8	16	X	11	24
17	SNU-4340T	23	14	17,18	8,11	17	X	9	24
18	SNU-4354T	17,18	14	17	11	14	X	10	20,25
19	SNU-4405	18,19	12,13	17,18	8	13	X	11	19,25
20	SNU-4482	23	13,13.2	18,19	8	16	X,Y	10,11	21,23
21	SNU-4492	18,23	13,14.2	18,19	8	15,16	X,Y	10,12	21,22
22	SNU-4525	18,23	13,15	18	8,10	14	X,Y	10,13	23,24
23	SNU-4733	22,23	13,14.2	18	8,11	15	X,Y	11,12	25
24	SNU-4771	22,24	13,14	14,16	11	19	X,Y	9,11	24
25	SNU-4866	17,23	13	16,18	11	13	X,Y	11,13	23,24
26	SNU-5177	19,25	14	18	8,11	13,15	X,Y	10,11	21.2,24

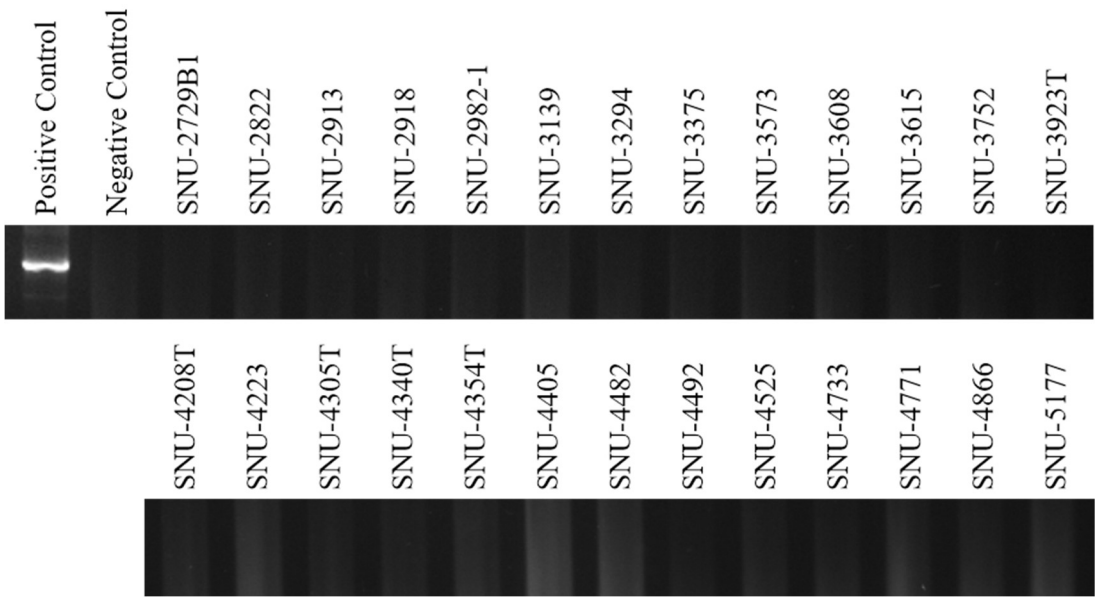

Figure 2. Mycoplasma test by the 16s-rRNA-gene-based polymerase chain reaction. PCR was performed to confirm the infection of mycoplasma. All cell lines were free of mycoplasma contamination.

3.2. Mutational landscape of 26 established pancreatic cancer

We sequenced cancer-related genes in 26 established pancreatic cancer cell lines. The mean read depth of exome sequencing was 100x. After filtering out non-significant variants, we identified 14,792 point mutations, including insertions and deletions, with at least 29 to 101 influential mutations in each cell line. First, we selected 5 representative genes of pancreatic cancer (KRAS, TP53, CDKN2A, BRAF, and MET), 7 SWI/SNP complexes and Chromatin remodeling (KDM6A, MLL3, PBRM1, ARID1A, ARID1B, and SMARCA4), 4 TGF-β signaling (SMAD4, TGFBR2, ACVR1B, and ACVR2A), 4 ROBO SLIT signaling (ROBO1, ROBO2, SLIT2, and MYCB2) and 5 DNA repair related genes (MLH1, MSH2, BRCA1, BRCA2, and MSH6) (6-8,14-18). Forty-seven missense mutations, 10 highly effective splice site mutations, 3 nonsense mutations, 4 amino-acid modifications, 1 deletion frameshift are founded (Figure 3). A list of major mutations of KRAS, TP53, SMAD4, CDKN2A, BRAS genes are summarized in Table 4.

KRAS codon G12 mutations were found in 85% of our cell lines, which is in line with reported statistics (6,8,19). In the type of alteration, G12D (Gly to Asp) mutations were found more frequently with G12D: 13, G12V: 5, G12R: 4, and Wild Type KRAS: 4. The SNU-2822, SNU-3752, SNU-4354T, and SNU-4223 without the KRAS mutation. Specifically, a small number mutations were found in the CDKN2A (8 %), BRAF genes (4 %), which are known to have more than 30% of patients (6-8,20,21). It has been reported that Asn486_Pro490delinsThr, Thr491del BRAF mutation is found in KRAS wild type pancreatic cancer (22-24), and these deletions are located in the tyrosine kinase domain with functional effects. However, in the TP53 gene belonging to the same cell cycle regulation with CDKN2A, more than two-thirds of missense mutations were found (25-27) in our cell lines. There are 5 types of TP53 mutations: codon 31 (SNU-3608), codon 72 (SNU-2913, SNU-2918, SNU-3294, SNU-3139, SNU-3573 and SNU-3752), codon 175 (SNU-4492), codon 248 (SNU-3608) and codon 285 (SNU-3294) and are reported to be pathogenic in most cases. The TP53 mutation at codon 72 was first reported in 1990 (28), subsequent studies have reported that it is not pathogenic but affects the reactivity and side effects of paclitaxel and platinum-based anti-cancer drugs in gastric

cancer (29), breast cancer (30), and ovarian cancer (31,32). The remaining four mutations (codon 31, 175, 248, 285) are all reported to be pathogenic, and in particular, the codon 285 mutation is located in the DNA binding domain (33).

Genes related to the TGF-β signaling pathway are known to have mutations in 47% of pancreatic cancer patients. Especially SMAD4 loss is associated with metastatic prognosis of cancer (34,35). In our cell lines, mutations were found in SMAD4 and TGFBR2, and only benign mutations were found in ACVR1B and ACVR2A genes (filtered). The TGFBR2 codon 315 mutation of SNU-3294 was known to be benign, but has recently been associated with colorectal cancer (36). SMAD4 codon 171 mutation has not been reported to ClinVar database, but the codon 445 nonsense mutation found in SNU-3615 has been reported as a pathogenic mutation associated with juvenile polyposis in colorectal cancer (37).

Among DNA repair related genes, there were benign and nonsense mutations in BRCA1, BRCA2, and MSH6. The cell lines had two types of mutations in BRCA2. The codon 372 mutations of SNU-2913, SNU-2918, SNU-2822, SNU-3294, SNU-4482, and SNU-3608 have been

reported as benign mutations (38), and the additional codon 2044 mutation of SNU-2822 has been reported as an uncertain significance in breast and ovarian cancer (39,40). Three types of BRCA1 mutations in codon 871, codon 1038, codon 1183 were found in eight cell lines (SNU-2822, SNU-2729B1, SNU-3139, SNU-3294, SNU-3752, SNU-4405, SNU-4223 and SNU-4482), but these were all reported to have benign effects (41).

In MSH6, effective mutations were observed. The missense mutations in SNU-3615 and SNU-3294 have been reported to have a benign effect (42), and the frameshift by deletion in codon 958 found in SNU-4492 is pathogenic and has been shown to be associated with lynch syndrome, particularly in colorectal cancer (43,44). Specifically, the frameshift due to insertion of SNU-4482 and SNU-3752 has not been reported to ClinVar, but it has been reported by COSMIC database with genomic mutation ID: COSV52273912 (45). This mutation is located at the C-terminus rather than the functional domain of MSH6, so the impact as a frameshift mutation is not expected to critical. In the Chromatin remodeling and ROBO/SLIT pathways, the SNU-4733 cell lines exclusively harbored nonsense mutation of MYCBP2 (Glu2506*) with

an effective variant.

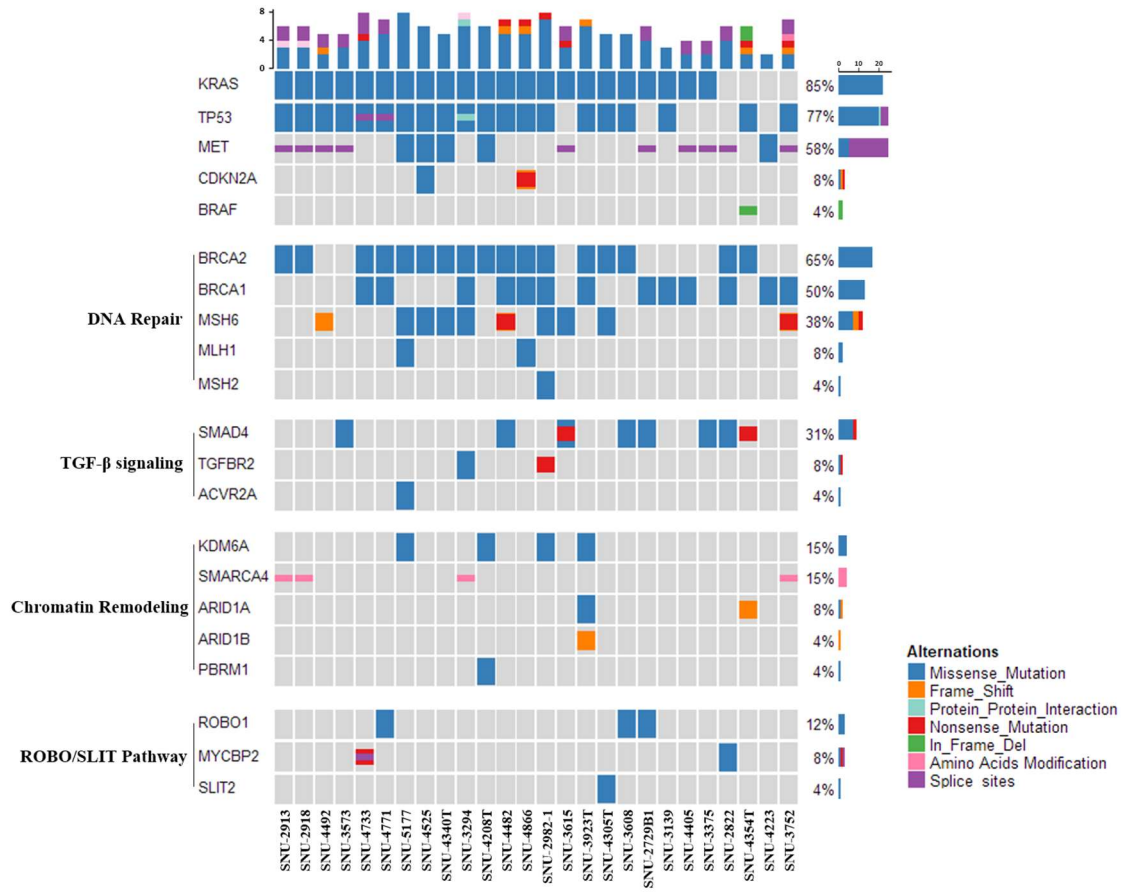

Figure 3. Mutations of key genes in 26 established pancreatic cancer.

The upper bar plots show non-silent single nucleotide variants and small insertions or deletions. The matrix shows alteration types: missense mutations (blue), frameshift (orange), nonsense mutation (yellow), protein-protein interactions related (sky), amino acid modifications (dark green), splice-site mutations (purple) and right bar plots show the proportion of mutations in the gene among all cell lines.

Table 4. Mutation list of 26 established pancreatic cancer cell lines.

	SNU-2729B1	SNU-2822	SNU-2913	SNU-2918	SNU-2982-1	SNU-3139	SNU-3294	SNU-3375	SNU-3573
KRAS	c.35G>T p.Gly12Val Pathogenic		c.35G>A p.Gly12Asp Pathogenic	c.35G>A p.Gly12Asp Pathogenic	c.35G>A p.Gly12Asp Pathogenic	c.35G>T p.Gly12Val Pathogenic	c.35G>A p.Gly12Asp Pathogenic	c.35G>A p.Gly12Asp Pathogenic	c.34G>C p.Gly12Arg Pathogenic
TP53			c.215C>G p.Pro72Arg U.S*	c.215C>G p.Pro72Arg U.S*	c.817C>T p.Arg273Cys Pathogenic	c.215C>G p.Pro72Arg U.S*	c.853G>A Protein protein contact Pathogenic c.215C>G p.Pro72Arg U.S*		c.215C>G p.Pro72Arg U.S*
SMAD4	c.513G>C p.Glu171Asp Not reported	c.513G>C p.Glu171Asp Not reported						c.513G>C p.Glu171Asp Not reported	c.513G>C p.Glu171Asp Not reported

continued

	SNU-3608	SNU-3615	SNU-3752	SNU-3923T	SNU-4208T	SNU-4305T	SNU-4340T	SNU-4354T
KRAS	c.34G>C p.Gly12Arg Pathogenic	c.34G>C p.Gly12Arg Pathogenic		c.35G>A p.Gly12Asp Pathogenic	c.35G>T p.Gly12Val Pathogenic	c.35G>A p.Gly12Asp Pathogenic	c.35G>T p.Gly12Val Pathogenic	
TP53	c.743G>A p.Arg248Gln Pathogenic c.91G>A p.Val31Ile U.S*		c.215C>G p.Pro72Arg U.S*	c.380C>T p.Ser127Phe Not reported	c.722C>A p.Ser241Tyr Not reported c.215C>G p.Pro72Arg U.S*	c.475G>C p.Ala159Pro Not reported c.215C>G p.Pro72Arg U.S*	c.659A>G p.Tyr220Cys Pathogenic	c.524G>A p.Arg175His C.I.P*
SMAD4	c.513G>C p.Glu171Asp Not reported	c.513G>C p.Glu171Asp Not reported						
CDKN2A								c.804G>A p.Trp268* Not reported
BRAF								c.1471_1473delACA p.Thr491del Not reported c.1457_1468delATGTGACAGCAC p.Asn486_Pro490delinsThr Not reported

continued

	SNU-4405	SNU-4482	SNU-4492	SNU-4525	SNU-4733	SNU-4771	SNU-4866	SNU-5177
KRAS	c.35G>A p.Gly12Asp Pathogenic	c.35G>T p.Gly12Val Pathogenic	c.35G>A p.Gly12Asp Pathogenic	c.35G>A p.Gly12Asp Pathogenic	c.34G>C p.Gly12Arg Pathogenic	c.35G>A p.Gly12Asp Pathogenic	c.35G>A p.Gly12Asp Pathogenic	c.35G>A p.Gly12Asp Pathogenic
TP53		c.215C>G p.Pro72Arg U.S*	c.524G>A p.Arg175His C.I.P *	c.524G>A p.Arg175His C.I.P* c.215C>G p.Pro72Arg U.S*	c.560-1G>T Intron variant Not reported c.215C>G p.Pro72Arg U.S*	c.994-1G>A Intron variant Pathogenic c.215C>G p.Pro72Arg U.S*	c.817C>T p.Arg273Cys Pathogenic	c.524G>A p.Arg175His C.I.P * c.215C>G p.Pro72Arg U.S*
CDKN2A				c.247C>T p.His83Tyr Likely pathogenic			c.131dupA p.Tyr44fs Not reported	
SMAD4		c.513G>C p.Glu171Asp Not reported						

(* C.I.P: Conflicting interpretations of pathogenicity, *U.S: Uncertain significance)

3.3. Enriched pathways of 26 established pancreatic cancer cell lines

Mutations were further analyzed for gene set enrichment analysis to find representative pathways that were mostly aberrated in the established pancreatic cancer cell lines. (Figure 4, Table 4-5). Overall, factors that were related to general disease were mostly affected, and therefore 'Diseases of signal transduction' had the highest count with low p-value. One of the most disrupted pathway was 'PI3K /AKT Signaling in Cancer' ($0.001<p$) and Constitutive Signaling by Aberrant PI3K in Cancer ($0.001<p$). There have been many reports that associates the "abnormal activation of Akt/mTOR pathway in cancer" (46,47). Associated genes in our cohort were FGFR4, PIK3CD, ERBB3, IRS2, KIT, ERBB2, and PIK3R2, which were all connected to signal transduction of Akt/mTOR pathway. With similar gene sets, "Constitutive Signaling by Aberrant PI3K in Cancer" pathway includes FGFR4, PIK3CD, PIK3R2, ERBB3, IRS2, KIT, ERBB2, PIK3R2 and SRC which were related to downstream cascade of Akt/mTOR pathway. This suggested that newly established pancreatic cancer cell lines were generally affected by alteration of Akt/mTOR signaling pathway and thus further research is

essential. Next, we identified genes that Oncogenic MAPK signaling (0.001<p) as well as KRAS pathway (0.001<p) were altered at a high rate, which included NF1, MAP2K2, BRAF (48,49).

Table 5. Pathway associated with 26 established pancreatic cancer cell lines

Related Pathway	Adjusted p value
Diseases of signal transduction	7.06E-05
Olfactory Signaling Pathway	0.000609944
Extracellular matrix organization	0.020561207
Diseases of glycosylation	0.001085179
DNA Double-Strand Break Repair	0.017567872
Degradation of the extracellular matrix	0.020561207
PI3K/AKT Signaling in Cancer	0.000373901
Negative regulation of the PI3K/AKT network	0.018037729
O-linked glycosylation	0.020561207
Diseases associated with O-glycosylation of proteins	7.06E-05
PI5P, PP2A and IER3 Regulate PI3K/AKT Signaling	0.018037729
Constitutive Signaling by Aberrant PI3K in Cancer	0.000609944
HDR through Homologous Recombination (HRR)	0.000412568
Oncogenic MAPK signaling	0.000770565
Signaling by BRAF and RAF fusions	0.001031236

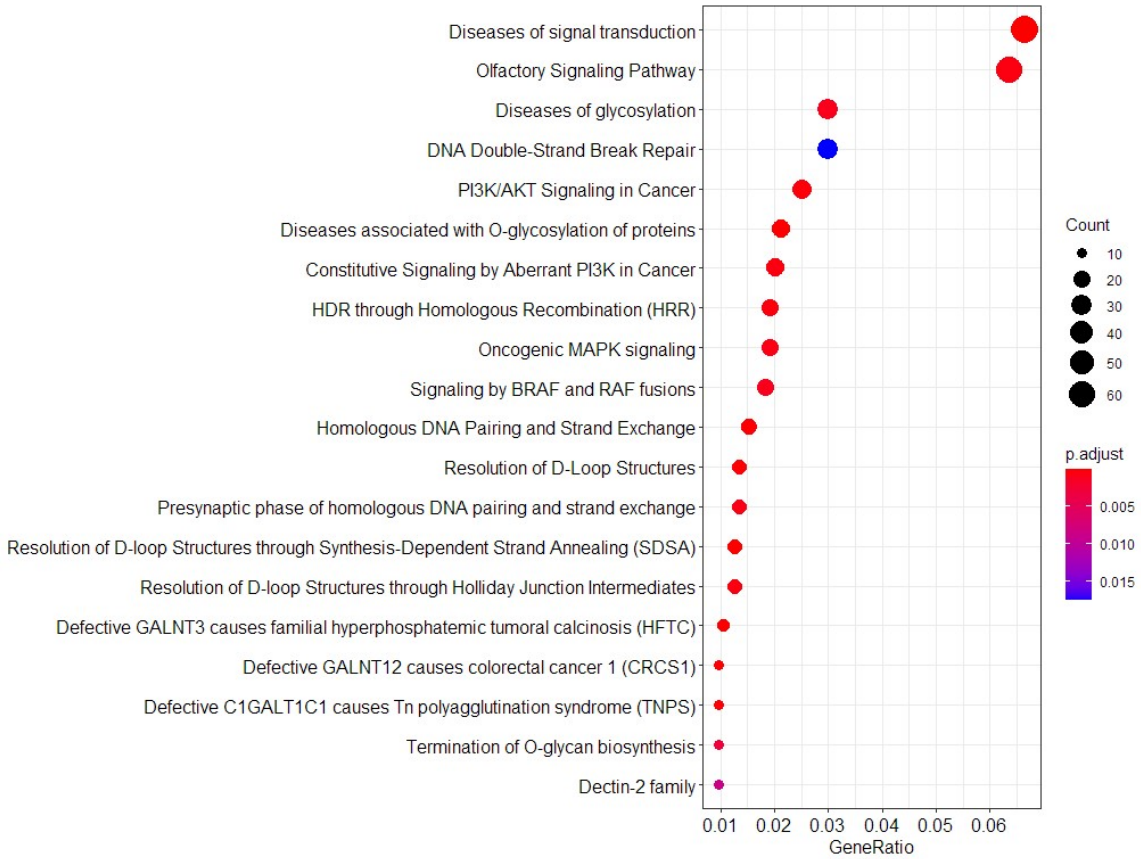

Figure 4. Pathway associated with mutations in 26 established pancreatic cancer cell lines. Enriched pathway of mutations in 26 established pancreatic cancers. The size of the circle refers to the number of genes found in cell lines.

3.4. Mutational signature of 26 established pancreatic cancer cell lines

Accumulation of mutations in cancer constructs different combinations of mutation types. The types of mutations formed by similar causes are collectively referred to as signatures (50). Having, therefore, such a mutation signature can reflect a similar mutation accumulation. It can be considered as influenced by the same risk factor, and this was analyzed in connection with the mutation characteristics (51). COSMIC signatures 1 is predominance of C>T transition with the age of patient at time of cancer diagnosis. The underlying proposed biological mechanism is the spontaneous deamination of 5-methylcytosine (50), COSMIC signatures 2, 13, APOBEC enzymes enriched for C>T and C>G substitutions. These are thought to arise from cytidine deaminase activity of the AID/APOBEC enzymes family. Both Signature 2 and Signature 13 are features with cytosine to uracil substitutions due to cytidine deaminases. COSMIC signatures 3, 6 is related to Homologous recombination deficiency and Mismatch repair deficiency (52), COSMIC signatures 5, has a predominance of T>C substitutions with transcriptional strand bias. Having the widest bar among the signatures displayed on each cell line

is considered to be the main cause of mutation in that cell line. Signature 5 is the most common in our cell line, Signature 5 contains mutations caused by errors during transcription, and it was frequently founded in most carcinomas (13).

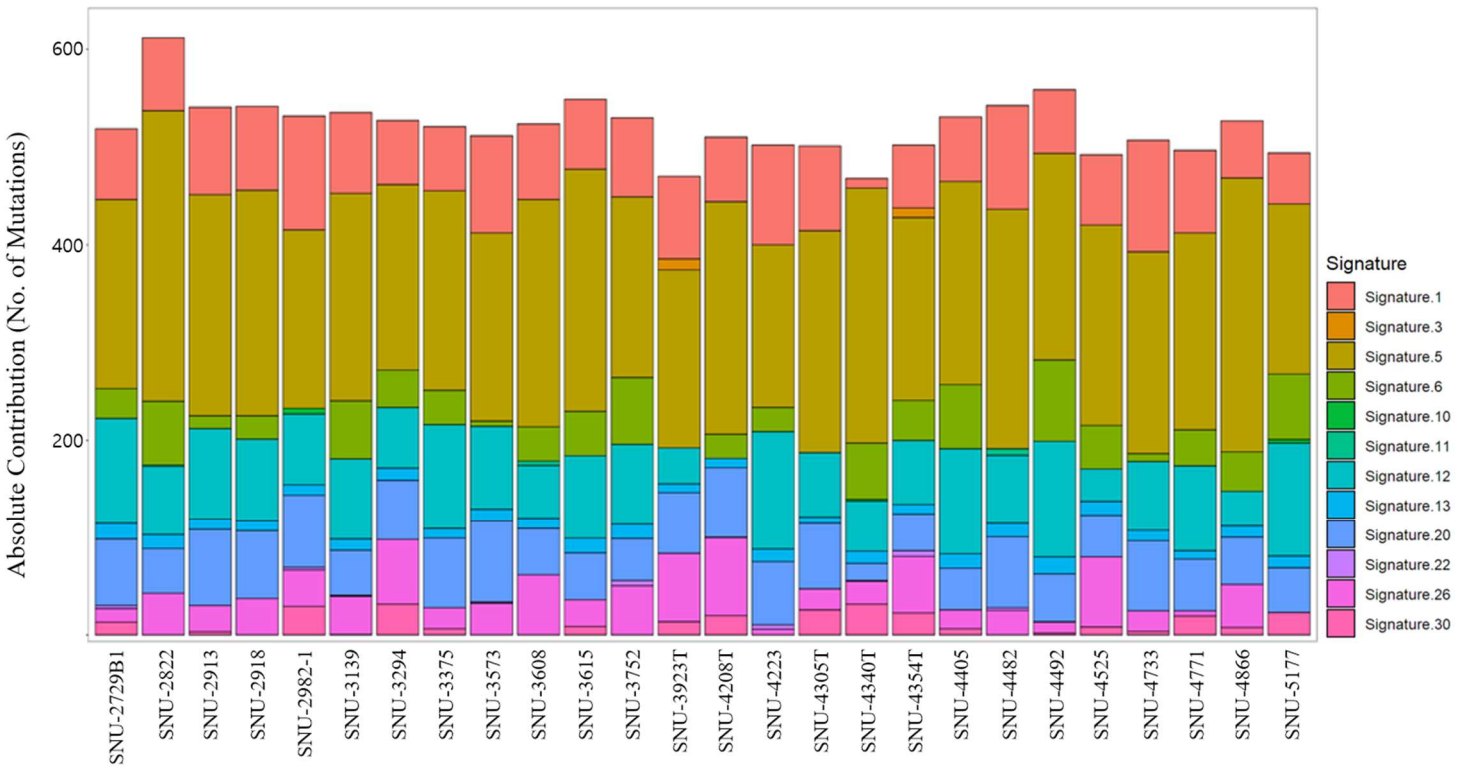

Figure 5. Mutational signature of 26 established pancreatic cancer cell lines.

3.5. Anti-cancer reagents response with mutational contexts

As a first validation, the grouping of drugs based on their AUC values identified a diverse range of sensitivities across the cell lines, and 3 major sub-groups in accordance with KRAS mutation status were separated (Table 6, Figure 5). One group (SNU-2822, SNU-3752, SNU-4223, and SNU-4354T) displayed wild type KRAS. Another group (SNU-2729B1, SNU-2913, SNU-3139, SNU-3294, SNU-3615, SNU-4305T, SNU-4405, SNU-4525, SNU-4733 and SNU-4866) exhibited mutated KRAS. The other group (SNU-3375, SNU-4208T, SNU-4340T, SNU-4482, SNU-4492, SNU-4771, and SNU-5177) displayed mutated KRAS with HER2 over-expression.

The KRAS wild-type group has been shown to generally have a high sensitivity to anti-cancer drugs except SNU-3752 cell lines. As a result of examining 12 genes specifically observed in wild type KRAS (BRAF, ERBB2, FGFR4, NF1, SPRED1, DUSP6, RREB1, GNAS, CTNNB1, JAK1, ATM, and PRSS1) (22), it was found that all

KRAS wild type cell lines had a pathogenic mutation in FGFR4 (Gly388Arg) except SNU-3752. In the SNU-4354T cell line, there was a deletion in the tyrosine kinase domain of BRAF, which was previously reported (22). In particular, SNU-2822 has low activity in the MAPK and Akt/mTOR pathways as much as more resistant to afatinib, sunitinib, erlotinib, and trametinib targeting RTK, EGFR, HER2, and its signaling proteins MEK1/2 than other cell lines. Therefore, it is thought that the tumor progression of SNU-2822 was induced through a non-canonical signaling pathway.

The KRAS mutant cell lines reflect the tumor heterogeneity of pancreatic cancer. Despite being a cell line that does not express HER2 protein, it shows various differences in reactivity for anti-cancer drugs. SNU-4866 is the most resistant to anti-cancer drugs in KRAS mutated cell lines. SNU-3294 and SNU-2729B1 also have similar resistance with SNU-4833, all of which are most sensitively response to trametinib, sunitinib, and erlotinib targeting EGFR, MEK1/2, and multiple receptor tyrosine kinases (RTKs). Therefore, the proliferation and replicating signals of these cell lines are expected to activate the non-canonical pathway by various RTKs. On the

other hand, it can be seen that the six cell lines (SNU-4525, SNU-3139, SNU-3573, SNU-2982-1, SNU-2913, and SNU-3615) with low anticancer drug resistance differ depending on the characteristics of each cell line.

HER2 over-expressing pancreatic cancer was reported as a lower survival rate than HER2 negative pancreatic cancer (53). Our HER2 over-expressing cell lines exhibit high anti-cancer drug resistance. SNU-4482 and SNU5177 have the highest resistance among all cell lines. Specifically, the expression of HER2 appears to have a great influence on the regulation of proliferation and replication, but the resistance to afatinib, a targeted inhibitor of EGFR and HER2, was shown to be different.

Table 6. List of Anti-cancer drugs

Names	Target	Effects
Everolimus	Mammalian target of rapamycin (mTOR).	Inhibition of mTORC1
Paclitaxel	Tubulin	Inhibition of mitotic spindle assembly, chromosome segregation, and cell division.
5-Fu	Thymidylate synthase	Interrupting synthesis of the pyrimidine, thymidine
Gemcitabine	Act as pyrimidine nucleosides	Competitive inhibition of DNA synthesis by masquerade as deoxycytidine triphosphate
Irinotecan	Topoisomerase I	Inhibition of DNA replication and transcription
Mitomycin C	Act as alkylating agent	DNA crosslinking, guanine nucleoside alkylation in DNA
Sunitinib	Multi-targeted receptor tyrosine kinase (RTK)	Reduces tumor vascularization and induces cancer cell apoptosis
Erlotinib	Epidermal growth factor receptor (EGFR)	Reversibly binding to the adenosine triphosphate (ATP) binding site of the receptor.
MK-5108	Aurora Kinase A	Inhibition of mitotic spindle assembly
Bupalisib	Pan-class I phosphoinositide 3-kinase (p110α/β/δ/γ)	Inhibiting the production of the secondary messenger
Apitolisib	Pan-class I phosphoinositide 3-kinase (p110α/β/δ/γ), mTOR	Inhibiting the production of the secondary messenger
Vorinostat	Histone deacetylases (HDAC)	Accumulation of acetylated histones and proteins
Belinostat	Histone deacetylases (HDAC)	Accumulation of acetylated histones and proteins
Trametinib	Mitogen-activated protein kinase kinase 1/2 (MEK1/2)	Inhibition of MAPK pathway
Afatinib	Epidermal growth factor receptor (EGFR), EGFR2	Decreasing phosphorylation between ErbB dimers, thus blocking the activity of downstream
Cyclopamine	Smoothened of sonic hedgehog (Shh) pathway	Inactivation and interrupts of hedgehog signaling
ICG-001	CREB binding protein (CBP)	Binding CREB-binding protein (CBP) and disruption of Wnt/β-catenin transcription
Olaparib	poly ADP ribose polymerase (PARP)	Inhibition of single-strand DNA breaks repairs

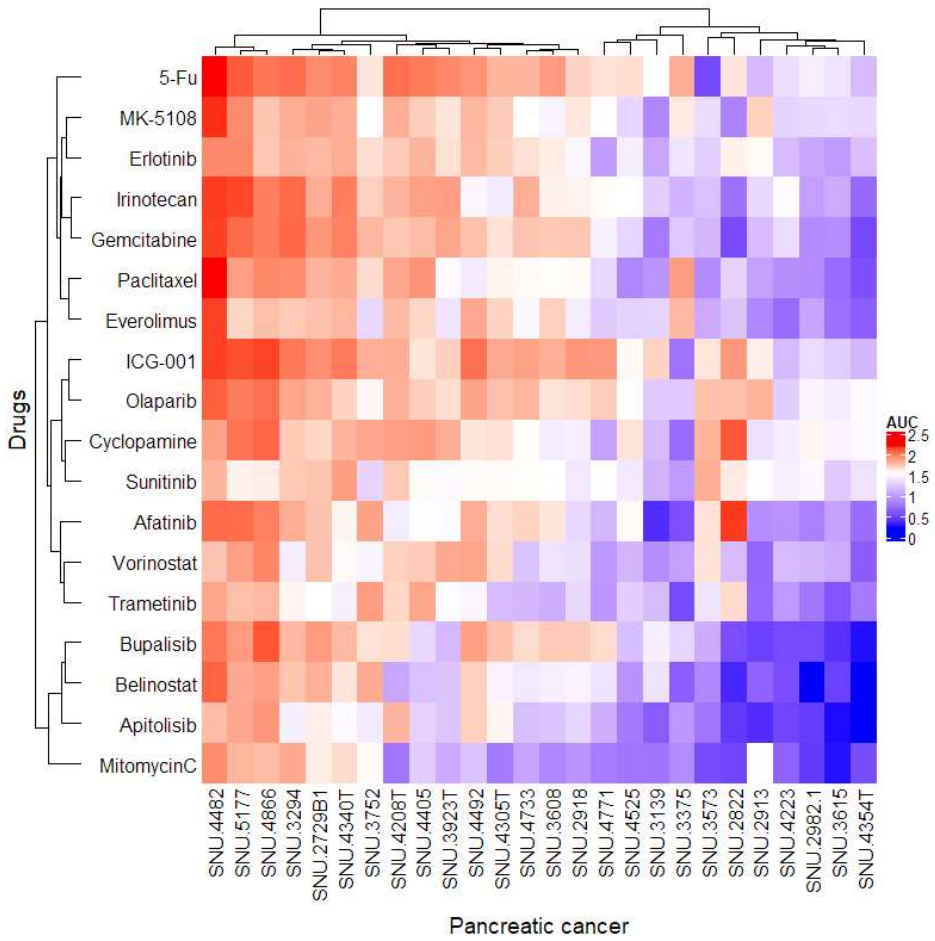

Figure 6. Mapped data from drug sensitivity of 26 established pancreatic cancer cell lines. Area under curve (AUC) was calculated and presented to the heatmap. The higher AUC revealed higher resistance in anti-cancer drugs.

3.6. Western blotting of 26 established pancreatic cancer cell lines

Protein expression was confirmed with western blotting. PTEN, Akt, p-Akt, mTOR, p-mTOR was detected for activity of Akt/mTOR pathway, KRAS, EGFR, p-EGFR, HER2, ERK, p-ERK was detected for the RAS-MEK-ERF pathway. Differences in protein expression in cell lines are indicated by tumor heterogeneity. In particular, in terms of pathway, highly activated EGFR cell lines are likely to have high expression levels of the lower cascade, p-ERK, but are unreliable. Likewise, the activity of p-mTOR is to be induced by p-Akt, but this also discordant. Perhaps there is another pathway activation via the non-canonical signal activation occurs. Representatively, it can be confirmed that the expressions of p-EGFR and HER2 of SNU-4340T, SNU-4492, SNU-4866, and SNU-2822 are not consistent with the activity of p-ERK and p-Akt.

Our KRAS wild-type cell lines have low activity of signaling proteins compared to other subgroups. This is prominent in the pattern of activation of p-EGFR, HER2, and p-mTOR by p-Akt.

Subgroups with KRAS mutated cell lines exhibit various inter-tumor heterogeneities representing pancreatic cancer. Among them, PTEN, an important tumor suppressor gene of the Akt/mTOR pathway, was expressed in four cell lines (SNU-2729B1, SNU-3139, SNU-4528, and SNU-4866), and showed significantly divergent resistance to everolimus, bupalisib, and apitolisib targeting the Akt/mTOR pathway. Only in this group, sensitivity to the MEK1/2 inhibitor trametinib appears to have a positive correlation with the expression level of phospho-ERK.

Unusually, Over-expressing HER2 cell lines were more frequently mutated rather than previous studies (6). The proportion is about 26% of 7 out of 26 cell lines, which is significantly higher than the reported percentage of 6%. This subgroup has higher activation of signaling proteins than the previous two groups. Since it has the expression of p-EGFR and HER2, the activity of the signal transduction proteins p-ERK and p-Akt and the basal expression level of mTOR are significantly higher. This is thought to be characteristic of cell lines with superior anti-cancer drug resistance.

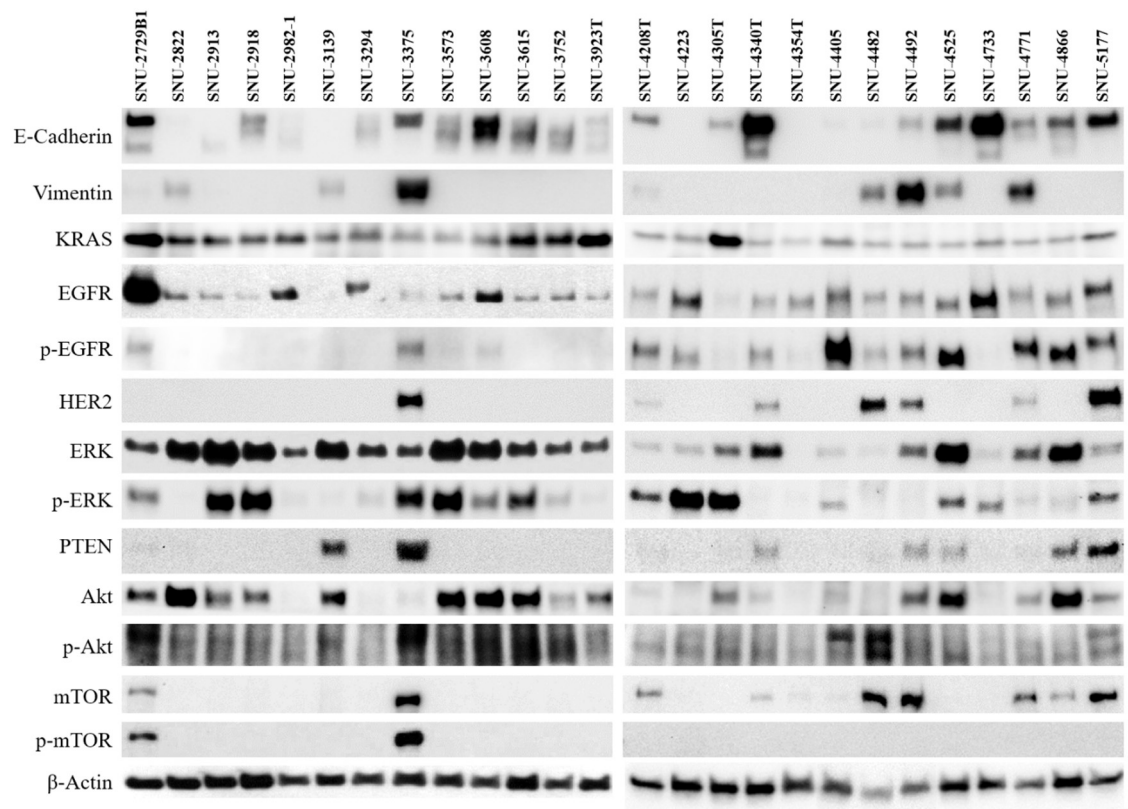

Figure 7. Western blotting of 26 established pancreatic cancer cell lines.

3.7. Fusion gene analysis of 26 established pancreatic cancer cell lines

In order to collect more genetic mutations, fusion gene was sorted from RNA sequencing results to detect NRG1 protein fusion that activates Akt and ERK pathways by replacing KRAS in recently discovered KRAS wild type pancreatic cancer (54,55). For analysis, the genes and junction types of the fusion genes found using FusionGDB were confirmed. As a result, no effective fusion gene was found. However, because translocation occurs frequently in carcinoma, the fusion genes found for each cell line are summarized in Table 7.

Table 7. Summary of discovered fusion genes in established 26 pancreatic cancer cell lines.

Sample	5'end fusion	3'end fusion	Fusion description	5'end fusion position	3'end fusion position
SNU-2729B1	STAT1	ACTB	cancer,m2	2:191863388:-	7:5567269:-
SNU-2729B1	TNRC18	JAK1	oncogene,cancer,m2	7:5352605:-	1:65363274:-
SNU-2913	ALK	ZNF587	oncogene,cancer,tumor,m2	2:29600117:-	19:58375744:+
SNU-3139	ACTB	ANXA1	cancer,m5,exon-exon	7:5566787:-	9:75772570:+
SNU-3139	FN1	CMBL	cancer	2:216250519:-	5:10279671:-
SNU-4305T	ACTB	GNE	cancer,m3,exon-exon	7:5580307:-	9:36227455:-
SNU-4340T	CNN2	SMAD3	cancer,exon-exon	19:1036999:+	15:67482751:+
SNU-4425T	ACTB	ANXA1	cancer,m5,exon-exon	7:5566787:-	9:75772570:+
SNU-4208T	ACTB	DNAJC13	cancer,exon-exon	7:5580307:-	3:132153382:+
SNU-4354T	ACTB	MYOF	cancer,m7,exon-exon	7:5580307:-	10:95211898:-
SNU-4354T	AHNAK	GATD1	N.A	11:62279338:-	11:767868:-
SNU-4405	ACTB	ADAM9	oncogene,cancer,exon-exon	7:5580307:-	8:38959382:+
SNU-4405	RAC1	ANXA1	oncogene,cancer,exon-exon	7:6442151:+	9:75772570:+
SNU-4482	S100A6	ITGB1	N.A	1:153507085:-	10:33276684:-
SNU-4492	ACTN4	METAP1	oncogene,exon-exon	19:39220784:+	4:99943923:+
SNU-4733	ACTB	F11R	cancer,m3	7:5575497:-	1:160967770:-
SNU-4733	ACTB	PDIA3	cancer,exon-exon	7:5566787:-	15:44038590:+
SNU-4866	ACTB	NSD1	oncogene,cancer,tumor,exon-exon	7:5566787:-	5:176692728:+
SNU-4866	AKT2	TJAP1	oncogene,cancer,tumor	19:40736935:-	6:43472140:+

4. Discussion

Advances in sequencing have made it possible to find variants that can be considered characteristic of carcinomas. Pancreatic cancer is well-known for early and aggressive invasion, high metastatic potential and poor prognosis. As a feature of the pancreas composed of both exocrine and endocrine structure, the configuration of the pancreas is complicated. Consequently, even with a surgical resection, the tumor often proceeds with recurrences or local metastasis, which leads to a poor prognosis. Another cause of poor prognosis is the heterogeneity of mutational characteristics. The high rates of KRAS mutations (>80%), inactivation of the tumor suppressor genes TP53, SMAD4, and CDKN2A occur at rates of >30% (6-8). The prevalence of mutations involved in chromatin modification, DNA damage repair, and other mechanisms important for carcinogenesis is known to be less than 10%. Even though molecular classification of pancreatic cancer is significant to precise diagnosis, and it has not been studied with Korean patients' cohorts. Overall characteristics of mutations in 26 newly established cell line were detected and validated. A total of 26,184 point mutations, insertions and

deletions were detected and filtered. After identifying the mutations of the well-known genes in pancreatic cancer by previously described methods, mutations in a total of 40 genes were observed to be significant (Figure 3). The KRAS codon 12 mutation, which is known to be the most representative of pancreatic cancer, was not found in four out of 26 cell lines (SNU-2822, SNU-3752, SNU-4223), This is a fairly similar ratio compared to what was previously reported (56). The signaling transductions regulated by KRAS are diverse (56,57). Typical examples include gene expression regulation through MAPK signaling, cell growth and survival through PI3K-Akt signaling, and cell mobility regulation through Rho and Rac proteins. Among them, it is known that cell proliferation signaling is related to cell growth rate. The fastest growing cell lines in our cell line are SNU-2913 with a doubling time of 22.6 hours and SNU-4492 with a doubling time of 37.9 hours. On the other hand, the slowest cell line is SNU-3752 with a doubling time of 113 hours and SNU-4405 with a doubling time of 119 hours, and has a similar expression pattern except for p-EGFR. As a result, it can be seen that the growth and division rate of cells are not predicted by KRAS alone, and it is necessary to predict through the activity of proteins that directly affect growth and division in pancreatic cancer with high

heterogeneity as controlled by various factors. In addition, KRAS is closely related to the immune response.

Cancer cells with a mutation in KRAS codon 12 are known to increase the secretion of granulocyte-macrophage colony-stimulating factor (GM-CSF) (58,59). This induces the accumulation of myeloid-derived suppressor cell (MDSC) in tumor tissue, which is known to be the main cause of tumor immune evasion mechanisms (60). Therefore, the development and growth of cancer also affects the interaction with the tumor microenvironment accompanied by immune cells.

Mutations were further analyzed for gene set enrichment analysis to find representative pathways that were mostly aberrated in the established pancreatic cancer cell lines. (Figure 4, Table 4-5). Overall, factors that were related to general disease were mostly affected, and therefore 'Diseases of signal transduction' had the highest count with low p-value. One of the most disrupted pathway was 'PI3K /AKT Signaling in Cancer' ($0.001<p$) and Constitutive Signaling by Aberrant PI3K in Cancer ($0.001<p$). There have been many reports that associates the "abnormal activation of Akt/mTOR pathway in cancer" (46,47). Associated genes in our cohort were FGFR4, PIK3CD, ERBB3, IRS2,

KIT, ERBB2, and PIK3R2, which were all connected to signal transduction of Akt/mTOR pathway.

As a result of comparing resistance and expression characteristics of 26 established pancreatic cancer cell lines through anti-cancer drug reactivity and direct protein expression comparison, cell lines could be divided into a total of 3 subgroups according to KRAS mutation characteristics.

One group (SNU-2822, SNU-3752, SNU-4223, and SNU-4354T) displayed wild type KRAS. Another group (SNU-2729B1, SNU-2913, SNU-3139, SNU-3294, SNU-3615, SNU-4305T, SNU-4405, SNU-4525, SNU-4733 and SNU-4866) exhibited mutated KRAS activation. The other group (SNU-3375, SNU-4208T, SNU-4340T, SNU-4482, SNU-4492, SNU-4771, and SNU-5177) displayed HER2 over-expression and mutated KRAS.

KRAS wild-type cell lines, except SNU-3752, showed a common characteristic of mainly low anticancer drug resistance. Compared to other KRAS wild-type cell lines, SNU-3752, which lacks the characteristics of mutant and signaling liver protein expression, requires further study.

KRAS mutated cell lines have broad anti-cancer resistance. The protein expression level and the related anticancer drug resistance tended to be inconsistent because additional mutation characteristics were not considered. It is thought that it is possible to have significant results by comparing the mutation and expression levels of all proteins related to each signaling.

Cell lines with HER2 expression have, on average, high anticancer drug resistance and protein expression. In addition, although it has a common activation of p-EGFR, it shows various resistances to sunitinib and erlotinib as targets. In particular, the resistance to afatinib, which inhibits the dimerization of HER2, appears different from the expectation that it will be consistent with the expression level of HER2. However, trastzumab and cetuximab, which are effectively known for HER2 overexpressing carcinoma, are expected to have low resistance because they inhibit the activation of Akt and induce the activation of the tumor suppressor gene CDKN1B (61,62).

In conclusion, we have newly established 26 pancreatic cancer cell lines from a varied samples and associated their wide-range of mutational characteristics with pathway analysis. In particular, KRAS wild-type cell

lines and HER2 over-expressing cell lines, which occur in small proportions around the world, have been established, and they can be continuously stored for studies on the veils of these two groups of cell lines. KRAS mutated cell lines also confirmed a variety of genetic heterogeneity and thus a broad spectrum of anticancer drug resistance.

Finally, the Korea Cell Line Bank deposits a total of 44 pancreatic cancer cell lines and accumulation of mutational properties of these cell lines contributes to the improvement of categorizing and treating pancreatic cancer. The therapeutic effect can be predicted by comparing the expression of specific protein to the anti-cancer drug response.

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국문 초록

췌장암은 환자의 5 년 평균 생존율에서 9 %의 낮은 생존율을 갖는 가장 치명적인 악성 종양 중 하나이다. 췌장암은 종양 간의 높은 이질성으로 인해 돌연변이 특성을 정의 하기 어려운데, 우리는 서로 다른 26 개의 췌장암 세포주를 수립하고 전장 엑솜 시퀀싱 및 RNA 시퀀싱과 western blotting 을 수행하여 각 세포주들의 돌연변이 특성과 단백질 발현량을 확인하고 항암제 반응성을 검사하였다. 췌장암의 대표적인 것으로 알려진 KRAS 코돈 12 번 돌연변이는 26 개 중 22 개의 세포주에서 발견되었으며, 4 개의 세포주에서 KRAS 돌연변이가 발견되지 않았다. KRAS 야생형을 가지는 췌장암은 SNU-3752 세포주를 제외하고 대체로 항암제에 낮은 저항성을 가지며 이러한 차이는 단백질 발현량과 서로 다른 유전적 요인에 의한 것으로 보인다. KRAS 야생형 의 돌연변이들은 세포 외 기질의 구성과 분해와 관련된 신호 전달과 관련이 있는 것으로 나타났다. KRAS 돌연변이 세포주들은 항암제 반응성과 단백질 발현량의 차이를 통해서 높은 종양 이질성을 확인하였으며 KRAS 돌연변이 세포주들에서 trametinib 과 phospho-ERK 1/2 단백질 발현량 간의

긍정적 상관관계를 확인하였다. HER2 과발현 췌장암 세포주들은 일반적으로 높은 항암제 저항성을 나타내었으며 특정 단백질들의 유전적 변이 및 발현의 차이에 따라서 다양한 결과를 나타내었다.

다양한 돌연변이 특성을 가지는 세포주 수립을 통해서 항암제 반응성과 돌연변이 특성을 비교하였고 이런 결과를 바탕으로 각 돌연변이 특성과 단백질 발현량을 연관시킴으로써 항암제 반응성을 예측할 수 있다.

주요어: 췌장암 세포주, 돌연변이 특성 분석, 항암제 반응성

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