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Establishment and Characterization of 10 Human Pancreatic Cancer Cell Lines Including a HER2 Overexpressed Cell Line

Soon-Chan Kim, BS, *† Young-Kyoung Shin, PhD, * Sun-Whe Kim, MD, PhD, ‡§ Ha-Young Seo, BS, *† Wooil Kwon, MD, ‡§ Hongbeom Kim, MD, ‡§ Youngmin Han, MS, ‡§ Ja-Oh Lee, MS, * Jin-Young Jang, MD, PhD, ‡§ and Ja-Lok Ku, DVM, PhD*†

Objective: The incidence of pancreatic adenocarcinoma (PA) approximates its prevalence, as the malignancy is almost consistently fatal within a year. Although the currently available adjuvant therapy seems to provide survival benefit, it is only moderate, and the standard regimen has not yet been established. Therefore, more biological resources to investigate the PA are needed.

Methods: Here, we established and characterized 10 human pancreatic cancer cell lines derived from primary tumor mass. Whole exome sequencing technique was used to identify driver mutations and aberrant pathways in each cell line.

Results: Five anticancer drugs were treated to find half maximal effective concentration (EC50), and the response was analyzed in reference to mutational status. Frame shift mutations in *ARID1A* gene and HER2 amplification were mutually related to better response to the anticancer drugs. In contrast, frame shift mutation in *MSH6* gene was associated with resistance to anticancer drugs.

Conclusions: In summary, we established 10 pancreatic cancer cell lines and integrated various molecular aberrations and features of pancreatic cancer cells. Our biological resources are expected to contribute to facilitating research on PA.

Key Words: pancreatic adenocarcinoma, drug response, characterization, HER2 overexpressed cell lines

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Pancreatic cancer is relatively infrequent worldwide, comprising only 2.5% of all forms of cancer.¹ Despite its low occurrence, its poor prognosis ranks pancreatic cancer as the fourth or fifth highest in malignancy mortalities.^{2,3} Surgery is still the mainstay of treatment for pancreatic cancer, but only 15% to 20% of pancreatic cancers are resectable at the time of diagnosis, and

From the *Korean Cell Line Bank, Laboratory of Cell Biology, Cancer Research Institute; †Department of Biomedical Sciences; ‡Cancer Research Institute; and §Department of Surgery, Seoul National University College of Medicine, Seoul, South Korea. Received for publication January 21, 2019; accepted September 8, 2019.

Address correspondence to: Ja-Lok Ku, DVM, PhD, Laboratory of Cell Biology, Cancer Research Institute, Seoul National University College of Medicine, 103 Daehak-ro, Jongno-gu, Seoul 03080, South Korea (e-mail: kujalok@snu.ac.kr); Jin-Young Jang, MD, PhD, Department of Surgery, Seoul National University College of Medicine, 103 Daehak-ro, Jongno-gu, Seoul 03080, South Korea (e-mail: jangjy4@snu.ac.kr).

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the prognosis after R0 resection is poor. Furthermore, although currently available adjuvant therapy seems to provide survival benefit, it is only moderate, and the standard regimen has not yet been established.^{4–6} Therefore, strategies to confirm therapeutic efficacy or identify additional options would be beneficial to both clinicians and patients. To address this need, we describe the establishment of a living biobank consisting of pancreatic cancer cell lines, which facilitates the integration of genomic data with drug screening of patients' tumor samples on an iterative platform. In previous work, we established and characterized 4 human pancreatic carcinoma cell lines.⁷ Here, we present 10 human pancreatic cancer cell lines (designated SNU-2466, SNU-2469, SNU-2485, SNU-2491, SNU-2543, SNU-2564, SNU-2570, SNU-2571, SNU-2608, and SNU-2617) and integrated various molecular aberrations and features of pancreatic cancer cells.

MATERIAL AND METHODS

Establishment of Cell Lines and Cell Culture Condition

Cell lines were established from pathologically proven pancreatic carcinomas. Detailed procedure was described previously.⁸ The locations and stages of original tumors of newly established pancreatic cancer cell lines are listed in Table 1.

DNA Finger Printing

The genomic DNA from each cell line was amplified using the AmpFISTR identifier PCR amplification kit (Applied Biosystems, Foster City, Calif). Detailed procedure was described previously.⁸

Cell Growth Properties

Cell growth rate was determined by procedures that were described previously.⁸ Mycoplasma contamination was tested by the 16S-rRNA-gene-based polymerase chain reaction (PCR) amplification method using the e-Myco Mycoplasma PCR detection kit (Intron Biotechnology, Gyeonggi, South Korea).

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, Carlsbad, Calif) and RNeasy Plus Mini Kit (Qiagen) according to the manufacturer's protocol. For cDNA synthesis, detailed procedure was described previously.⁸

Quantitative Real-Time PCR

Detailed procedure was described previously.⁸ The primers that were used in this study are listed in the Supplementary Table 1, <http://links.lww.com/MPA/A749>.

Colony-Forming Assay

Cells were seeded using 300, 500, and 1000 cells onto 60-mm diameter tissue culture dishes containing 5 mL of culture medium

TABLE 1. Clinicopathologic Characteristics of 10 Pancreatic Cancer Cell Lines

	In Vitro				In Vivo			
	Growth Pattern	Doubling Time	Cell Morphology	Sex	Age	Cultured Tumor Site	Tumor Size, cm	Degree of Differentiation
SNU-2466	Adherent	45	Fusiform	F	75	Primary	2.5 × 2.5 × 2.3	M/D
SNU-2469	Adherent	48	Polygonal	F	79	Primary	3.3 × 2.5 × 2.0	M/D
SNU-2485	Adherent	45	Polygonal	F	61	Primary	3.0 × 2.8 × 2.4	
SNU-2491	Adherent/floating	29	Round/Polygonal	F	61	Primary	2.5 × 2.0 × 2.1	M/D
SNU-2543	Adherent	55	Polygonal	M	70	Primary	4.6 × 3.5 × 3.5	M/D
SNU-2564	Adherent	58	Polygonal	F	64	Primary	4.5 × 3.5 × 3.5	
SNU-2570	Adherent	49	Polygonal	F	68	Primary	3.5 × 3.0 × 3.0	M/D
SNU-2571	Adherent/floating	36	Round/Polygonal	F	74	Primary	2.8 × 2.5 × 2.0	P/D
SNU-2608	Adherent	48	Polygonal	F	77	Primary	3.6 × 3.0 × 2.2	M/D
SNU-2617	Adherent	17	Polygonal	M	68	Primary	4.0 × 2.5 × 2.0	W/D

M/D indicates moderate differentiated; P/D, poorly differentiated.

and incubated for 7 days. The dishes were stained with cell stain solution (Cell Biolabs, Inc, San Diego, Calif), and the number of colonies containing more than 10 cells was counted. Gastric cancer cell line, SNU-1, was used as a control as it has proven clonogenic ability.

Protein Isolation and Western Blotting

Entire procedure was described previously.⁸ Antibodies used in Western blotting are listed in Supplementary Table 2, <http://links.lww.com/MPA/A749>.

Drug Sensitivity Test

At 70% to 80% of confluency, cells were detached from a T75 flask with 2× trypsin. After centrifugation, the cells were re-suspended with a culture medium. According to various growth rates, 2 to 8 × 10⁵ 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. Anticancer 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 Ez-Cytox (Dea-II biotech, Gyeonggi-do, South Korea) was added to each well. After 2 hours of incubation at 37°C, the optical density was measured at 450 nm using Multiskan FC Microplate Photometer (Thermo Fisher Scientific, Waltham, Mass).

Microsatellite Instability and Mutation Analyses of DNA Mismatch Repair Gene

For microsatellite instability analysis, BAT-25 and BAT-26 were evaluated using a capillary-based sequencing analysis. Polymerase chain reaction was performed as described previously, with the exception that the forward primers were labeled with a fluorescent dye, and the labeled samples were run on an ABI 3730 genetic analyzer (Applied Biosystems). GeneMapper software v4.0 (Applied Biosystems) was used to calculate the size of each fluorescent PCR product. For gel-based microsatellite instability analysis, the desired fragments were amplified in the presence of [α-³²P] deoxycytidine triphosphate. The PCR products were denatured and separated on 6 M urea/7% polyacrylamide gels run at 60 W.

Statistical Analysis

Statistical analysis was performed using R program Version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria)

with various packages including maftools, PerformanceAnalytics, survminer, survival, iplot, gplot, and lattice. The relationship between gene mutation or expression and clinical data of pancreatic adenocarcinoma (PA) was analyzed by the χ^2 test and Pearson correlation method. Fisher exact test was used to analyze Gene Ontology analysis of various genes. All tests were performed using bilateral 95% confidence intervals. A value of $P < 0.05$ was considered statistically significant.

RESULTS

General Characteristics of the Cell Lines

On in vitro cultivation, 8 cell lines (SNU-2466, SNU-2469, SNU-2485, SNU-2543, SNU-2564, SNU-2570, SNU-2608, and SNU-2617) grew as monolayer of substrate-adherent cells, and 2 cell lines (SNU-2491 and SNU-2571) formed floating and adherent aggregates. Most tumor cells displayed a polygonal shape and had round-to-oval nuclei with prominent single-to-double nucleoli (Fig. 1). Each cell line was passaged at least 3 times before characteristic analysis. Population doubling times ranged from 47 to 135 hours. Clinicopathologic information is listed in Table 1. Patients' history of preoperative/postoperative adjuvant therapy and overall survival are listed in Table 2. All cell lines were confirmed to be free of bacterial and mycoplasma contamination (Supplementary Fig. 1, <http://links.lww.com/MPA/A749>). Fifteen tetranucleotide repeat loci and Amelogen sex-determining markers were heterogeneously distributed in each cell line and were not cross-contaminated (Table 3).

Whole Exome Sequencing Analysis

To establish the mutational context of the established pancreatic cancer cell lines, whole exome sequencing (WES) was performed. To further analyze WES data, 434 genes that have been involved in PA were selected (Supplementary Table 3, <http://links.lww.com/MPA/A749>), and mutations that occurred in the sorted genes were screened. The general information, such as variant classification and single nucleotide variants class, are summarized in Figure 2A. SNU-2491 had the largest number of variants, whereas SNU-2571 had the smallest number of variants. The median number of variants per sample was 176.5. Mutations were further analyzed for gene set enrichment analysis to find representative pathways that were aberrated in the established PA cell lines. Genes consisting of MAPK family signaling

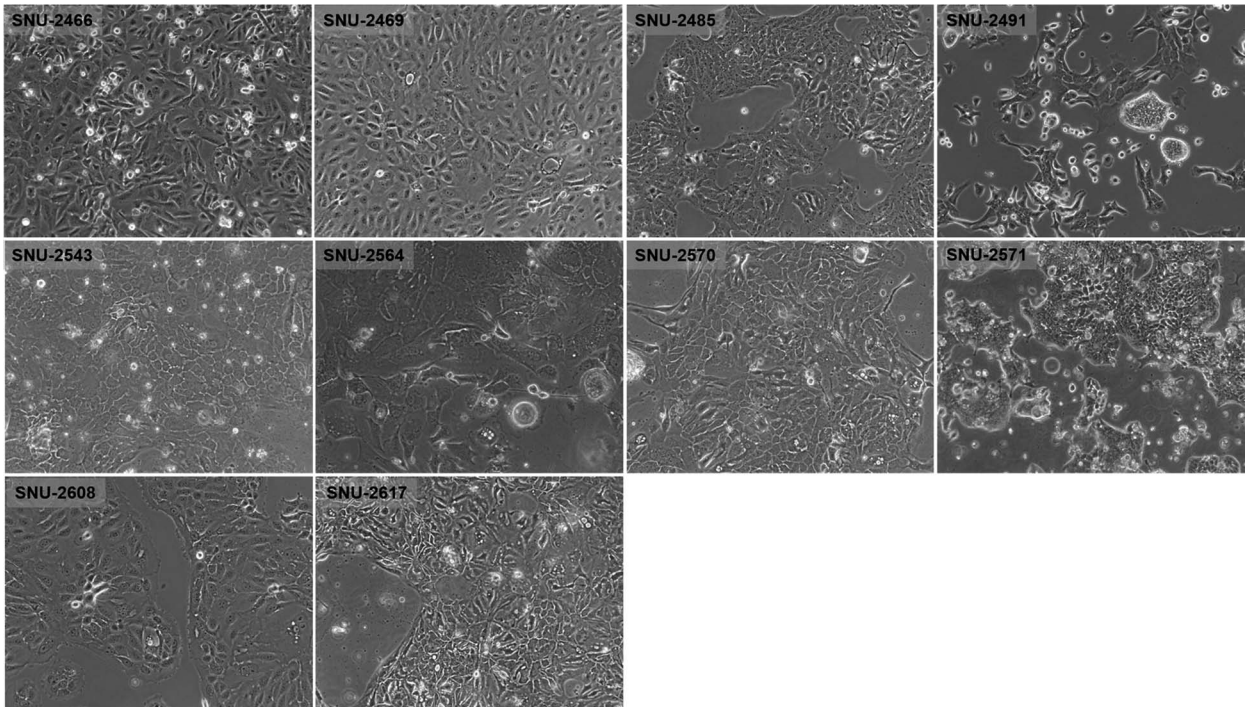


FIGURE 1. Phase-contrast microscopy of PA cell lines. On in vitro cultivation, 8 cell lines (SNU-2466, SNU-2469, SNU-2485, SNU-2543, SNU-2564, SNU-2570, SNU-2608, and SNU-2617) grew as monolayer of substrate-adherent cells, and 2 cell lines (SNU-2491 and SNU-2571) formed floating and adherent aggregates. Most tumor cells displayed a polygonal shape and had round-to-oval nuclei with prominent single-to-double nucleoli.

cascade and interleukin-20 family signaling were mostly mutated (Fig. 2B). The prevalence of aberrations in key driver genes is categorized into 5 groups as indicated in Figure 2C. The mutational statuses and proposed functions of such genes are summarized in Supplementary Table 4, <http://links.lww.com/MPA/A749>. Many such driver genes in cancer are co-occurring, or show exclusiveness in their mutation patterns, and can be detected using somatic interactions function in Maftools, which performs pair-wise Fisher exact test to detect such significant pair of genes. For instance, mutations in *PIK3R1* and *CDH1* genes are co-occurring, whereas mutations in *HIF-1A* and *BIRC5* genes are exclusive (Fig. 2D). Mutational signatures characterized by a specific pattern of nucleotide substitutions were extracted by decomposing a matrix

of nucleotide substitutions and were then compared with the public database presented by Alexandrov et al.⁷ Newly established pancreatic cancer cell lines showed a pattern of signature 5 (Fig. 2E). Drug-gene interactions and gene druggability information can be extracted from drug-gene interaction database using drug interactions function in Maftools. The result showed that kinase and DNA repair pathways were potential druggable gene categories (Fig. 2F).

Anticancer Drug Response With Mutational Contexts

Five anticancer drugs (5-Fu, cisplatin, paclitaxel, gemcitabine, and cetuximab) were used to estimate the drug sensitivities of the

TABLE 2. Patients' History of Preoperative/Postoperative Adjuvant Therapy

	Preoperative		Postoperative		OS, mo
	Chemotherapy	Radiotherapy	Chemotherapy	Radiotherapy	
SNU-2466	No	No	Yes (Gem/Cap)	Yes	7
SNU-2469	No	No	Yes (Gem/Cap)	No	10
SNU-2485	No	No	Yes (Gem, Cap, FOLFOX)	Yes	9
SNU-2491	No	No	Yes (Cap, FOLFOX)	No	40
SNU-2543	No	No	Yes (Gem, FOLFOX)	Yes	11
SNU-2564	No	No	Yes (5-FU, FL, Gem, FOLFOX, iFAM)	Yes	36
SNU-2570	No	No	Yes (Gem, FOLFOX)	No	10
SNU-2571	No	No	Yes (Gem, FOLFOX)	No	5
SNU-2608	No	No	Yes (Gem)	No	131
SNU-2617	No	No	Yes (Gem, FOLFOX)	No	12

Cap indicates capecitabine; Gem, gemcitabine; iFAM, infusional 5-fluorouracil, doxorubicin, and mitomycin-C; OS, overall survival.

TABLE 3. Short Tandem Repeat Profile of 10 Pancreatic Cancer Cell Lines

	SNU-2466	SNU-2469	SNU-2485	SNU-2491	SNU-2543	SNU-2564	SNU-2570	SNU-2571	SNU-2608	SNU-2617
D8S1179	12,13	13,14	13	12	11,12	14,15	15,16	13	13,14	11,13
D21S11	29,31.2	29,30	29,31	29	29,30	30	30,31	30,32.2	31	30
D7S820	8,13	8	8,11	9,11	10,11	11	11	8,11	10,12	11
CSFIPO	12	11,12	11,12	10,12	13	9,13	10	10	9,12	10
D3S1358	16	14,16	15	14,15	15,16	15	17	15,20	15,17	16
THO1	8,9	7	6,2,8,10	9	9	9	8,9	7,9	9,3	6,9
D13S317	9,12	10	8,11	8	11,12	9,11	11	11	9,10	8
D16S539	10,12	9,14	9,10	9,13	9,12	9	12	11,12	10	9,10
D2S1338	19,20	19,20	17,24	18,24	24,25	17,20	18,19	17,19	20	24,25
D19S433	13,14.2	14,15	13	14.2, 15.2	13,15.2	13,15	13,14	12,16.2	11,13	14
vWA	18,19	14,20	16,18	16,19	17,18	16,18	14	16,18	14, 17	14,15
TPOX	8	9,11	8,11	8	8,11	11	8,11	8,11	9,11	11,12
D18S51	13	15	13	13	15	16	17	15	16	18
D5S818	10,13	7,10	10,11	10,11	9	11,12	11	11	11	7,13
FGA	22	24	23	22	23	23,26	24,25	23,24	21,23	22,25
Amelogenin	X	X	X	X	X,Y	X	X	X	X	X

established pancreatic cancer cell lines. SNU-2617 was excluded because of its tardy growth rate. The SNU-2571 and SNU-2491 cell lines were relatively sensitive to 5-Fu. Of the remaining 8 cell lines, SNU-2608 was the most resistant to 5-Fu, where its half maximal inhibitory concentration (IC50) was 3.993 μ M. The remaining cell lines had IC50 of 5-Fu ranging from 1.653 to 2.678 μ M (Fig. 3A). IC50 for cisplatin was relatively consistent in the established pancreatic cancer cell lines where its range was from 2.664 to 3.265 μ M. Similar to 5-Fu, the most sensitive and resistant cell lines for cisplatin were SNU-2571 and SNU-2608, respectively

(Fig. 3B). IC50 for paclitaxel was also relatively constant, except for SNU-2608. Similar to 5-Fu and cisplatin, the most sensitive and resistant cell lines for paclitaxel were SNU-2571 and SNU-2608, respectively (Fig. 3C). Cells were clustered in 2 groups for sensitivities of gemcitabine. SNU-2570, SNU-2564, and SNU-2608 were comparatively resistant, whereas SNU-2466, SNU-2469, SNU-2485, SNU-2491, and SNU-2543 were relatively sensitive (Fig. 3D). The sigmoidal dose response of SNU-2571 to gemcitabine was not obtained because its EC50 was not converged to numeric value. The results are summarized in Table 4. IC50 value for cetuximab

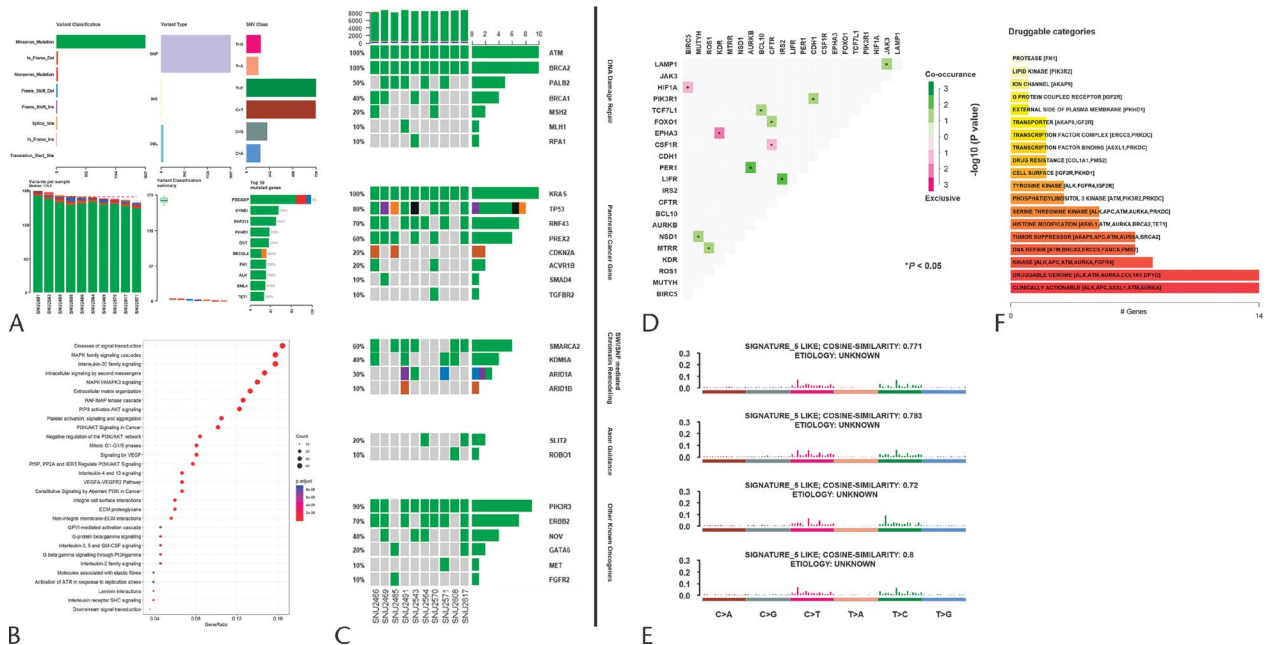


FIGURE 2. Mutational context of the established pancreatic cancer cell lines. A, Summarization of variants. B, Gene set enrichment analysis to find representative pathways that were aberrated in the established PA cell lines. C, The prevalence of aberrations in key driver genes with 5 categories. D, Co-occurring or exclusiveness in the mutation patterns of pancreatic cancer cell lines. E, Mutational signatures characterized by a specific pattern of nucleotide substitutions. F, Targetable genes were identified in reference with the number of genes that are involved in drug target pathways.

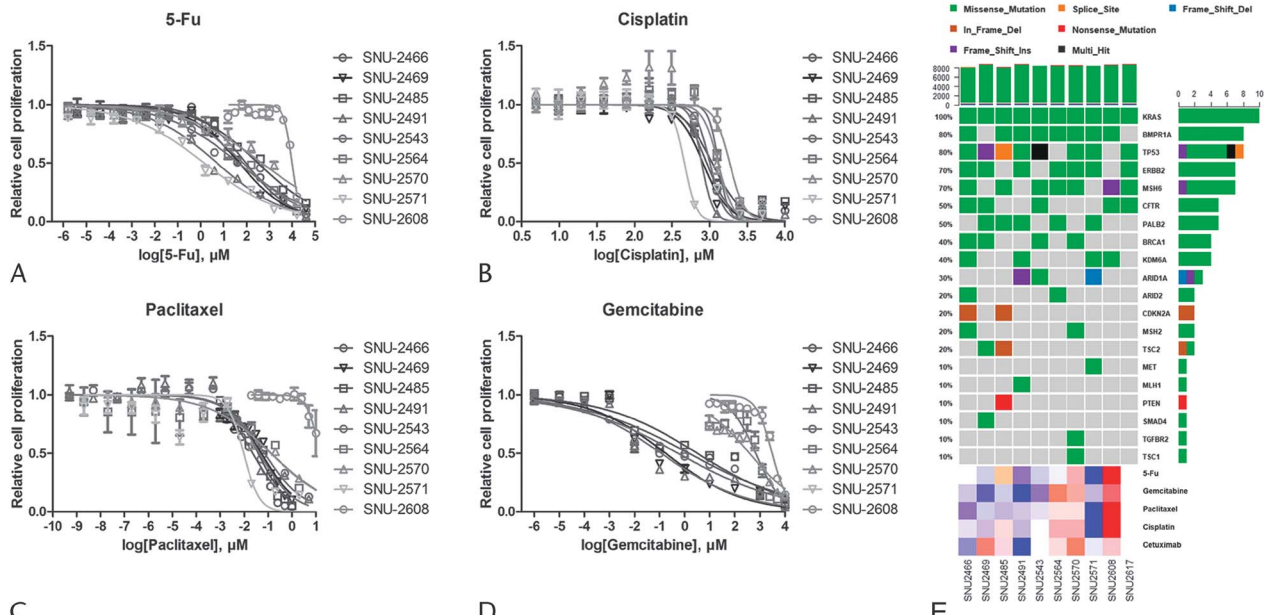


FIGURE 3. Anticancer drug response with mutational contexts. Four anticancer drugs (5-Fu [A], cisplatin [B], paclitaxel [C], and gemcitabine [D]) were used to estimate drug response of the established pancreatic cancer cell lines. SNU-2564, SNU-2570, and SNU-2608 cell lines were the most resistant group for the anticancer agents. SNU-2608 was the most resistant cell line for all anticancer drugs tested in this study. E, Mutational status of 20 genes that have been reported to be involved in PA was screened with regard to the calculated response of 5 anticancer drugs.

was not able to be computed because all cell lines except for SNU-2491 did not respond to the maximum concentration of cetuximab. Therefore, IC₅₀ unit was converted to area under curve to compare different molar concentration of 5 anticancer drugs. Mutational status of 20 genes that have been reported to be involved in PA were screened with regard to the calculated response of 5 anticancer drugs. Interestingly, SNU-2491 and SNU-2571 cell lines had frameshift mutation in *ARID1A* gene and were sensitive to all anticancer drugs tested in this study. In contrast, SNU-2608 cell line had frameshift mutation in *MSH6* gene and was the most resistant cell line for all anticancer drugs tested in this study (Fig. 3E).

Anticancer Drug Response With Expression of Molecular Markers

The mRNA expressions of various genes were verified by reverse transcription PCR (Figs. 4A, B). The functional roles of the examined genes are categorized into the following 4 groups: angiogenesis, cell cycle regulation, epithelial to mesenchymal transition (EMT), and stemness. MIA PaCa-2 and PANC-1 cell lines were obtained from the Korean Cell Line Bank to compare gene expression in widely used pancreatic cancer cell lines. The mRNA levels of vascular endothelial growth factor A (VEGF-A), VEGF-C, and hypoxia-inducible factor 1 α (HIF-1 α) were screened to examine the angiogenesis-related ability of each cell line. Vascular endothelial growth factor A was weakly or not expressed in all pancreatic cancer cell lines. The expression patterns of VEGF-C and HIF-1 α were analogous in all cell lines with the exception of the SNU-2608 and SNU-2617 cell lines. The SNU-2564, SNU-2570, and SNU-2571 cell lines did not express all angiogenesis-related genes tested. The mRNA levels of cyclin A, cyclin B1, and cyclin D were screened to examine the cell cycle-related genes. Cyclin A was strongly expressed in MIA PaCa-2 and PANC-1, whereas newly established pancreatic cell lines had weak expression. Cyclin B1 was not expressed in the SNU-2543 and

SNU-2564 cell lines. All cell line expressed Cyclin D with the exception of SNU-2608. Among the EMT markers, N-cadherin was expressed in all cell lines with the exception of SNU-2570. The mRNA levels of E-cadherin and N-cadherin did not have inverse correlation. Various stemness-related genes were also examined. Markedly, *SOX9* was expressed in all established pancreatic cancer cell lines, whereas *SOX4* was not expressed in all established pancreatic cancer cell lines. The expression of EMT-related proteins was examined by Western blotting (Fig. 4C). Vimentin was exclusively expressed in SNU-2466. The protein expressions of epidermal growth factor receptor (EGFR), HER2, ERK1, ERK2, pERK1/2, PTEN, and SMAD4 were also screened. Among the 10 pancreatic cancer cell lines, HER2 was exceptionally expressed in SNU-2491. The patterns of mRNA expression were analyzed in comparison with drug response, and the mRNA expressions of VEGF-C and HIF-1A were inversely related to overall drug response (Fig. 4D).

erbB2 Amplification Influences Colony-Forming Ability and Response to Cetuximab in Pancreatic Cancer Cells

Since the exclusive erbB2 amplification (Figs. 4C, 5B), positive response to cetuximab (Figs. 3E, 5C) and negative response to trastuzumab (Fig. 5D) of SNU-2491 cell line were intriguing, we further screened whether there is phenotypical difference between SNU-2491 and other cell lines. Each cell line was tested for anchorage-dependent colony-forming assay. The SNU-1 cell line was obtained from Korean Cell Line Bank as the positive control for the colony formation. Among the 10 pancreatic cancer cell lines, only SNU-2491 formed an average of 17 colonies 28 days after seeding (Fig. 5A). Anchorage-dependent colony-forming assay was performed again with maximum concentrations of trastuzumab (100 μ g/mL) and cetuximab (1000 μ g/mL) to consolidate the positive response of SNU-2491 to cetuximab instead of trastuzumab. SNU-2491-containing PBS yielded an average of

TABLE 4. IC50 Values of 10 Established Pancreatic Cancer Cell Lines for 4 Anticancer Drugs

	SNU-2466	SNU-2469	SNU-2485	SNU-2491	SNU-2543	SNU-2564	SNU-2570	SNU-2571	SNU-2608
5-Fu, μ M	1.98 (0.079)	1.69 (0.094)	2.51 (0.094)	0.72 (0.089)	1.65 (0.114)	1.85 (0.088)	2.68 (0.151)	0.16 (0.102)	3.99 (0.022)
Gemcitabine, μ M	0.18 (0.224)	-0.76 (0.153)	0.51 (0.183)	-0.93 (0.177)	-0.26 (0.148)	2.83 (0.077)	2.59 (0.062)	NA*	3.49 (0.035)
Paclitaxel, μ M	0.023 (0.055)	0.053 (0.05)	0.039 (0.034)	0.044 (0.118)	0.071 (0.084)	0.136 (0.247)	0.129 (0.092)	0.01 (0.145)	14.29 (0.029)
Cisplatin, μ M	2.96 (0.015)	2.91 (0.028)	3.03 (0.031)	2.9 (0.014)	3 (0.012)	3.12 (0.018)	3.11 (0.044)	2.66 (0.032)	3.27 (0.019)

Data are presented as mean (standard deviation).

*The dose response of SNU-2571 to gemcitabine was not converged to numeric value and indicated as NA.

41 colonies 45 days after seeding. When treated with trastuzumab, there were an average of 35 colonies. When treated with cetuximab, there were an average of 13 colonies. Dual treatment of cetuximab and trastuzumab yielded only 6 colonies (Fig. 5E).

DISCUSSION

The incidence of PA approximates its prevalence, as the malignancy is almost consistently fatal, frequently within a year.¹⁰ This high mortality rate is mostly due to its aggressiveness, resistance to chemotherapy, and difficulty in early detection. The disease often progresses to an advanced stage at the time of diagnosis, making surgical resection impractical.¹¹ Moreover, the molecular basis of the aggressiveness remains elusive because of its biological complexity, and more detailed investigation of PA is indispensable to improve strategies for prevention and treatment.¹² A major predicament in researching PA biology arises from the peculiar morphologic characters of the tumor. In vivo, PA stimulates desmoplastic reactions that widen the spaces among tumor cells with reactive nontumor elements, such as inflammatory cells and fibroblasts, and consequently reduce the number of viable tumor cells available per single macroscopic field.^{13,14} With this biological nature, there are only 53 pancreatic cancer cell lines available in the research field. Besides, those available cell lines are somewhat outdated. For example, the SW-1990 line, which has been widely used in PA study, was established in 1978 from a spleen metastasis of a grade II PA and passaged numerous times. Accumulation of genetic aberrations of cancer cell lines that occurs with increasing passage numbers has limited their clinical correlation. Genetically altered cancer cell lines under in vitro conditions do not truly represent clinical scenarios. There is a wide range of variability in patient response toward the same drugs used on tumors that are identical in their genetic aberration. The derivation and short-term culture of primary cells from solid tumors have thus gained significant importance in personalized cancer therapy. Therefore, newly established pancreatic cancer cell lines may provide adequate models for studying a wider spectrum of biological and molecular characters of PA.¹⁵ Here, we present 10 new human pancreatic cancer cell lines (designated SNU-2466, SNU-2469, SNU-2485, SNU-2491, SNU-2543, SNU-2564, SNU-2570, SNU-2571, SNU-2608, and SNU-2617) derived from a primary tumor mass. All cell lines introduced in this study will be deposited to Korean Cell Line Bank (<http://cellbank.snu.ac.kr>) at initial passages to be distributed to researchers worldwide.

Various molecular aberrations have been documented in PA involving several oncogenes and oncosuppressor genes, such as *KRAS*, *TP53*, *CDKN2A*, *SMAD4*, and *ARID1A*.^{10,16} Classical progression of pancreatic carcinogenesis has been classified into early (telomere shortening and activating mutations in *KRAS*), intermediate (inactivating mutations or epigenetic silencing of *CDKN2A2*), and late (inactivating mutations of *TP53* and *SMAD4*) events.¹⁷ Activating mutations of *KRAS* is frequently reported in PA. Most (>98%) *KRAS* mutations in PA are point mutations at codon G12, whereas codon G13 and Q61 comprise lower portions.^{18,19} All of our PA cell lines harbored point mutations at codon G12. Forty-nine percent of pancreatic cancers in the COSMIC (Catalogue Of Somatic Mutations In Cancer) database harbor *TP53* mutations.²⁰ Except for SNU-2608, our PA cell lines had *TP53* mutation either in the splice site or in the exonic region. All missense mutations and one frameshift insertion in the *TP53* gene were previously reported in COSMIC. *CDKN2A* is known to be a tumor suppressor gene,²¹ and germline mutation of this gene is associated with increased occurrence of PA.²² SNU-2466 had a p.Thr18_Ala20del in-frame deletion, and

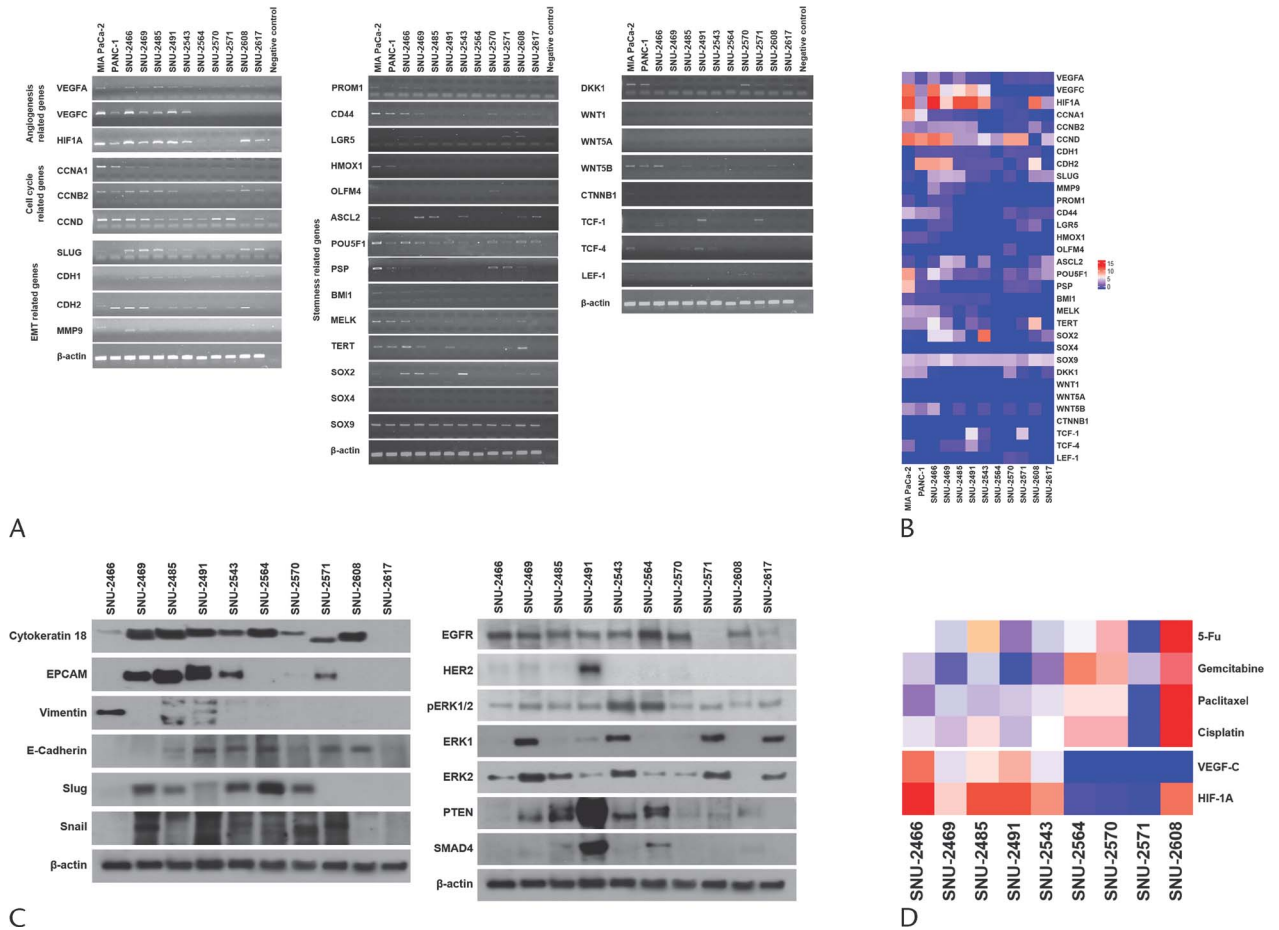


FIGURE 4. Gene expression patterns of PA cell lines. A, The mRNA levels of various fundamental genes that account for angiogenesis, cell cycle regulation, EMT, and stemness were examined. B, The mRNA level is converted to numeric value to be expressed in heatmap. C, The expression of EMT-related proteins and key proteins to develop pancreatic cancer were examined by Western blotting. D, mRNA expressions of VEGF-C and HIF-1A were inversely related to overall drug response.

SNU-2485 harbored p.Pro70_Asn71del disruptive in-frame deletion on the *CDKN2A* gene, and both mutations were homozygous. Average survival time of 10 enrolled pancreatic cancer patients was 27 months. Follow-up record of SNU-2466 and SNU-2485 patients was lost after 7 months and 9 months, respectively, because of the markedly aggressive relapse. This suggested that the in-frame deletion of *CDKN2A* may affect the recurrence rate after the surgery.

Among the cell lines, only SNU-2491 formed a colony during the anchorage-dependent colony-forming assay. Colony formation may imply stronger tumorigenic behavior, which can lead to further speculation of poorer prognosis.²³ The patient developed liver metastasis only 43 days after surgery, which may reflect the aggressive behavior of SNU-2491. Chemotherapy (Gemzar and Xeloda) was performed, but the disease continuously progressed and folinic acid, fluorouracil, and oxaliplatin (FOLFOX) was additionally administered with little success. The follow-up record of the patient after 6 months was lost. Although the prognosis of this patient seems unfavorable, additional cases need to be investigated to draw a conclusion on the clinical implication of colony formation assay.

It was observed that HER2 was exclusively amplified in SNU-2491. HER2 amplification is known to occur in 2% of PA and has distinct features, such as a preponderance of lung and brain metastases.²⁴ Although the connection between augmented

HER2 expression and colony-forming efficacy has not been documented in pancreatic cancer cells, Alajati et al²⁵ reported that an amplified HER2 increased the colony-forming capacity of breast cancer cells. Our data suggested that HER2 amplification in PA may cause more aggressive behavior.

The exclusive HER2 amplification and colony-forming ability rendered the SNU-2491 as the suitable model for the trastuzumab (a monoclonal antibody that targets the HER2 receptor) treatment. Against expectations, inhibition of HER2 with trastuzumab did not impede proliferation of SNU-2491 as in other PA cell lines (Fig. 5D). Remarkably, cetuximab, a monoclonal antibody that targets anti-EGFR, seemed exclusively effective on the SNU-2491 compared with other cell lines (Fig. 5B), although the protein level of EGFR was analogous among the PA cell lines (Fig. 4C). The positive response of cetuximab was confirmed again with anchorage-dependent colony-forming assay (Fig. 5E). The poor response of pancreatic cancer cells to trastuzumab had been reported in multiple studies.^{26–28} Besides, the use of cetuximab in treating PA has been previously reported.²⁹ Our data reinforced that the use of cetuximab in HER2-overexpressed pancreatic cancer has better outcomes than the use of trastuzumab. Nevertheless, the clinical importance and the molecular inhibitory mechanism of cetuximab in HER2-amplified pancreatic cancer still need to be elucidated.

Five generally used anticancer drugs (5-Fu, cisplatin, paclitaxel, gemcitabine, and cetuximab) in pancreatic cancer³⁰ were

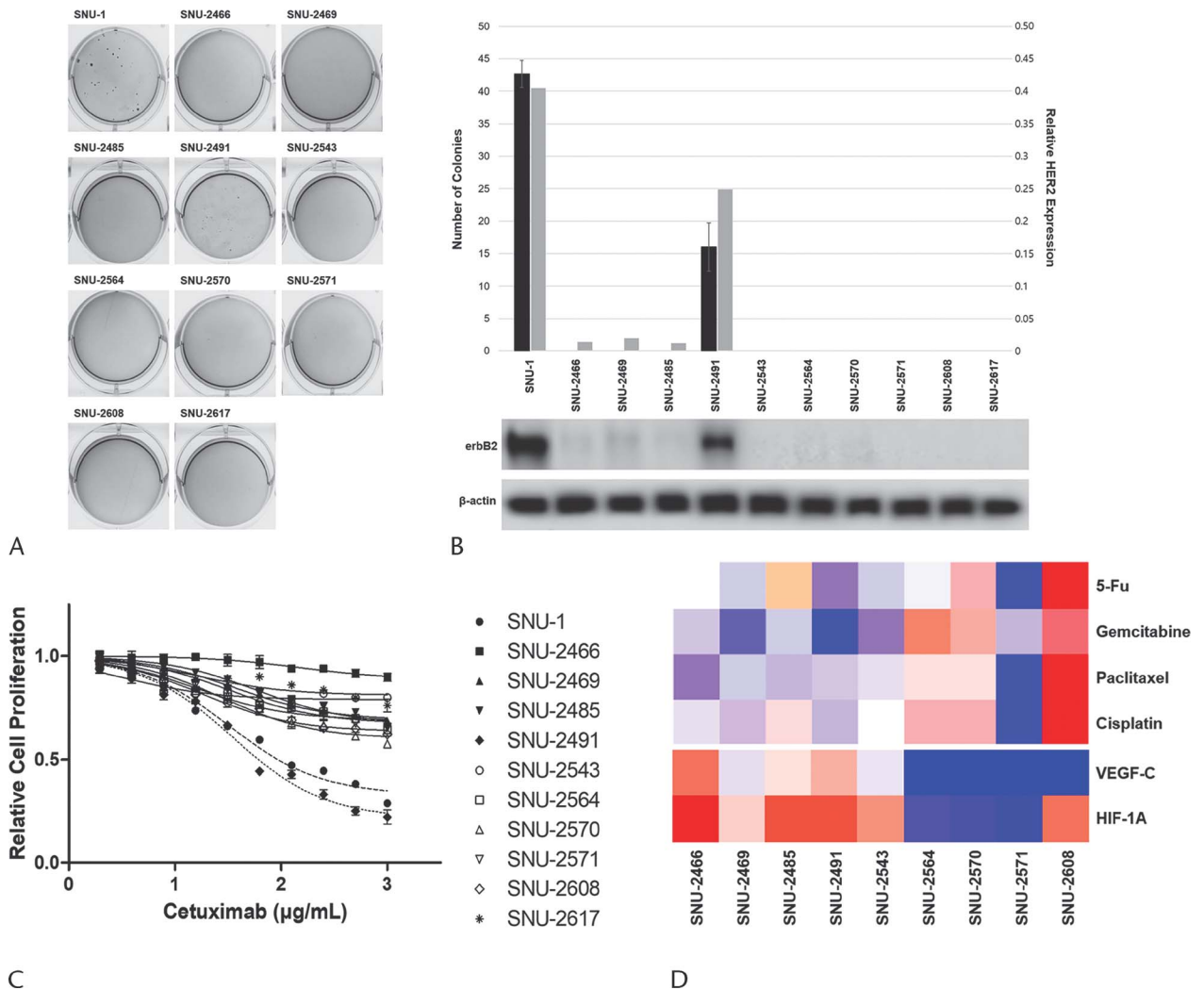


FIGURE 5. erbB2 amplification influences colony-forming ability and response to cetuximab in pancreatic cancer cells. A, Among 10 pancreatic cancer cell lines, only SNU-2491 formed average 17 colonies 28 days after seeding. B, SNU-2491 was the only cell line that had evidently upregulated HER2 protein level compared with other established cell lines. C, Each cell lines were tested for the response of cetuximab. SNU-2491 was the only cell line that showed significantly inhibited growth rate in accordance with cetuximab treatment. D, SNU-2491 did not respond to trastuzumab despite of amplified HER2. E, The efficiency of cetuximab compared with trastuzumab in SNU-2491 was re-encapsulated with anchorage-dependent colony-forming assay.

used for estimating the drug sensitivities of 10 newly established pancreatic cancer cell lines. The SNU-2564, SNU-2570, and SNU-2608 cell lines were the most resistant group for the anticancer agents. Mutations that were exclusively present in 3 drug-resistant cell lines were screened (Supplementary Table 5, <http://links.lww.com/MPA/A749>). Among them, the *MYL1* gene had frameshift mutation at the very first codon in all 3 drug-resistant cell lines. Further investigations regarding exclusive mutations are suggested. SNU-2608 was the most resistant cell line for all anticancer drugs tested in this study. Interestingly, SNU-2608 had relatively low numbers of driver mutations compared with other established cell lines (Figs. 2C, 3E), and the survival time of SNU-2608 patient after surgery was 131 months, which was longest among the 10 enrolled patients in this study.

Impaired drug delivery pathways have been shown to be another reason for the drug resistance in pancreatic cancer.³¹ It is believed that solid tumor masses appear within pancreatic tumors

that lack adequate vasculature with intensive desmoplastic reaction.²⁵ The patterns of mRNA expression were analyzed in comparison with drug response, and the mRNA expressions of VEGF-C and HIF-1A were inversely related to overall drug response (Fig. 4D). It has been reported that the expression of HIF-1A and VEGF-C was directly related to lymphatic vessel density,³² and level of lymphatic vessel density influences direct targeting of tumor vasculature in the clinic with Food and Drug Administration-approved drugs, such as bevacizumab, sorafenib, and sunitinib that target kinase activity of VEGF family receptors.³³ Our data may suggest that mRNA expressions of VEGF-C and HIF-1A affect other anticancer drugs, such as 5-Fu, cisplatin, paclitaxel, and gemcitabine. Nevertheless, drawing a correlation between the HIF-1A and VEGF-C mRNA expression and drug resistance on a 2-dimensional model was inadequate. The approach using the 3-dimensional culture model should be accessed to further investigate the stroma marker expression of the tumor and its effect on drug delivery.

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