의학 석사 학위논문

# Establishment and characterization of breast cancer patient-derived cell lines, xenograft, and organoids

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# Abstract

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Breast cancer is the most common cancer in woman, and although many targeted agents have been developed for last 2 decades, breast cancer still remains one of the leading causes of death in women. Breast cancer is heterogeneous disease which could be divided into three major subtypes and endocrine treatment and many targeted agents are currently developing to control this disease. Twenty pleural effusion-derived breast cancer cell lines and one ascites-derived breast cancer cell line were newly established. In addition, three pairs of cell line-organoid were established from the tumor tissue of breast cancer patient derived xenograft (PDX). Cellular and molecular properties of a total of new 24 cell lines and 3 organoids were analyzed. Genetic characteristics were revealed through screening for mutant

genes that are found to have a large variation in breast cancer such as *TP53, PIK3CA, PTEN, ERBB2*(HER2), *BRCA1/2, RB1*, *MAP3K.* The hormone receptor expression status including estrogen receptor (ER), progesterone receptor (PR) and human epithelial growth factor receptor 2 (HER2) were confirmed. and the new breast cancer cell lines and organoids were grouped and divided according to ER, PR, and HER2 status. Next, we measured sensitivity to various drugs that are widely used in breast cancer treatment, research and clinical trials. The results showed corresponding outcomes with the presence or absence of target mutations known to affect the reactivity or expression of drug targets previously identified. Even if they were from the same patient origin, each cell line showed some different results, and two pairs of cell line–organoid showed a noticeable difference in reactivity despite having similar mutational profiles. The results of gene screening based on the database proved that pleural effusion could be used for constructing an experimental model in breast cancer. In addition, the differences between patients and the similarity and diversity between cell lines from same patient can be the basis for clinical treatment planning. These results suggest that the accumulation of various

characters and types of experimental models will greatly contribute to improving the accuracy and suitability of the database.

Keywords : Pleural effusion, Ascites, patient-derived xenograft (PDX), breast cancer cell line, breast cancer organoid, characterization analysis, Drug sensitivity, Fusion gene

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# 1. Introduction

For both sexes combined, Breast cancer is a type of cancer with a high incidence rate, which is almost the same as that of lung cancer with the most frequently diagnosed cancer (11.6%). Among females, breast cancer is the most common cancer and leading cause of cancer death [1]. Human breast cancers are heterogeneous, and breast cancer patient outcomes and responses to therapy are extremely varied.

Subtype of breast cancer was based on the following basic classification criteria : histological type, tumor grade, lymph node status, the presence or/and absence of hormonal (estrogen, progesterone) receptors (ER, PR) and human epidermal growth factor receptor2 (HER2), expression of a marker of proliferation. Advances in immunohistochemical techniques and molecular biological methods have enabled in-depth studies of various forms identification of breast cancer. The genetic features of breast cancer are also highly diverse. Genes that are most frequently mutated in breast cancer include *TP53, PIK3CA, MYC, CCND1, PTEN, ERBB2, GATA3, RB1* and *MAP3K1* [2].

HER2 is encoded by the *ERBB2* gene. In particular, HER2 is

amplified in 20% of breast cancers. It is known that an intrinsic activating mutation leads to a valine-to-leucine substitution at codon 777 within the HER2 kinase domain (HER2 V777L), which induces trastuzumab resistance [3]. The tumor suppressor genes *BRCA1* and *BRCA2* are mutated in hereditary breast-ovarian cancer syndrome. Patients with this variant are likely to have a higher probability of lifetime risk, but no conclusive conclusion has yet been reached [4]. High-throughput molecular profiling studies have confirmed that breast cancer has spatial and temporal intra-tumor heterogeneity beyond our expectations [5]. Clinical approaches and management of the disease comprises are tailored to these various characteristics, including morphological assessments (size, grade), three subtypes based on immunohistochemical staining of breast cancer tissues and genomic features [6, 7]. Naturally, the coexistence of multiple subclone with different genetic variations and relative difference of drug sensitivities might not be effective against anticancer drugs that target predominant aberrations [5]. Therefore, the accumulation of data on as many and various characteristics as possible is necessary, and the more diverse the breast cancer research platform is, the more effective the

study is expected to be applied to clinical applications.

Various types of cancer research models have been developed and used. Immortalized cell lines are used commonly to study breast cancer, are easy to manipulate, and are ideal for examining the molecular mechanism of tumor cell biology [8]. Patient-derived xenograft (PDX) models have an advantage in that it preserves heterogeneity and microenvironment included. Organoid culture not only retains heterogeneity, but also enables high-throughput screening [9]. In this way, each platform has its own advantages and limitations, and experimenters use a suitable model for their research purpose. Patient-derived cell lines, PDX-derived cell lines and organoids were established and characterization was performed for various type of breast cancer research and proceeding to present the direction of personalized treatment. Several types of experimental models with various characteristics are expected to be greatly helpful in future breast cancer research.

## 2. Materials and Methods

### *2.1. Establishment and maintenance of breast cancer cell lines*

Twenty Cell lines were established from malignant pleural effusion and one cell line was established from malignant ascites sample. Three cell lines were gained from patient derived xenograft (PDX) tumor tissue sample. All samples were from patients in Seoul National University Hospital. Suspended cells were gathered by spinning down. Gathered cell pellet were seeded into T-25㎠ or T-75㎠ flasks. Cancer cells were initially cultured in Opti-MEM I (Thermo Fisher Scientific, MA, USA) with 5% fetal bovine serum (FBS). Confined-area trypsinization or scraping method was used to attain achieve a pure tumor cells when stromal cells like mesothelial cells or fibroblasts grew in the initial culture. After primary culture, established cell lines were sustained in RPMI 1640 (Thermo Fisher Scientific, MA, USA) with 10% fetal bovine serum and 1% (v/v) penicillin and streptomycin (10,000U/ml). Incubated flasks in humidified incubators at 37℃ in an atmosphere of 5% CO2 and 95% air.

### *2.2. Establishment and maintenance of breast cancer organoids*

#### *2.2.1 Tumor isolation and culture*

PDX tumor tissue was cut finely with scissors for about 5 min. The enzyme solution consisting of CollagenaseⅡ(1.5mg/ml), Hyaluronidase (20ug/ml) and Ly27632 (10㎛M) was added to the chopped tissue and incubated for at least 4hr at 37℃ while spinning. FCS was added for neutralization and the mixture was filtered through 100㎛M cell strainer (SPL, #93100) to remove large chunks and impurities that were not cut well. Spun down pellet at 1,000 rpm for 3 min. In the case of a pleural effusion sample, centrifugation was performed immediately without any enzyme digestion. The supernatant was suctioned and then plated in an appropriate amount of BME gel (Gibco, A14132-02). When the BME gel hardened, the HBEC medium (Basal culture medium with 50% Wnt conditioned medium, 20% R-Spondin conditioned medium, 10% Noggin conditioned medium, 1x B27 (Gibco, 17504-044), 1.25mM n-Acetyl cysteine (Sigma, A7250), 5mM Nicotinamide, 5nM Neuregulin(Peprotech, 100-03), 500nM A83-01, 500nM SB202190(Sigma, S7067), 5mM Ly27632, 5ng/ml human EGF (Peprotech,AF-100-15), 20ng/ml human FGF-10 (Peprotech, 100-26), 5ng/ml human FGF-

7(Peprotech, 100-19) and 50㎍g/ml Primocin (Thermo, ant-pm-1))was added and incubated at 37℃.

### *2.2.2 Organoid Culture*

When passaging, first remove the media and pipet off the BME gel. Collect the loose gel in a tube. Mixture of the organoids and gel was centrifuged at 1,000 rpm for 3min and the medium suctioned. About 5 ml Triple Express (Invitrogen) was added and the mixture was incubated at 37℃ for approximately 10 min. After 5 minutes, the size of organoids was checked and the gel was removed every minute. Care should be taken not to treat organoids in the Triple Express for too long. To neutralize, FCS and medium were added and loose cells were spun down at 1,500 rpm for 3 min. After mixing the pellet with the appropriate amount of gel, mixture was plated in droplets of 50-100㎕L each. Leave for 10 min to allow the BME gel to solidify and then fill the HBEC medium. The media is usually changed every week.

### *2.3. Growth properties and morphology in vitro*

To acquire each tumor population' s doubling time, 5x$10^4$ to 2x$10^5$ viable cells from each cell line were seeded into 12-24

identical well of 96 well plate and cell viability was calculated daily for 5-12 days. Since the first cell seeding, in every 24 hours, 10ul EZ-Cytox solution (Daeil Lab, Seoul, Korea) was added to well of each seeded lung cancer cells in triplicate. After 2 hour-incubation at 37℃, Optical density of EZ-Cytox-treated cells was calculated by Multiskan™ GO Microplate Spectrophotometer (Thermo Fisher Scientific, MA, USA). Growth rate values were measured by GraphPad Prism 5 (GraphPad Software, CA, USA). Growth rates are values to multiply 10 by days that cell population were duplicated. To observe cell line's morphology, each cell line was cultured in 75$cm^2$ culture flasks and then pictured daily by phase-contrast microscopy.

### *2.4. Genomic DNA extraction and DNA fingerprinting analysis*

Genomic DNA (gDNA) extraction was performed by using QIAamp DNA Mini kit (Qiagen). gDNA extracted from each breast cancer cell line and organoid 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).

### *2.5. Genomic DNA Mycoplasma detection test*

gDNA extracted from each breast cancer cell line and organoid was amplified using an TaKaRa Polymerase Chain Reaction (PCR) *Mycoplasma* Detection Set (TAKARA BIO INC., Shiga, JAPAN). a primer set was designed to detect the presence of *Mycoplasma* which might contaminate biological materials such as cultured cells. This primer set allows sensitive and specific detection of several different species of *Mycoplasma* (*M. fermentans, M. hyorhinis, M. arginini, M. orale, M. salivarium, M. hominis, M. pulmonis, M. arthritidis, M. neurolyticum, M. hyopneumoniae, M. capricolum)* and one species of *Ureaplasma* (*U. urealyticum*). Amplify the spacer regions in the rRNA operon (for example, the region between the 16S and 23S genes) using two primers (F1

and R1), which were designed based on the DNA encoding the 16S and 23S rRNAs. Perform Nested PCR using two primers, F2, based on the conserved region, and R2, based on the 23S gene.

### *2.6. Drug sensitivity test*

#### *2.6.1 2D cell lines seeding/treatment procedure*

2x$10^5$ to 4x$10^5$viable cells from each cell line were seeded into well of 96 well white plate (SPL, #30196) in triplicate to measure sensitivity of several drugs. A day after, all cell lines and organoids were respectively treated for proper concentration of Drugs. If the cell line was adherent cell, adherent state was confirmed. After 72 hours-incubation at 37 ° C, 10ul Cell-titer glo solution was added to well of each seeded breast cancer cells and organoids. After 20 minute-incubation at 37° C, optical density of Cell-titer glo treated cells was calculated by Multiskan™ Ascent Microplate Luminometer (Thermo Fisher Scientific). These steps were repeated in duplicate.

### *2.6.1 3D organoids seeding/treatment procedure*

Cleaved organoids were placed around the rim of the well of 96 well white plates (SPL, #30196) in a 1:1 mixture of HBEC medium and RGF basement membrane matrix (Gibco, A14132-02). Plates are incubated at 37℃ with 5% CO2 for 15 minutes to solidify the gel. After solidation, 20 ul of pre-warmed HBEC medium to each well. 96 hours later, 20 ul of serial diluted drug solution is added to each well. The mixture of HBEC medium and drug-solvent solution is added for the control well. After 20 minute-incubation at 37° C, optical density of Cell-titer glo treated cells was calculated by Multiskan™ Ascent Microplate Luminometer (Thermo Fisher Scientific). These steps were repeated in duplicate.

## *2.7. Protein extraction and Western blotting*

Cultivated cells that had full confluency were harvested with cell scrapper. Cell pellet was treated by EzRIPA buffer (ATTO Co., Tokyo, Japan) after washed by cool PBS. Whole protein was extracted by this step. Protein concentration of each cell line was determined by Pierce™ BCA Protein Assay Kit (Thermo

Scientific). Proteins that fixed into equal concentration were loaded on a 4-15% Polyacrylamide gel (Bio-Rad) at 50 volts for 3 hours and then proteins on loaded gel were transferred to a Trans-Blot®Turbo™ Transfer Pack PVDF membrane (Bio-rad) by Trans-Blot®Turbo™ Transfer system (Bio-Rad). Proteins of transferred membrane was blocked by incubating in 1.5% to 2.0% skim milk and 0.05% Tween 20-TBS buffer including 1mM $MgCl_2$ for an hour at room temperature. Primary antibodies were used against E-cadherin, N-cadherin, Snail, ER-$\alpha$, PR A/B, EGFR, phospho-EGFR, HER2, pan Akt, phospho-Akt, mTOR, phospho-mTOR, IGF-1R$\beta$, MEK 1/2, phospho-MEK1/2, PTEN and B-actin. Those antibodies were Abcam products (Abcam, Cambridge, UK) and CST products (Cell Signaling Technology, MA, USA) except for exon 19 E746-A750 deleted EGFR that was Cell signaling product (Cell signaling, MA, USA). Mouse or rabbit IgG $2^{nd}$ antibody (Jackson Immunoresearch, PA, USA) (1:5000) conjugated with peroxidase that matched with used $1^{st}$ antibody was added to membrane. After chemiluminescent working solution, SuperSignal™ West Pico PLUS Chemiluminescent Substrate (Thermo Scientific) was treated to the membrane, the membrane was exposed to Fuji RX film

(Fujifilm, Tokyo, Japan) for 0.5 – 10 minutes.

### *2.8. Confocal analysis of immunofluorescence staining*

Cells were seeded on chambered coverglass (Thermo Fisher Scientific, MA, USA) with a desirable cell confluency. The chambered coverglass was designed to be hydrophilic and no ECM component was treated before seeding. 72 hours after cell seeding, cells were washed with cold DPBS for 15 minutes three times. Then, cells were fixed and permeabilized with BD Cytofix/Cytoperm™ (BD science, CA, USA). After cells were washed with washing solution (BD science), DPBS containing 2% FBS (GE Healthcare Life Sciences, Buckinghamshire, UK) was applied for an hour for blocking. After cells were washed with cold DPBS, HER2 (Santa Cruz Biotechnology, CA, USA) and E-cadherin antibody (Abcam, Cambridge, United Kingdom) diluted in 0.05% of PBS.T was applied for an 1.5 hours in room temperature. Thereafter, cells were washed with 0.05% of PBS.T, and Alexa 488 and Alexa 594 secondary antibodies (Thermo Fisher Scientific, MA, USA) diluted in 0.05% of PBS.T were applied for an hour in room temperature. 1x DAPI (Sigma-Aldrich, MO, USA) were diluted in distilled water and applied

for 30 minutes in room temperature. The cells were washed with DPBS three times, and pictured under confocal microscope. LSM800 Confocal Laser Scanning Microscope and ZEN software (Carl Zeiss, Oberkochen, Germany) was used to examine cells. Diverse magnifications were used for different growth patterns and sizes of cells. The intensity of each channel was fixed for the comparison of target protein expression between samples. Digital resolution, scan speed and the number of pictures averaged were set to 1024 x 1024, 40 seconds per one channel, and 8 pictures respectively. The pictures were focused on the very bottom of the fixed cells for investigating protruding region of cell colonies and the location of HER2 and E-cadherin.

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### *2.9. FFPE block production and H&E staining*

The gel dome containing the organoid was scraped out the flask floor with a pipette tip that has been cut off. PBS was added and transferred to the 1.5mL tube. After a short centrifugation, the process of suctioning the supernatant is repeated so that the gel disappears and only the organoids remain as much as possible. Collected pellets were embedded into low melting (2% diluted in

PBS) agarose gel (iNtRON Biotechnology, Seongnam, Korea). Solidified agarose gel was fix 10% paraformaldehyde (PFA) for 30 minutes at room temperature. The agarose blocks were processed before being embedded into paraffin. Sections (4 $\mu$m) of organoids were subjected to routine haematoxylin and eosin (H&E) staining.

### *2.10. Whole Exome Sequencing*

#### *2.10.1. Analysis of 3 organoids*

SureSelect sequencing libraries were prepared according to manufacturer’s instructions (Agilent sureselect all Exon kit 50 Mb) using the Bravo automated liquid handler. Three micrograms of genomic DNA were fragmented to a median size of 150 bp using the Covaris-S2 instrument (Covaris, Woburn, MA). The adapter ligated DNA was amplified by PCR, and the PCR product quality was assessed by capillary electrophoresis (Bioanalyzer, Agilent). The hybridization buffer and DNA blocker mix were incubated for 5 minute at 95° C and then for10 minutes at 65° C in a thermal cycler. The hybridization mixture was added to the bead suspension and incubated for 30 minutes at RT while mixing. The beads were washed, and DNA was

eluted with 50 ml SureSelect elution buffer (Agilent). The flow cell loaded on HISEQ 2500 sequencing system (Illumina).

### *2.10.2. Analysis of 24 cell lines*

DNA should be as intact as possible, with an OD260/280 ratio of 1.8–2. we checked quality of DNA by 1% agarose gel electrophoresis and PicoGreen® dsDNA Assay(Invitrogen). SureSelect sequencing libraries were prepared according to the manufacturer’s instructions (Agilent SureSelectXT Human All Exon V4) using The Bravo automated liquid handler. 200ng of genomic DNA in 50 ul EB buffer was fragmented to a median size of 150 bp using the Covaris-S2 instrument (Covaris) with the following settings: duty cycle 10%, intensity 5, cycles per burst 200, and mode frequency sweeping for 360 s at 4℃. The fragmentation efficiency was evaluated by capillary electrophoresis on DNA1000 chips (Bioanalyzer, Agilent). Sequencing adapters were ligated on the DNA fragments following the manufacturer’s protocol (Agilent). The adapter ligated DNA was amplified by PCR. The quality of the PCR products was assessed by capillary electrophoresis (Bioanalyzer, Agilent). SureSelect hyb #1, #2, #3, and #4 reagents (Agilent) were mixed to prepare the hybridization buffer. The amplified

DNA fragments were concentrated 750 ng in 3.4 ul. SureSelect block #1, #2, and #3 reagents (Agilent) were added to the 750 ng of DNA. The hybridization buffer and the DNA blocker mix were incubated for 5 min at 95℃ and then for 10 min at 65℃ in a thermal cycler. RNase block (Agilent) was added to the SureSelect oligo capture library (Agilent). The capture library was incubated for 2 min at 65℃. First the hybridization buffer, and then the DNA blocker mix were added to the capture library and the mixture was incubated for 24 hours at 65℃ in a thermal cycler. Fifty ul of streptavidin coated the Dynal MyOne Streptavidin T1 (Invitrogen) were washed three times with 200 ml SureSelect binding buffer (Agilent) and resuspended in 200 ml of the binding buffer. The hybridization mixture was added to the bead suspension and incubated for 30 min at RT with mixing. The beads were washed with 500 ml SureSelect wash buffer #1 (Agilent) for 15 min at RT, and three times with 500 ml SureSelect wash buffer #2 (Agilent) for 10 min at 65℃. DNA was eluted with 30 ul nuclease-free water. The captured library was amplified to add index tags using Herculase II Fusion DNA Polymerase (Finnzymes). The quality of the amplified libraries was verified by capillary electrophoresis (Bioanalyzer, Agilent).

After QPCR using SYBR Green PCR Master Mix (Applied Biosystems), we combined libraries that index tagged in equimolar amounts in the pool. Sequencing is performed using an Illumina Novaseq 6000 system following provided protocols for 2x100 sequencing.

### *2.10. RNA Sequencing – Fusion gene analysis*

Both Fusionmap (Version:8.0.2.32) and Chimerascan (version0.4.5) were used to analyze fusion genes. The Fusionmap data were needed for the data filtering step based on the recommended filtering options in the sample: Seed Count >= 3, splice Pattern Class = Canonical Pattern [Major] or Canonical Pattern [Minor], Filter = Empty. The Chimerascan data were already filtered according to the recommended filtering option. All the RNA sequencing data were visualized using the integrative genomics viewer (IGV) version 2.4 and the fusion gene candidates were confirmed, respectively. The candidates of fusion gene were sorted reliably based on the intersection of gene sets obtained using Defuse, Fusionmap and Chimerascan methods.

Table 1. Chemotherapeutic agents and targeted agents for breast cancer used in this study

| No. | Drug | Type | Target / Mechanism of action |
|---|---|---|---|
| 1 | **Tamoxifen** | Molecular Targeted Drug | Selective Estrogen Receptor Modulator(SERM) |
| 2 | **Anastrozole** | Molecular Targeted Drug | Aromatase Inhibitor |
| 3 | **Fulvestrant** | Molecular Targeted Drug | Selective Estrogen Receptor Degrader(SERD) |
| 4 | **Trastuzumab** | Molecular Targeted Drug | Anti-HER2 antibody |
| 5 | **Lapatinib** | Molecular Targeted Drug | Anti-HER2, EGFR tyrosine kinase inhibitor |
| 6 | **DS-8201a** | Molecular Targeted Drug | Anti-HER2 (trastuzumab deruxtecan)drug conjugate |
| 7 | **Olaparib** | Molecular Targeted Drug | DNA damage repair inhibitor (DDRi)<br>Poly ADP-ribose polymerase : PARP Inhibitor |
| 8 | **Talazoparib** | Molecular Targeted Drug | DDRi (PARP Inhibitor) |
| 9 | **BAY1895344** | Molecular Targeted Drug | DDRi (ATR Inhibitor)) |
| 10 | **Palbociclib** | Molecular Targeted Drug | CDK4/6 Inhibitor |
| 11 | **Abemaciclib** | Molecular Targeted Drug | CDK4/6 Inhibitor |
| 12 | **Afatinib** | Molecular Targeted Drug | EGFR tyrosine kinases Inhibitor |
| 13 | **Erlotinib** | Molecular Targeted Drug | EGFR tyrosine kinases Inhibitor |
| 14 | **Gefitinib** | Molecular Targeted Drug | EGFR tyrosine kinases Inhibitor |
| 15 | **Trametinib** | Molecular Targeted Drug | MEK 1/2 Inhibitor |
| 16 | **Everolimus** | Molecular Targeted Drug | mTOR Inhibitor |
| 17 | **Akt1/2 kinase inhibitor** | Molecular Targeted Drug | Akt1/2 kinase Inhibitor |
| 18 | **5-FU** | Anti-Metabolites | Pyrimidine Analogue |
| 19 | **Pemetrexed** | Anti-Metabolites | Multi-targeted anti-Folate |
| 20 | **Gemcitabine** | Anti-Metabolites | Pyrimidine Analogue |
| 21 | **Cisplatin** | Alkylating agents | interferes with DNA replication |
| 22 | **Docetaxel** | Anti-Microtubule Agent | preventing microtubule disassembly |
| 23 | **Paclitaxel** | Anti-Microtubule Agent | preventing microtubule disassembly |
| 24 | **Irinotecan** | Topoisomerase Inhibitor | Topo I Inhibitor |
| 25 | **Doxorubicin** | Topoisomerase Inhibitor | Topo Ⅱ Inhibitor, Anthracycline |
| 26 | **Epirubicin** | Topoisomerase Inhibitor | Topo Ⅱ Inhibitor, Anthracycline |

* DDR : DNA Damage Response

# 3. Results

## 3.1. Sample origin and identity verification

Within the 24 newly established cell lines, 20 were derived from pleural effusion, one was established from ascites, and 3 were from PDX tissues. Three organoids were established from three identical PDX tissues each derived from a cell line. For convenience, patient 1 (a set of SNU-3223, SNU-3224, SNU-3230), Patient 2 (a set of SNU-3380, SNU-3393), Patient 3 (a set of SNU-3698A, SNU-3698B, SNU-3698C, SNU-3705, SNU-3716, SNU-3730) and patient 4 (a set of SNU-5188, SNU-5226B) were named. In addition, the cell line-organoid pairs were referred to as set 1 (a pair of SNU-4842, SNU-4842-TO), set 2 (a pair of SNU-4856, SNU-4856-TO) and set 3 (a pair of SNU-5126, SNU-5126-TO). DNA fingerprinting revealed a heterogeneous distribution of 15 tetranucleotide repeat loci and an amelogenin gender determining marker in each cell line and organoids. This confirmed 15 unique and unrelated cell lines, and that it was a different cell line between same patients. It also proved that the 3 cell lines and organoids are from the same PDX tissue.

Table 2. Origin and *in vivo* characteristics of 21 human breast cancer cell lines and 3 paired cell line–organoid sets derived from same patient–derived xenograft (PDX) tumor

| No. | SNU Name | Culture initiation date | Origin | biopsy of PDX | Sex/Age | Tumor type | Stage | Histology subtype |
|---|---|---|---|---|---|---|---|---|
| 1 | SNU-2480 | 2010-05-18 | Pleural effusion | - | F/37 | Primary | IV | IDC |
| 2 | SNU-2532A | 2010-10-21 | Pleural effusion | - | F/32 | Metastatic | IV | IDC |
| 3 | SNU-2924 | 2014-07-11 | Pleural effusion | - | F/46 | Primary | IV | IDC |
| 4 | SNU-3129 | 2015-05-07 | Pleural effusion | - | F/53 | Metastatic | IV | IDC |
| 5 | SNU-3171 | 2015-06-19 | Ascites | - | F/56 | Metastatic | IV | Other |
| 6 | SNU-3196 | 2015-07-20 | Pleural effusion | - | F/70 | Metastatic | IV | Other |
| 7 | SNU-3223 | 2015-08-11 | | | | | | |
| 8 | SNU-3224 | 2015-08-13 | Pleural effusion | - | F/41 | Metastatic | IV | IDC |
| 9 | SNU-3230 | 2015-08-20 | | | | | | |
| 10 | SNU-3351 | 2015-11-05 | Pleural effusion | - | F/65 | Metastatic | IV | ILC |
| 11 | SNU-3380 | 2015-11-25 | Pleural effusion | - | F/63 | Metastatic | IV | IDC |
| 12 | SNU-3393 | 2015-12-04 | | | | | | |
| 13 | SNU-3698A | 2016-05-23 | | | | | | |
| 14 | SNU-3698B | 2016-05-23 | | | | | | |
| 15 | SNU-3698C | 2016-05-23 | Pleural effusion | - | F/36 | Metastatic | IV | IDC |
| 16 | SNU-3705 | 2016-05-26 | | | | | | |
| 17 | SNU-3716 | 2016-06-01 | | | | | | |
| 18 | SNU-3730 | 2016-06-23 | | | | | | |
| 19 | SNU-4842 | 2018-03-23 | PDX tissue | Breast | F/44 | Primary | IV | IDC |
| 20 | SNU-4842-TO | | | | | | | |
| 21 | SNU-4856 | 2018-03-29 | PDX tissue | Liver | F/44 | Metastatic | IV | IDC |
| 22 | SNU-4856-TO | | | | | | | |
| 23 | SNU-5126 | 2018-07-20 | PDX tissue | Breast | F/53 | Metastatic | IV | IDC |
| 24 | SNU-5126-TO | | | | | | | |
| 25 | SNU-5188 | 2018-08-10 | Pleural effusion | - | F/62 | Metastatic | IV | IDC |
| 26 | SNU-5226B | 2018-08-28 | | | | | | |
| 27 | SNU-5884B | 2019-05-13 | Pleural effusion | - | F/48 | Metastatic | IV | IDC |

* - : not recorded ** IDC : Invasive Ductal Carcinoma *** ILC : Invasive Lobular Carcinoma

*Continued*

| No. | SNU Name | Subtype | ER | PR | HER2 | Ki-67 |
|---|---|---|---|---|---|---|
| 1 | SNU-2480 | HR-HER2- | Negative | Negative | Negative | 60 |
| 2 | SNU-2532A | HR-HER2- | Negative | Negative | 1+ | - |
| 3 | SNU-2924 | HR-HER2- | Negative | Negative | Negative | 50 |
| 4 | SNU-3129 | HR-HER2- | Negative | Negative | Negative | 5 |
| 5 | SNU-3171 | HR+HER2- | 60 | Negative | 2+(FISH-) | - |
| 6 | SNU-3196 | HR+HER2- | Focal weak | - | Negative | - |
| 7 | SNU-3223 | | | | | |
| 8 | SNU-3224 | HR-HER2+ | Negative | Negative | 3+ | 60 |
| 9 | SNU-3230 | | | | | |
| 10 | SNU-3351 | HR+HER2- | 90 | 50 | 2+ | - |
| 11 | SNU-3380 | HR-HER2+ | Negative | Negative | 3+ | - |
| 12 | SNU-3393 | | | | | |
| 13 | SNU-3698A | | | | | |
| 14 | SNU-3698B | | | | | |
| 15 | SNU-3698C | HR-HER2+ | Negative | Negative | 3+ | - |
| 16 | SNU-3705 | | | | | |
| 17 | SNU-3716 | | | | | |
| 18 | SNU-3730 | | | | | |
| 19 | SNU-4842 | HR-HER2+ | Negative | Negative | Negative | 30 |
| 20 | SNU-4842-TO | | | | | |
| 21 | SNU-4856 | HR+HER2- | Negative | 70 | Negative | 10 |
| 22 | SNU-4856-TO | | | | | |
| 23 | SNU-5126 | HR+HER2- | 95 | Negative | Negative | - |
| 24 | SNU-5126-TO | | | | | |
| 25 | SNU-5188 | HR+HER2- | 80 | 5 | Negative | 7 |
| 26 | SNU-5226B | | | | | |
| 27 | SNU-5884B | HR-HER2- | <1% | Negative | Negative | - |

Table 3. DNA fingerprinting analysis using 15 STR loci and amelogenin for newly established 21 breast cancer cell lines and 3 matched patient-derived xenograft (PDX) cell line-organoid pairs

| No. | Cell-Name | D8S1179 | D21S11 | D7S820 | CSF1PO | D3S1358 | TH01 | D13S317 | D16S539 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | SNU-2480 | 10, 13 | 30, 31 | 9, 11 | 11 | 15 | 7 | 8 | 11 |
| 2 | SNU-2532A | 13 | 29 | 10, 12 | 9, 11 | 15, 16 | 6, 9 | 8 | 11 |
| 3 | SNU-2924 | 12 | 30, 33.2 | 10 | 12 | 15 | 7 | 8 | 9, 11 |
| 4 | SNU-3129 | 8, 12 | 31.2 | 8, 10 | 10 | 15 | 7 | 11 | 9, 10 |
| 5 | SNU-3171 | 14, 15 | 30 | 11 | 11 | 15 | 9 | 8 | 11, 12 |
| 6 | SNU-3196 | 11, 12 | 30, 31 | 11, 12 | 12 | 15, 18 | 10 | 9 | 9 |
| 7 | SNU-3223 | 13,16 | 30 | 10, 12 | 12 | 15, 16 | 9 | 8, 10 | 10, 11 |
| 8 | SNU-3224 | 13, 16 | 30 | 10, 12 | 12 | 15, 16 | 9 | 8, 10 | 10, 11 |
| 9 | SNU-3230 | 13, 16 | 30 | 10, 12 | 12 | 15, 16 | 9 | 8, 10 | 10, 11 |
| 10 | SNU-3351 | 13 | 31.2, 32.2 | 11, 12 | 11, 12 | 14 | 6, 8 | 8, 11 | 13 |
| 11 | SNU-3380 | 10, 13 | 30, 31 | 11 | 12 | 15 | 9.3 | 11 | 11 |
| 12 | SNU-3393 | 10, 13 | 30, 31 | 11 | 12 | 15 | 9.3 | 11 | 11 |
| 13 | SNU-3698A | 11, 16 | 30 | 11 | 12 | 15 | 9 | 8 | 9 |
| 14 | SNU-3698B | 11, 16 | 30 | 11 | 12 | 15 | 9 | 8 | 9 |
| 15 | SNU-3698C | 11, 16 | 30 | 11 | 12 | 15 | 9 | 8 | 9 |
| 16 | SNU-3705 | 11, 16 | 30 | 11 | 12 | 15 | 9 | 8 | 9 |
| 17 | SNU-3716 | 11, 16 | 30 | 11 | 12 | 15 | 9 | 8 | 9 |
| 18 | SNU-3730 | 11, 16 | 30 | 11 | 12 | 15 | 9 | 8 | 9 |
| 19 | SNU-4842 | 14, 15 | 31, 32.2 | 10, 12 | 12 | 17 | 9 | 8 | 10, 11 |
| 20 | SNU-4842-TO | 14, 15 | 31, 32.2 | 10, 12 | 12 | 17 | 9 | 8 | 10, 11 |
| 21 | SNU-4856 | 10, 12 | 29, 30 | 10, 12 | 11, 12 | 15, 16 | 8, 9 | 8, 10 | 12 |
| 22 | SNU-4856-TO | 10, 12 | 29, 30 | 10, 12 | 11, 12 | 15, 16 | 8, 9 | 8, 10 | 12 |
| 23 | SNU-5126 | 10, 12 | 29, 32.2 | 11, 12 | 10 | 15, 16 | 8, 9 | 8, 10 | 12 |
| 24 | SNU-5126-TO | 10, 12 | 29, 32.2 | 11, 12 | 10 | 15, 16 | 8, 9 | 8, 10 | 12 |
| 25 | SNU-5188 | 12, 16 | 30 | 11, 12 | 9, 10 | 16, 17 | 9, 9.3 | 12 | 9 |
| 26 | SNU-5226B | 12, 16 | 30 | 11, 12 | 9, 10 | 16, 17 | 9, 9.3 | 12 | 9 |
| 27 | SNU-5884B | 13, 14, 16 | 31.2 | 11 | 10, 11 | 15, 17 | 9 | 10 | 9, 10 |

*Continued*

| No. | Cell-Name | D2S1338 | D19S433 | VWA | TPOX | D18S51 | Amelogenin | D5S818 | FGA |
|---|---|---|---|---|---|---|---|---|---|
| 1 | SNU-2480 | 17 | 13, 14 | 16, 17 | 11 | 12, 16 | X, X | 12 | 26 |
| 2 | SNU-2532A | 20 | 14.2 | 20 | 10, 11 | 13 | X, X | 12 | 26 |
| 3 | SNU-2924 | 17 | 13 | 16 | 11, 12 | 18 | X, X | 9 | 21 |
| 4 | SNU-3129 | 19, 25 | 12, 14.2 | 18 | 8, 11 | 11, 13 | X, X | 13 | 22 |
| 5 | SNU-3171 | 19 | 13, 13.2 | 14 | 8, 9 | 10, 21.2 | X, X | 10 | 23 |
| 6 | SNU-3196 | 23, 24 | 14 | 14, 18 | 11 | 19 | X, X | 10 | 24 |
| 7 | SNU-3223 | 17, 20 | 14, 14.2 | 14 | 9 | 13, 16 | X, X | 11 | 20, 21 |
| 8 | SNU-3224 | 17, 20 | 14, 14.2 | 14 | 9 | 13, 16 | X, X | 11 | 20, 21 |
| 9 | SNU-3230 | 17, 20 | 14, 14.2 | 14 | 9 | 13, 16 | X, X | 11 | 20, 21 |
| 10 | SNU-3351 | 19 | 14, 15.2 | 14, 19 | 8, 11 | 14 | X, X | 11, 12 | 22, 22 |
| 11 | SNU-3380 | 23, 25 | 13, 15.2 | 14, 19 | 8, 11 | 16 | X, X | 11, 12 | 22, 23 |
| 12 | SNU-3393 | 23, 25 | 13, 15.2 | 14, 19 | 8, 11 | 16 | X, X | 11, 12 | 22, 23 |
| 13 | SNU-3698A | 23 | 13, 14.2 | 16, 17 | 8, 11 | 15 | X, X | 11, 13 | 22 |
| 14 | SNU-3698B | 23 | 13, 14.2 | 16, 17 | 8, 11 | 15 | X, X | 11, 13 | 22 |
| 15 | SNU-3698C | 23 | 13, 14.2 | 16, 17 | 8, 11 | 15 | X, X | 11, 13 | 22 |
| 16 | SNU-3705 | 23 | 13, 14.2 | 16, 17 | 8, 11 | 15 | X, X | 11, 13 | 22 |
| 17 | SNU-3716 | 23 | 13, 14.2 | 16, 17 | 8, 11 | 15 | X, X | 11, 13 | 22 |
| 18 | SNU-3730 | 23 | 13, 14.2 | 16, 17 | 8, 11 | 15 | X, X | 11, 13 | 22 |
| 19 | SNU-4842 | 23 | 14 | 16, 17 | 9, 11 | 14, 16 | X, X | 10 | 22, 23 |
| 20 | SNU-4842-TO | 23 | 14 | 16, 17 | 9, 11 | 14, 16 | X, X | 10 | 22, 23 |
| 21 | SNU-4856 | 19 | 12, 13 | 14, 18 | 8, 11 | 13, 17 | X, X | 10, 13 | 19, 25 |
| 22 | SNU-4856-TO | 19 | 12, 13 | 14, 18 | 8, 11 | 13, 17 | X, X | 10, 13 | 19, 25 |
| 23 | SNU-5126 | 17 | 12, 15.2 | 16 | 9, 11 | 13 | X, X | 12 | 22, 24 |
| 24 | SNU-5126-TO | 17 | 12, 15.2 | 16 | 9, 11 | 13 | X, X | 12 | 22, 24 |
| 25 | SNU-5188 | 17 | 13, 15.2 | 14, 19 | 8, 10 | 13, 20 | X, X | 10, 12 | 24 |
| 26 | SNU-5226B | 17 | 13, 15.2 | 14, 19 | 8, 10 | 13, 20 | X, X | 10, 12 | 24 |
| 27 | SNU-5884B | 17, 24 | 15.2 | 14, 18 | 11 | 12, 17 | X, X | 11, 12 | 19, 25 |

### 3.1. Culture characteristics

Majority of the established cell lines were in adherent form or coexisted with floating cells. Although derived from pleural effusion and ascites, only SNU-2532A, SNU-3698C cell lines maintained floating morphology. Of the three cell lines derived from PDX tissues, SNU-4842 was only floating pattern, the cells were tightly aggregated into a single lump like a matched organoid. The morphology of the whole cell line was classified into four types: polygonal, oval, fibroblast-like, round. Even in the same patient-derived cell lines, the more floating and clumped form, the slower it grew. As described above, growth patterns and characteristics of cell lines of the same origin were slightly different. SNU-3393, SNU-3698B and SNU-5188 were particularly slow in growth rate than other cell lines of the same patient. Overall, it was SNU-3196 with the longest doubling time and SNU-3129 with the shortest. It has been observed that all three organoids grow in various forms: dense and round, hollow, polygonal. Organoid sizes were mostly around 100-200㎛. The results of FFPE slide H&E staining also showed well-organized round-shaped organoids. In the initial passage, the size was larger, but over the passage, it did not

appear to be greatly larger than a certain level. When taking three organoid confocal images, it was noticeable that SNU-4842-TO only HER2 green was overexpressed under the same conditions and brightness correction. All cell lines and organoids were confirmed to be free of contamination from either bacteria or mycoplasma and without cross-contamination.

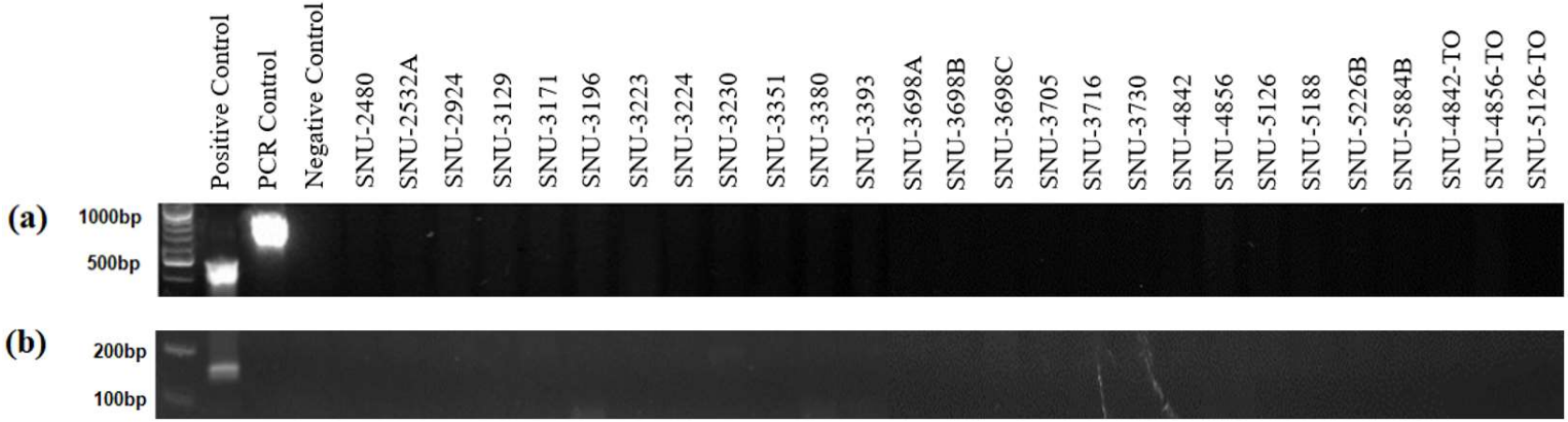
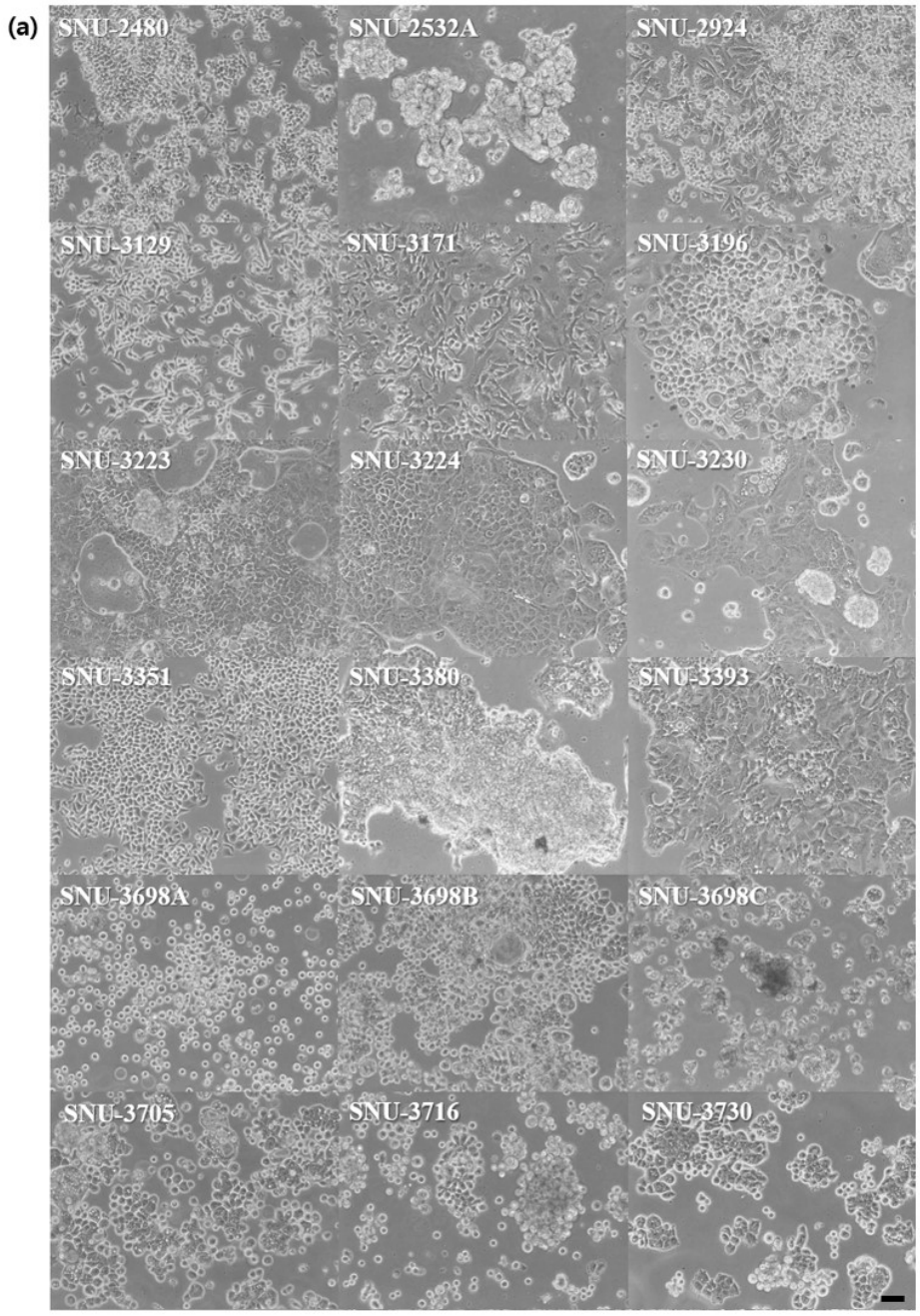



Figure 1. Mycoplasma detection test

Mycoplasma was not detected in all cell lines and organoids. (a) 1st PCR result – Sample control : 810bp, Mycoplasma detection : 700–300bp (b) 2nd PCR result – Sample control : 590bp, Mycoplasma detection : 250–150bp


(a)
SNU-2480
SNU-2532A
SNU-2924
SNU-3129
SNU-3171
SNU-3196
SNU-3223
SNU-3224
SNU-3230
SNU-3351
SNU-3380
SNU-3393
SNU-3698A
SNU-3698B
SNU-3698C
SNU-3705
SNU-3716
SNU-3730

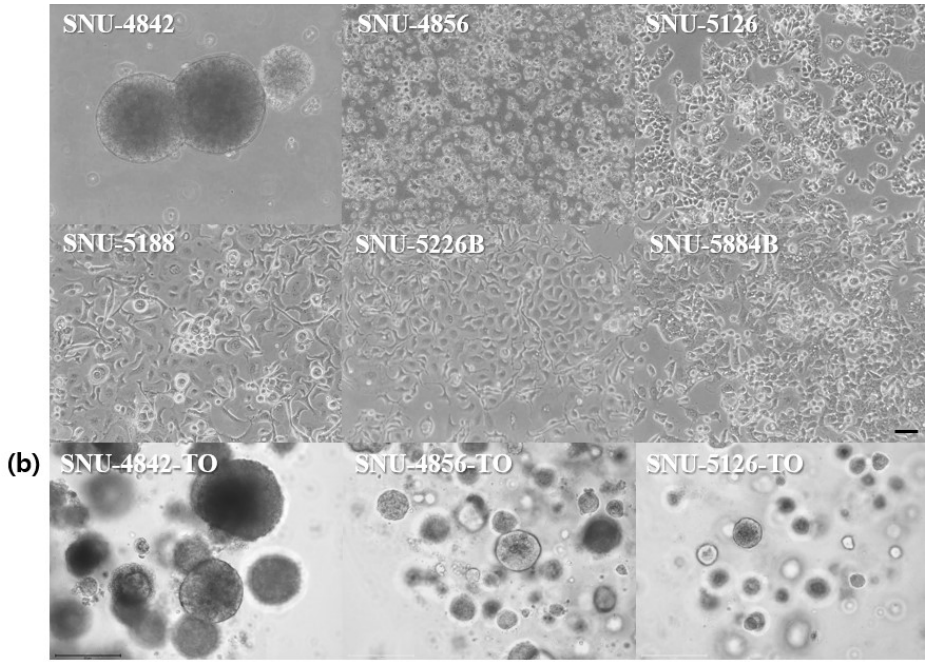



Figure 2. Microscopic images of 24 breast cancer cell lines and 3 breast PDX organoids

(a) Phase-contrast microscopy of newly established 24 breast cancer cell lines. The magnification is 100X and scale bar is 50 ㎛. (b) Morphology of 3 breast cancer organoids taken through EVOS™ FL Auto 2 Imaging System (Thermo Fisher Scientific, MA, USA). The magnification is 200X and scale bar is 200㎛.

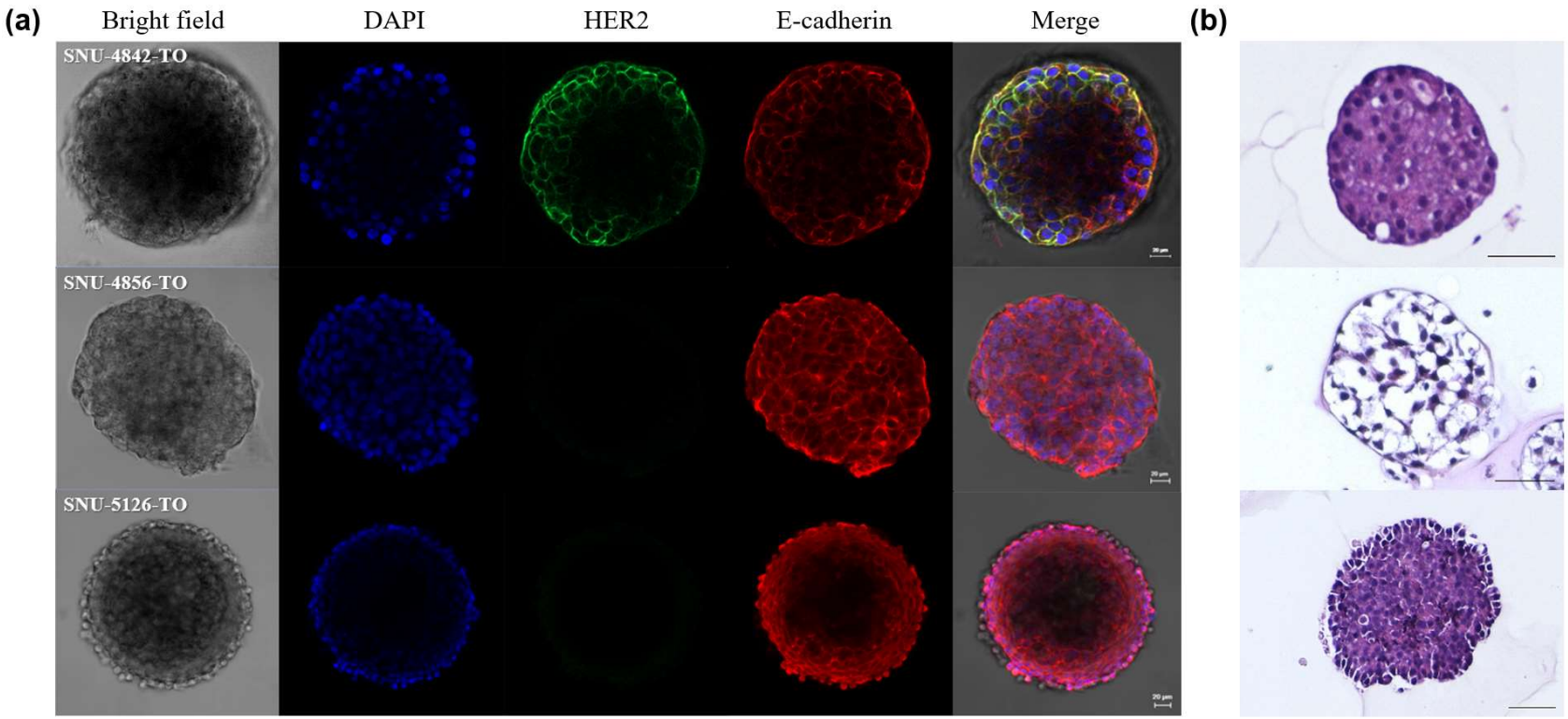



Figure 3. Images of confocal microscope and FFPE H&E staining of 3 organoids derived from PDXT

(a) Confocal analysis of immunofluorescence staining of 3 organoids. The scale bar size is 20㎛. Blue : DAPI, Green : HER2, Red : E-cadherin (b) H&E images from FFPE block sections of 3 organoids. The magnification is 100X and taken by EVOS™ FL Auto 2 Imaging System (Thermo Fisher Scientific, MA, USA). The scale bar size is 100㎛.

Table 4. *in vitro* characteristics of 24 breast cancer cell lines

| No | Cell Name | Growth patterns | Doubling time | Morphology |
|---|---|---|---|---|
| 1 | SNU-2480 | Adherent/Floating | 17.6 | Oval |
| 2 | SNU-2532A | Floating | 9.9 | Round |
| 3 | SNU-2924 | Adherent/Floating | 6.0 | Round / Polygonal |
| 4 | SNU-3129 | Adherent | 2.3 | Fibroblast-like / Oval |
| 5 | SNU-3171 | Adherent | 8.7 | Fibroblast-like / Polygonal |
| 6 | SNU-3196 | Adherent/Floating | 163.7 | Round / Polygonal |
| 7 | SNU-3223 | Adherent | 6.0 | Round / Polygonal |
| 8 | SNU-3224 | Adherent | 7.6 | Round / Polygonal |
| 9 | SNU-3230 | Adherent/Floating | 18.9 | Round / Polygonal |
| 10 | SNU-3351 | Adherent | 19.0 | Polygonal |
| 11 | SNU-3380 | Adherent | 8.0 | Round / Polygonal |
| 12 | SNU-3393 | Adherent | 76.5 | Polygonal |
| 13 | SNU-3698A | Adherent/Floating | 8.0 | Round |
| 14 | SNU-3698B | Adherent/Floating | 23.4 | Round |
| 15 | SNU-3698C | Floating | 5.1 | Round |
| 16 | SNU-3705 | Adherent/Floating | 5.8 | Round |
| 17 | SNU-3716 | Adherent/Floating | 10.9 | Round |
| 18 | SNU-3730 | Adherent/Floating | 6.7 | Round |
| 19 | SNU-4842 | Floating | 10.1 | Round |
| 20 | SNU-4856 | Adherent | - | Round |
| 21 | SNU-5126 | Adherent | 4.7 | Polygonal / Oval |
| 22 | SNU-5188 | Adherent | 60.5 | Fibroblast-like / Round |
| 23 | SNU-5226B | Adherent/Floating | 12.0 | Fibroblast-like / Polygonal |
| 24 | SNU-5884B | Adherent | 4.3 | Round / Polygonal |

### 3.2. Mutational traits

Whole-exome sequencing (WES) was performed for genetic characterization of newly established breast cancer cell lines and organoids. Among the overall results, 52 genes known for many genetic aberrations in breast cancer were analyzed. In the mutations reported in the Clinvar database (https://www.ncbi.nlm.nih.gov/clinvar), the results for the effect predictable mutations and *BRCA*, *ERBB2* genes considered important in breast cancer are summarized in Table 5. Out of the 52 screened genes, organoids tend to have more mutations than cell lines. The least mutation burden was SNU-2532A. Throughout all cell lines and organoids, the gene with the highest number of mutations was *WNK2*, and in almost all cases, the gene with aberrations was *TP53*. On the other hand, *CHEK2*, *PBXW7*, *MEN1*, *NIBEAL2*, *NOTCH1*, *PBRM1*, *PTEN*, and *USP9X* were meaningful mutant genes that appeared only in one cell line or organoid. More than half of the many genetic variations in breast cancer mentioned above were also identified in out samples: *TP53, PIK3CA, PTEN, HER2, RB1* and *MAP3K*. Different cell lines from one patient were found to have the same mutational profiles overall. In patient 1 (SNU-3223, SNU-3224, SNU-

3230) cell lines, some genes, such as *MAP3K1* or *FOXO3*, had different mutational status, but most of them were known to have no significant effect, and the aberrations of the most important *BRCA* gene in breast cancer was completely the same. Patient 2 cell lines (SNU-3380, SNU-3393), Patient 3 cell liens (SNU-3698A, SNU-3698B, SNU-3698C, SNU-3705, SNU-3716, SNU-3730) and patient 4 cell lines (SNU-5188, SNU-5226B) were analyzed to have the same major mutational status within each set. On the other hand, the distinction between cell line and organoid sets seemed to be clearly different. However, as with differences between cell lines of the same origin, most were not considered to have a major impact. There were some notable genes in the mutant state with some differences. In particular, set 2 (SNU-4856, SNU-4856-TO) had a lot of mutations in organoids, some of which are still controversial but thought to be pathogenic or to have a specific effect. Set 3 organoid (SNU-5126-TO) had *TP53* aberration which is thought to be likely pathogenic. But, this mutation was not found in set 3 cell line (SNU-5126). This result was confirmed by the IGV (Integrative Genomics Viewer, Broad Institute and the Regents of the University of California) program, as the mutation detection may

be due to the difference in the number of nucleotide transition copies. Although the HER2 mutation that was immediately identified in the set 1 organoid (SNU-4842-TO) was not immediately identified in the cell line (SNU-4842), it was confirmed that the same mutation exists even though the number of copies was small in the cell line using the above program. In western blot, there was no significant difference between samples from the same patient as a whole. Patient 3 cell lines (SNU-3698A, SNU-3698B, SNU-3698C, SNU-3705, SNU-3716, SNU-3730), however, are clearly differentiated within the same patient origin set. Among ER, PR and HER2, which are widely used as criteria for dividing breast cancer, the whole cell lines and organoids were grouped based on ER and HER2, which clearly showed results. The results are summarized in Table6. Set 1 (SNU-4842, SNU-4842-TO) was classified as ER+HER2+ (ER positive, HER2 positive). Patient 1 cell lines (SNU-3223, SNU-3224, SNU-3230), Patient 2 cell lines (SNU-3380, SNU-3393) and Patient 3 cell lines (SNU-3698A, SNU-3698B, SNU-3698C, SNU-3705, SNU-3716, SNU-3730) were categorized in ER-HER2+ (ER negative, HER2 positive). ER+HER2- (ER positive, HER2 negative) includes

set2 and patient 4 cell lines (SNU-5188, SNU-5226B). In addition, when the overall results of western blot were analyzed, SNU-3129 was not able to confirm the expression of most proteins or the expression level was quite low, whereas SNU-3716 was fairly high in protein expression in the set or as a whole.

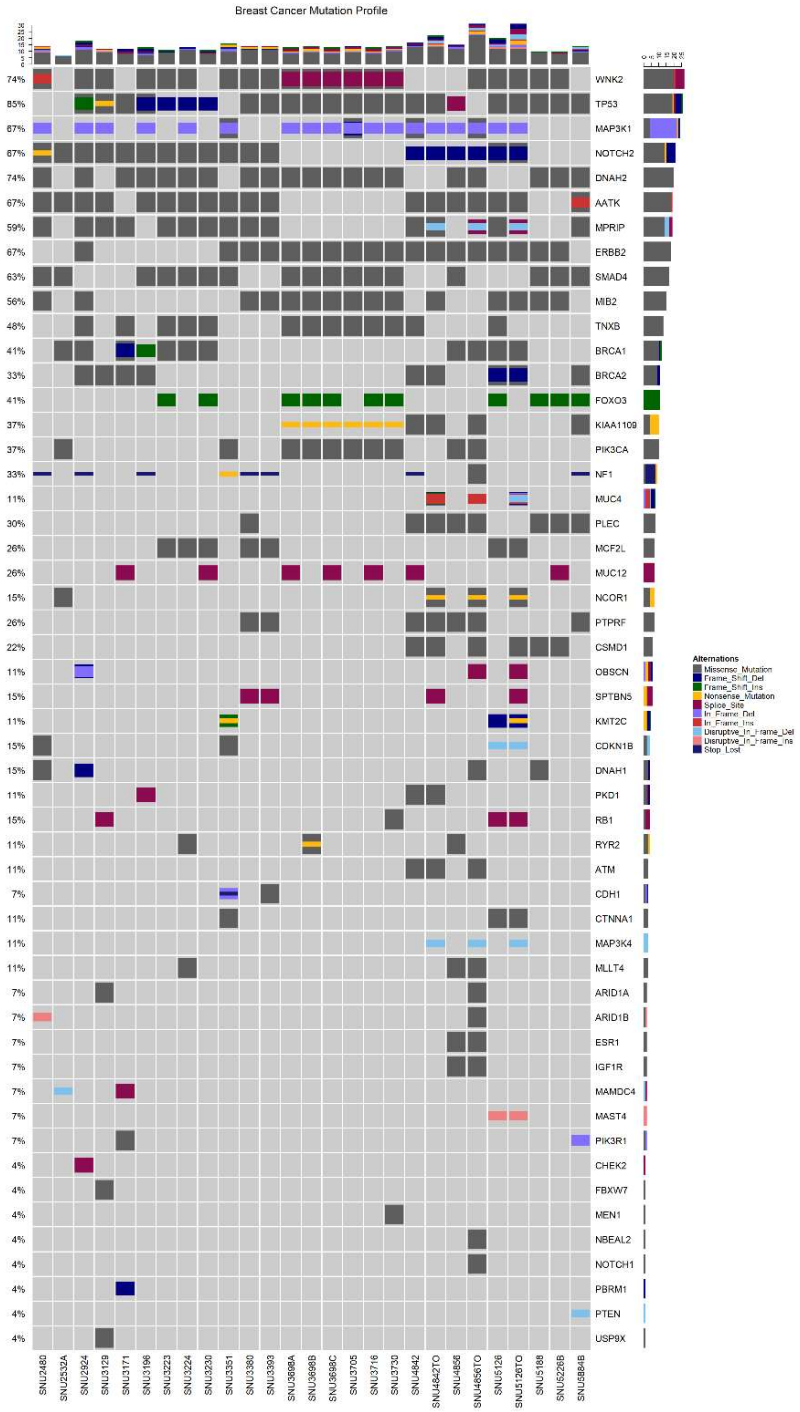



Figure 4. Mutational landscape of the established breast cancer cell lines and PDX organoids

Table 5. Major mutational profile table

| | Gene | nt change | a.a. change | Effect | Gene | nt change | a.a. change | Effect |
|---|---|---|---|---|---|---|---|---|
| SNU-2480 | CDKN1B | 443G>T | Cys148Phe | Uncertain significance | | | | |
| SNU-2532A | BRCA1 | 3548A>G | Lys1183Arg | Uncertain significance | PIK3CA | 3140A>G | His1047Arg | Pathogenic |
| | | 3113A>G | Glu1038Gly | CIP | | | | |
| | | 2612C>T | Pro871Leu | CIP | | | | |
| SNU-2924 | BRCA1 | 3548A>G | Lys1183Arg | Uncertain significance | TP53 | 215C>G | Pro72Arg | Uncertain significance |
| | | 3113A>G | Glu1038Gly | CIP | TNXB | 9946G>A | Ala3316Thr | Uncertain significance |
| | | 2612C>T | Pro871Leu | CIP | ERBB2 | 3508C>G | Pro1170Ala | - |
| SNU-3129 | BRCA2 | 1114A>C | Asn372His | - | TP53 | 215C>G | Pro72Arg | Uncertain significance |
| SNU-3171 | BRCA1 | 3548A>G | Lys1183Arg | Uncertain significance | TP53 | 742C>T | Arg248Trp | CIP |
| | | 3113A>G | Glu1038Gly | CIP | | 215C>G | Pro72Arg | Uncertain significance |
| | | 2612C>T | Pro871Leu | CIP | | | | |
| SNU-3196 | TP53 | 215C>G | Pro72Arg | Uncertain significance | BRCA2 | 1114A>C | Asn372His | - |
| | BRCA1 | 3627dupA | Glu1210fs | Pathogenic | | | | |

* nt : nucleotide ** a.a. : amino acid *** CIP : Conflicting interpretations of pathogenicity **** - : not reported

*Continued*

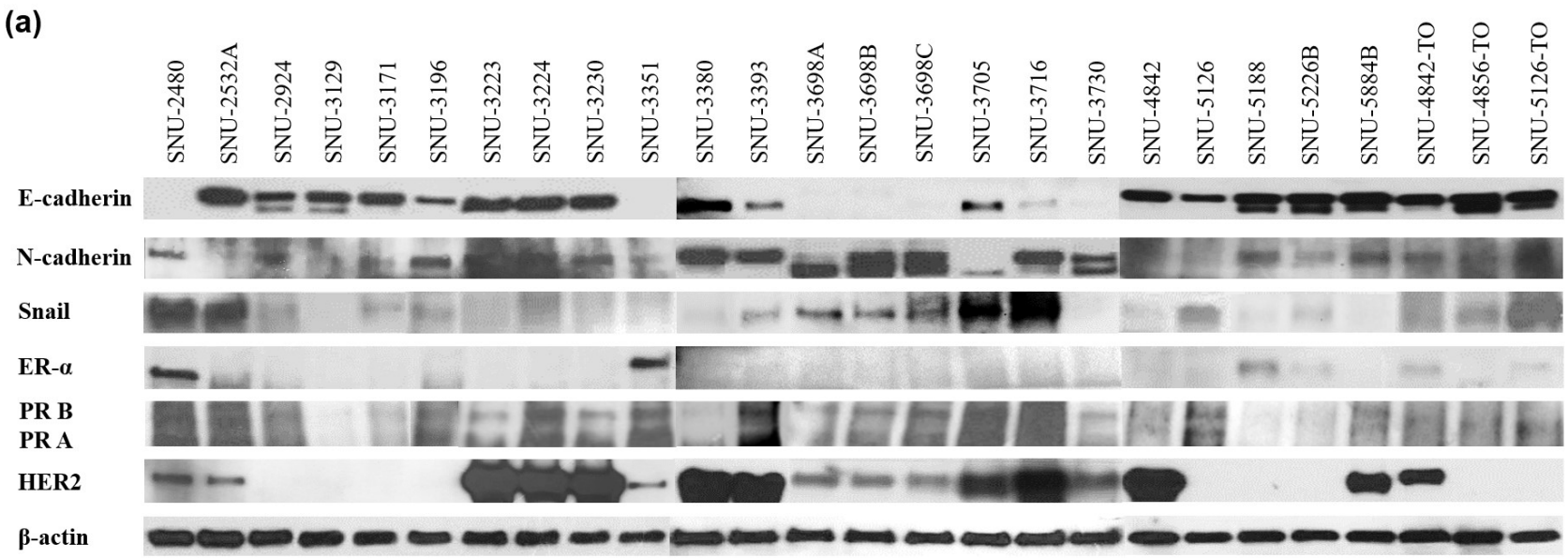
| | Gene | nt change | a.a. change | Effect | Gene | nt change | a.a. change | Effect |
|---|---|---|---|---|---|---|---|---|
| SNU-3223 | BRCA1 | 3548A>G | Lys1183Arg | Uncertain significance | BRCA1 | 2612C>T | Pro871Leu | CIP |
| | | 3113A>G | Glu1038Gly | CIP | | | | |
| SNU-3224 | BRCA1 | 3548A>G | Lys1183Arg | Uncertain significance | BRCA1 | 2612C>T | Pro871Leu | CIP |
| | | 3113A>G | Glu1038Gly | CIP | | | | |
| SNU-3230 | BRCA1 | 3548A>G | Lys1183Arg | Uncertain significance | BRCA1 | 2612C>T | Pro871Leu | CIP |
| | | 3113A>G | Glu1038Gly | CIP | | | | |
| SNU-3351 | PIK3CA | 3140A>G | His1047Arg | Pathogenic | ERBB2 | 3508C>G | Pro1170Ala | - |
| | CTNNA1 | 770A>G | Asn257Ser | Uncertain significance | CDKN1B | 326T>G | Val109Gly | CIP |
| SNU-3380 | TP53 | 743G>A | Arg248Gln | Likely pathogenic | ERBB2 | 3508C>G | Pro1170Ala | - |
| SNU-3393 | TP53 | 743G>A | Arg248Gln | Likely pathogenic | ERBB2 | 3508C>G | Pro1170Ala | - |
| SNU-3698A | PIK3CA | 3145G>C | Gly1049Arg | Likely pathogenic | ERBB2 | 3508C>G | Pro1170Ala | - |
| SNU-3698B | PIK3CA | 3145G>C | Gly1049Arg | Likely pathogenic | ERBB2 | 3508C>G | Pro1170Ala | - |
| SNU-3698C | PIK3CA | 3145G>C | Gly1049Arg | Likely pathogenic | ERBB2 | 3508C>G | Pro1170Ala | - |
| SNU-3705 | PIK3CA | 3145G>C | Gly1049Arg | Likely pathogenic | ERBB2 | 3508C>G | Pro1170Ala | - |
| SNU-3716 | PIK3CA | 3145G>C | Gly1049Arg | Likely pathogenic | ERBB2 | 3508C>G | Pro1170Ala | - |
| SNU-3730 | PIK3CA | 3145G>C | Gly1049Arg | Likely pathogenic | ERBB2 | 3508C>G | Pro1170Ala | - |

*Continued*

| | Gene | nt change | a.a. change | Effect | Gene | nt change | a.a. change | Effect |
|---|---|---|---|---|---|---|---|---|
| SNU-4842 | TP53 | 215C>G | Pro72Arg | Uncertain significance | BRCA2 | 5608T>G | Phe1870Val | - |
| SNU-4842-TO | TP53 | 737T>G | Met246Arg | Likely pathogenic | NOTCH2 | 17_18delCC | Pro6fs | Uncertain significance |
| | | 215C>G | Pro72Arg | Uncertain significance | ERBB2 | 2329G>T | Val777Leu | Likely pathogenic |
| SNU-4856 | BRCA1 | 3548A>G | Lys1183Arg | Uncertain significance | PIK3CA | 1636C>A | Gln546Lys | Pathogenic/Likely pathogenic |
| | | 3113A>G | Glu1038Gly | CIP | PLEC | 2947G>A | Gly983Ser | CIP |
| | | 2612C>T | Pro871Leu | CIP | ERBB2 | 3508C>G | Pro1170Ala | - |
| | | 2056G>A | Glu686Lys | - | | | | |
| SNU-4856-TO | BRCA1 | 3548A>G | Lys1183Arg | Uncertain significance | NOTCH2 | 17_18delCC | Pro6fs | Uncertain significance |
| | | 3113A>G | Glu1038Gly | CIP | PIK3CA | 1636C>A | Gln546Lys | Pathogenic/Likely pathogenic |
| | | 2612C>T | Pro871Leu | CIP | PIK3R1 | 961G>A | Gly321Ser | Uncertain significance |
| | BRCA2 | 956A>G | Asn319Ser | Likely pathogenic | APC | 3512G>A | Arg1171His | CIP |
| | | 943A>G | Arg315Gly | Uncertain significance | | 7778A>G | Asn2593Ser | CIP |
| | | 8092G>A | Ala2698Thr | CIP | PLEC | 2947G>A | Gly983Ser | CIP |
| | | 8117A>G | Asn2706Ser | CIP | NOTCH1 | 5189C>T | Pro1730Leu | Uncertain significance |
| | ERBB2 | 3110C>T | Pro1037Leu | - | ATM | 370A>G | Ile124Val | CIP |
| | | 3115G>A | Ala1039Thr | - | RYR3 | 11296G>A | Val3766Ile | Uncertain significance |
| | | 3508C>G | Pro1170Ala | - | NF1 | 2191C>T | Leu731Phe | CIP |

*Continued*

| | Gene | nt change | a.a. change | Effect | Gene | nt change | a.a. change | Effect |
|---|---|---|---|---|---|---|---|---|
| SNU-5126 | BRCA1 | 3548A>G | Lys1183Arg | Uncertain significance | BRCA2 | 1114A>C | Asn372His | - |
| | | 3113A>G | Glu1038Gly | CIP | | 5576_5579delTTAA | Ile1859fs | Pathogenic |
| | | 2612C>T | Pro871Leu | CIP | ERBB2 | 3508C>G | Pro1170Ala | - |
| | TP53 | 215C>G | Pro72Arg | Uncertain significance | | | | |
| SNU-5126-TO | BRCA1 | 3548A>G | Lys1183Arg | Uncertain significance | NOTCH2 | 17_18delCC | Pro6fs | Uncertain significance |
| | | 3113A>G | Glu1038Gly | CIP | BRCA2 | 1114A>C | Asn372His | - |
| | | 2612C>T | Pro871Leu | CIP | | 5576_5579delTTAA | Ile1859fs | Pathogenic |
| | TP53 | 404G>A | Cys135Tyr | Likely pathogenic | ERBB2 | 3508C>G | Pro1170Ala | - |
| | | 215C>G | Pro72Arg | Uncertain significance | | | | |
| SNU-5188 | TP53 | 215C>G | Pro72Arg | Uncertain significance | ERBB2 | 3508C>G | Pro1170Ala | - |
| | APC | 5465T>A | Val1822Asp | Uncertain significance | | | | |
| SNU-5226B | TP53 | 215C>G | Pro72Arg | Uncertain significance | ERBB2 | 3508C>G | Pro1170Ala | - |
| | APC | 5465T>A | Val1822Asp | Uncertain significance | | | | |
| SNU-5884B | BRCA2 | 1114A>C | Asn372His | - | TP53 | 215C>G | Pro72Arg | Uncertain significance |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SNU-5884B
SNU-4842-TO
SNU-4856-TO
SNU-5126-TO
E-cadherin
N-cadherin
Snail
ER-α
PR B
PR A
HER2
β-actin

(b)

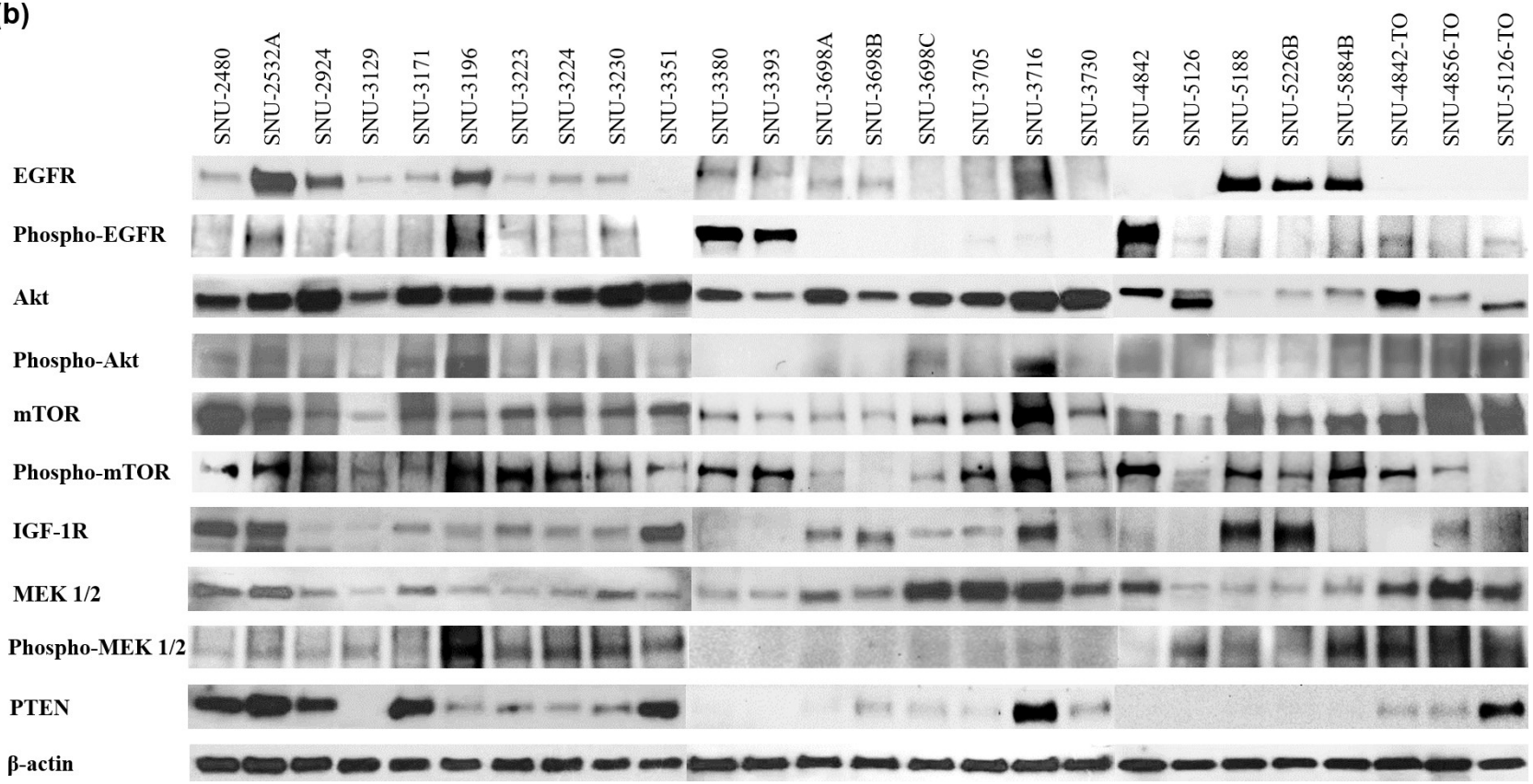

SNU-2480
SNU-2532A
SNU-2924
SNU-3129
SNU-3171
SNU-3196
SNU-3223
SNU-3224
SNU-3230
SNU-3351
SNU-3380
SNU-3393
SNU-3698A
SNU-3698B
SNU-3698C
SNU-3705
SNU-3716
SNU-3730
SNU-4842
SNU-5126
SNU-5188
SNU-5226B
SNU-5884B
SNU-4842-TO
SNU-4856-TO
SNU-5126-TO
EGFR
Phospho-EGFR
Akt
Phospho-Akt
mTOR
Phospho-mTOR
IGF-1R
MEK 1/2
Phospho-MEK 1/2
PTEN
β-actin

Figure 5. Western blot analysis

(a) Expression levels of EMT-related markers and breast cancer subtype markers. (b) Expression levels of various proteins involved in signaling pathways that may be associated with anticancer drugs for breast cancer.

Expressions of EGFR, HER2, Akt, mTOR, IGF-1R, MEK 1/2, PTEN in 23 cell lines and 3 organoids were analyzed using Western Blotting. In SNU-2532A, SNU-2924, SNU-3196, SNU-3698B, SNU-5188, SNU-5226B and SNU-5884B, the expression level of EGFR was relatively high. On the other hand, in the case of phospho-EGFR, the expression level was significantly high in patient 2 cell lines (SNU-3380, SNU-3393), and in the case of the cell line and organoid set, the expression level was relatively low in SNU-4842-TO, whereas it was highly overexpressed in SNU-4842. Patient 1 set (SNU-3223, SNU-3224, SNU-3230) showed similar aspects amongst each other, but overall displayed reasonably low expression levels. Patient 3 set (SNU-3698A, SNU-3698B, SNU-3698C, SNU-3705, SNU-3716, SNU-3730) and patient 4 set cell lines (SNU-5188, SNU-5226B) had hardly any expression level. Akt expression levels were relatively low in SNU-3129, patient 4 cell lines (SNU-5188, SNU-5226B), SNU-5884B and SNU-4856-TO. IGF-1R was almost negative in patient 2 cell lines (SNU-3380, SNU-3393), set 1 (SNU-4842, SNU-4842-TO) and SNU-5126-TO. Phospho-mTOR, unlike the case of phospho- EGFR, there was some difference in expression level

in the corresponding set (patient 1~4, set 1/3). MEK 1/2 expression was relatively high in SNU-3698C, SNU-3705, SNU-3716, set 1 (SNU-4842, SNU-4842-TO), SNU-4856-TO and SNU-5126-TO. Phospho- MEK1/2 expression level also did not show a large difference between sets, but the expression in SNU-4842 was extremely low and in SNU-4842-TO was greatly high. Similarly, PTEN showed a relative difference. At SNU-2480, SNU-2532A, SNU-2924, SNU-3171, SNU-3351, SNU-3716 and SNU-5126-TO, the expression level was significantly higher than the rest. In the case of HER2, it was confirmed that the expression levels in patient 1 (SNU-3223, SNU-3224, SNU-3230), Patient 2 (SNU-3380, SNU-3393), SNU-3705, SNU-3716, set 1 (SNU-4842, SNU-4842-TO) and SNU-5884B were greatly high. On the other hand, in SNU-2924, SNU-3129, SNU-3171, SNU-3196, set 3 (SNU-5126, SNU-5126-TO), patient 4 cell lines (SNU-5188, SNU-5226B) and SNU-4856-TO, there was no HER2 detected within the exposure time. SNU-2480, SNU-3351, patient 4 cell lines, SNU-4842-TO and SNU-5126-TO were showed as ER positive, and SNU-4842, SNU-5126 were significantly weak, but the band was confirmed several times.

Although it was difficult to analyze the results despite repeated PRs, SNU-3351 and SNU-5884B determined that positive bands were detected at the correct location. N-cadherin appeared in SNU-2480 and SNU-3351, but there was no expression in E-cadherin. The expression of N-cadherin was high in patient 2 (SNU-3380, SNU-3393) and patient 3 (SNU-3698A, SNU-3698B, SNU-3698C, SNU-3705, SNU-3716) and SNU-3730), while E-cadherin expression was relatively low.

Table 6. Classification of cell lines and organoids based on ER and HER2 expression

| | cell lines & organoids |
|---|---|
| ER+HER2+ | SNU-2480, SNU-3351, SNU-4842, SNU-4842-TO |
| ER-HER2+ | SNU-2532A, SNU-3223, SNU-3224, SNU-3230, SNU-3380, SNU-3393, SNU-3698A, SNU-3698B, SNU-3698C, SNU-3705, SNU-3716, SNU-3730, SNU-5884B |
| ER+HER2- | SNU-5126, SNU-5126-TO, SNU-5188, SNU-5226B |
| ER-HER2- | SNU-2924, SNU-3129, SNU-3171, SNU-3196, SNU-4856-TO |

### 3.3. Anticancer drug response

A total of 26 Drugs were screened. Among them, DS-8201a only partially drew results due to the problem of drug supply and demand, which can be confirmed in Table 7. Most of drugs were selected among the materials introduced by NIH (https://www.cancer.gov/about-cancer/treatment/drugs/breast) as breast cancer medical supplies, and by adding several known molecular-targeted drugs or some other drugs used as universal anticancer drugs. Amongst several units that represents drug sensitivity, Area Under the Curve (AUC) was used in this study. The higher the AUC value, the more resistant the cell lines and organoids were to the drug. In comparison, the lower the AUC value, the more responsive they were to the drug. Figure 6 is the heat maps of the average of AUC results carried out in repeated experiments. Patient 3 set (SNU-3698A, SNU-3698B, SNU-3698C, SNU-3705, SNU-3716, SNU-3730) was the most resistant in the majority of the drugs. On the other hand, set 1 (SNU-4842, SNU-4842-TO) was the significantly responsive to most of the drugs. The pair, SNU-4842, also had good reactivity on average. The trends of SNU-4842-TO and SNU-4842, which are set 1, were generally similar, but the cell lines

SNU-4842 were relatively inactive in Gemcitabine, Irinotecan, Paclitaxel, and Talazoparib. Contrary to SNU-5226B, which was then highly reactive, SNU-5188 was generally resistant to these drugs. Both results showed similar tendencies themselves, but SNU-5188 was particularly resistant to Gemcitabine, Irinotecan, and Paclitaxel. Set 3, SNU-5126 and SNU-5126-TO showed highly different responsiveness to the same drug. SNU-5126 is resistant to most drugs, while SNU-5126-TO has been shown to be greatly reactive, especially with some drugs like Doxorubicin, Epirubicin, Lapatinib, Tamoxifen, Trametinib. SNU-5884B responded most sensitively to Doxorubicin and died to the point where few live cells remained, and showed almost complete resistance to Palbociclib. The cell lines of patient 1 (SNU-3223, SNU-3224, SNU-3230) showed similar tendencies, and the cell lines of Patient 2 (SNU-3380, SNU-3393) and patient 3 (SNU-3698A, SNU-3698B, SNU-3698C, SNU-3705, SNU-3716, SNU-3730) showed similar trends, respectively. Patient 3 cell lines were found to be exceedingly resistant to most drugs, especially 5-FU screened highly responsively as a whole with little reactivity. They showed meaningful differences in reactivity in Afatinib, Akt1/2 kinase

inhibitor, Epirubicin and Lapatinib. In addition, SNU-3351, SNU-3171, SNU-3129 and SNU-3196 were almost unresponsive to the drugs screened this time except for 5-FU and one or two drugs.

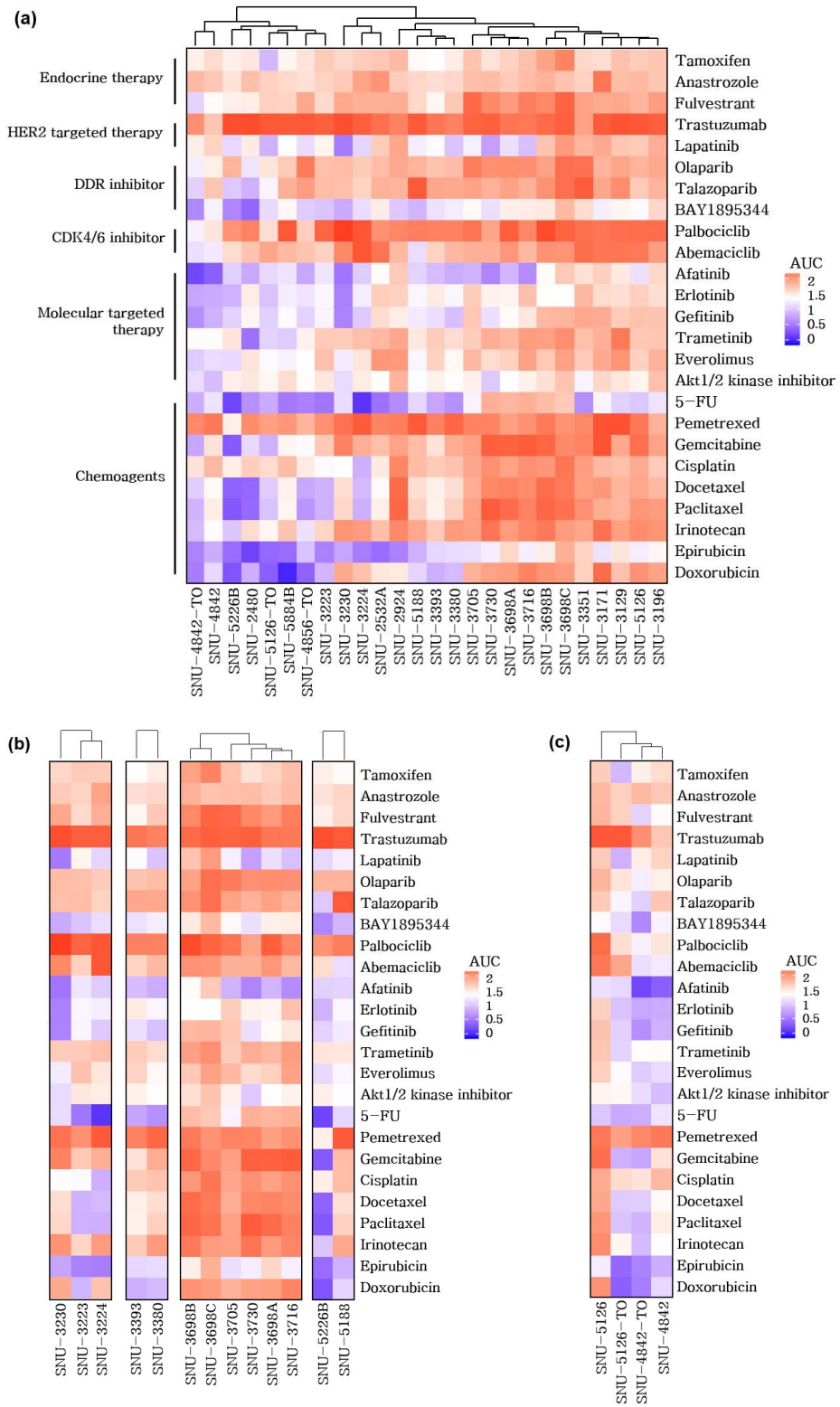
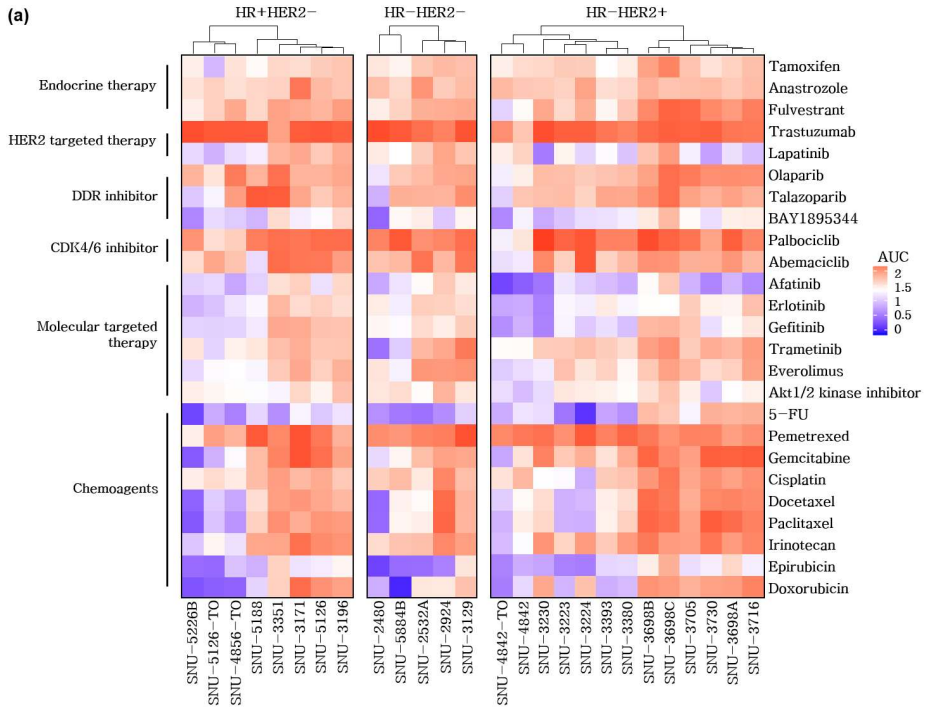

(a)
Endocrine therapy
HER2 targeted therapy
DDR inhibitor
CDK4/6 inhibitor
Molecular targeted therapy
Chemoagents
Tamoxifen
Anastrozole
Fulvestrant
Trastuzumab
Lapatinib
Olaparib
Talazoparib
BAY1895344
Palbociclib
Abemaciclib
Afatinib
Erlotinib
Gefitinib
Trametinib
Everolimus
Akt1/2 kinase inhibitor
5−FU
Pemetrexed
Gemcitabine
Cisplatin
Docetaxel
Paclitaxel
Irinotecan
Epirubicin
Doxorubicin
AUC
2
1.5
1
0.5
0
SNU−4842−TO
SNU−4842
SNU−5226B
SNU−2480
SNU−5126−TO
SNU−5884B
SNU−4856−TO
SNU−3223
SNU−3230
SNU−3224
SNU−2532A
SNU−2924
SNU−5188
SNU−3393
SNU−3380
SNU−3705
SNU−3730
SNU−3698A
SNU−3716
SNU−3698B
SNU−3698C
SNU−3351
SNU−3171
SNU−3129
SNU−5126
SNU−3196
(b)
Tamoxifen
Anastrozole
Fulvestrant
Trastuzumab
Lapatinib
Olaparib
Talazoparib
BAY1895344
Palbociclib
Abemaciclib
Afatinib
Erlotinib
Gefitinib
Trametinib
Everolimus
Akt1/2 kinase inhibitor
5−FU
Pemetrexed
Gemcitabine
Cisplatin
Docetaxel
Paclitaxel
Irinotecan
Epirubicin
Doxorubicin
AUC
2
1.5
1
0.5
0
SNU−3230
SNU−3223
SNU−3224
SNU−3393
SNU−3380
SNU−3698B
SNU−3698C
SNU−3705
SNU−3730
SNU−3698A
SNU−3716
SNU−5226B
SNU−5188
(c)
Tamoxifen
Anastrozole
Fulvestrant
Trastuzumab
Lapatinib
Olaparib
Talazoparib
BAY1895344
Palbociclib
Abemaciclib
Afatinib
Erlotinib
Gefitinib
Trametinib
Everolimus
Akt1/2 kinase inhibitor
5−FU
Pemetrexed
Gemcitabine
Cisplatin
Docetaxel
Paclitaxel
Irinotecan
Epirubicin
Doxorubicin
AUC
2
1.5
1
0.5
0
SNU−5126
SNU−5126−TO
SNU−4842−TO
SNU−4842

Figure 6. Drug sensitivity AUC result heatmap of breast cancer cell lines and organoids

(a) A heatmap for the drug sensitivity of 23 breast cancer cell lines and 3 organoids. The values used for the heatmap are the AUC results. (b) The result is a set of different cell lines derived from four patients. (c) This is the result of paired cell line and organoid derived from two PDX tissues.


(a)
HR+HER2−
HR−HER2−
HR−HER2+
Endocrine therapy
HER2 targeted therapy
DDR inhibitor
CDK4/6 inhibitor
Molecular targeted therapy
Chemoagents
Tamoxifen
Anastrozole
Fulvestrant
Trastuzumab
Lapatinib
Olaparib
Talazoparib
BAY1895344
Palbociclib
Abemaciclib
Afatinib
Erlotinib
Gefitinib
Trametinib
Everolimus
Akt1/2 kinase inhibitor
5−FU
Pemetrexed
Gemcitabine
Cisplatin
Docetaxel
Paclitaxel
Irinotecan
Epirubicin
Doxorubicin
AUC
2
1.5
1
0.5
0
SNU−5226B
SNU−5126−TO
SNU−4856−TO
SNU−5188
SNU−3351
SNU−3171
SNU−5126
SNU−3196
SNU−2480
SNU−5884B
SNU−2532A
SNU−2924
SNU−3129
SNU−4842−TO
SNU−4842
SNU−3230
SNU−3223
SNU−3224
SNU−3393
SNU−3380
SNU−3698B
SNU−3698C
SNU−3705
SNU−3730
SNU−3698A
SNU−3716

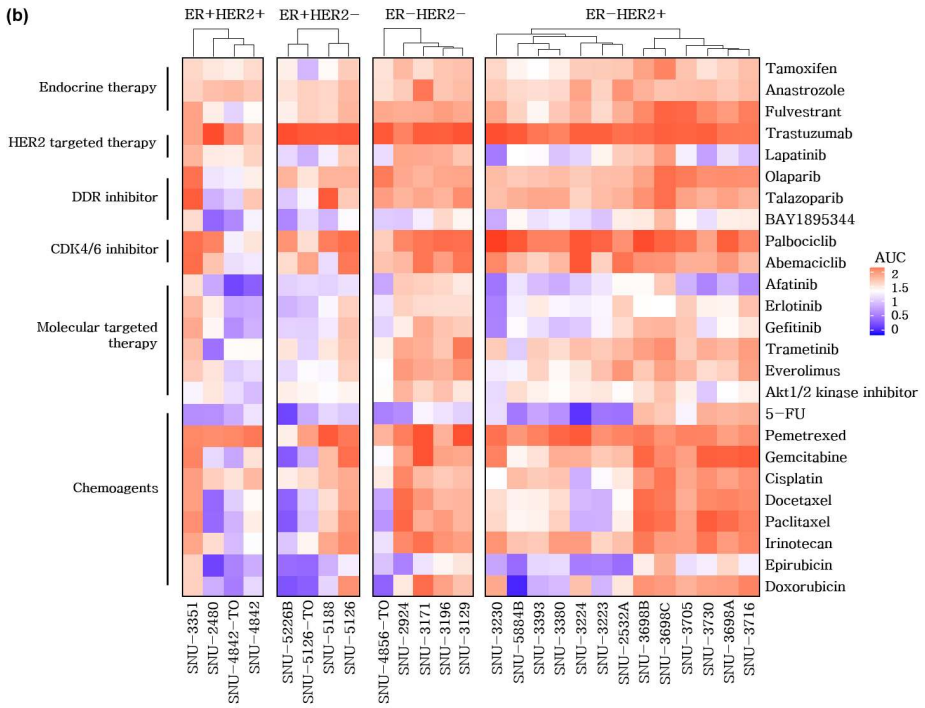

(b)
ER+HER2+
ER+HER2−
ER−HER2−
ER−HER2+
Endocrine therapy
HER2 targeted therapy
DDR inhibitor
CDK4/6 inhibitor
Molecular targeted therapy
Chemoagents
Tamoxifen
Anastrozole
Fulvestrant
Trastuzumab
Lapatinib
Olaparib
Talazoparib
BAY1895344
Palbociclib
Abemaciclib
Afatinib
Erlotinib
Gefitinib
Trametinib
Everolimus
Akt1/2 kinase inhibitor
5−FU
Pemetrexed
Gemcitabine
Cisplatin
Docetaxel
Paclitaxel
Irinotecan
Epirubicin
Doxorubicin
AUC
2
1.5
1
0.5
0
SNU−3351
SNU−2480
SNU−4842−TO
SNU−4842
SNU−5226B
SNU−5126−TO
SNU−5188
SNU−5126
SNU−4856−TO
SNU−2924
SNU−3171
SNU−3196
SNU−3129
SNU−3230
SNU−5884B
SNU−3393
SNU−3380
SNU−3224
SNU−3223
SNU−2532A
SNU−3698B
SNU−3698C
SNU−3705
SNU−3730
SNU−3698A
SNU−3716

Figure 7. AUC heat map divided into groups according to patient information and ER/HER2 expression.

(a) Drug screening result of cell lines and organoids corresponding to the subtype of the derived patient. (b) Drug heat map grouped by ER and HER2 expression status in our western blot results.

Table 7. AUC of 23 breast cancer cell lines and 3 organoids

| SNU Name | Tamoxifen | Anastrozole | Fulvestrant | Trastuzumab | Lapatinib | DS-8201a |
|---|---|---|---|---|---|---|
| SNU-2480 | 1.436 | 1.666 | 1.399 | 2.235 | 1.418 | 2.255 |
| SNU-2532A | 1.617 | 1.902 | 1.745 | 2.085 | 1.588 | 1.946 |
| SNU-2924 | 1.697 | 1.591 | 1.768 | 1.991 | 1.806 | 2.110 |
| SNU-3129 | 1.654 | 1.686 | 1.790 | 2.209 | 1.629 | 2.105 |
| SNU-3171 | 1.499 | 2.050 | 1.780 | 2.177 | 1.783 | 2.171 |
| SNU-3196 | 1.630 | 1.627 | 1.854 | 2.154 | 1.759 | 2.162 |
| SNU-3223 | 1.602 | 1.552 | 1.518 | 2.154 | 1.370 | - |
| SNU-3224 | 1.587 | 1.817 | 1.751 | 2.160 | 1.068 | 2.052 |
| SNU-3230 | 1.531 | 1.605 | 1.796 | 2.229 | 0.556 | 1.846 |
| SNU-3351 | 1.529 | 1.557 | 1.825 | 1.828 | 1.721 | - |
| SNU-3380 | 1.409 | 1.524 | 1.614 | 1.999 | 0.975 | 1.856 |
| SNU-3393 | 1.305 | 1.485 | 1.349 | 2.056 | 1.276 | - |
| SNU-3698A | 1.561 | 1.607 | 1.872 | 2.059 | 1.129 | 2.278 |
| SNU-3698B | 1.822 | 1.743 | 1.961 | 2.114 | 1.640 | 2.215 |
| SNU-3698C | 1.982 | 1.639 | 2.138 | 2.157 | 1.867 | 2.118 |
| SNU-3705 | 1.641 | 1.668 | 2.118 | 2.145 | 1.210 | 2.223 |
| SNU-3716 | 1.664 | 1.684 | 2.019 | 2.044 | 0.942 | 2.119 |
| SNU-3730 | 1.466 | 1.685 | 1.960 | 2.156 | 0.768 | 2.026 |
| SNU-4842 | 1.515 | 1.614 | 1.324 | 1.625 | 1.548 | - |
| SNU-5126 | 1.588 | 1.693 | 1.720 | 2.197 | 1.604 | 2.223 |
| SNU-5188 | 1.329 | 1.539 | 1.527 | 2.183 | 1.200 | - |
| SNU-5226B | 1.391 | 1.466 | 1.385 | 2.227 | 1.078 | - |
| SNU-5884B | 1.364 | 1.520 | 1.547 | 2.179 | 1.301 | - |
| SNU-4842-TO | 1.391 | 1.694 | 1.050 | 1.932 | 1.401 | 0.414 |
| SNU-4856-TO | 1.467 | 1.478 | 1.786 | 2.187 | 1.100 | 1.799 |
| SNU-5126-TO | 0.900 | 1.576 | 1.570 | 2.190 | 0.869 | - |

*Continued*

| SNU Name | Olaparib | Talazoparib | BAY1895344 | Palbociclib | Abemaciclib | Afatinib |
|---|---|---|---|---|---|---|
| SNU-2480 | 1.154 | 0.864 | 0.440 | 1.989 | 1.640 | 0.817 |
| SNU-2532A | 1.676 | 1.738 | 1.401 | 1.923 | 2.067 | 1.326 |
| SNU-2924 | 1.782 | 1.729 | 0.995 | 1.976 | 1.705 | 1.604 |
| SNU-3129 | 1.813 | 1.939 | 1.353 | 2.088 | 2.055 | 1.419 |
| SNU-3171 | 1.727 | 1.801 | 1.208 | 2.057 | 2.058 | 1.570 |
| SNU-3196 | 1.793 | 1.735 | 1.524 | 2.099 | 1.848 | 1.527 |
| SNU-3223 | 1.672 | 1.679 | 0.978 | 2.134 | 1.563 | 1.162 |
| SNU-3224 | 1.599 | 1.550 | 1.113 | 2.198 | 2.199 | 1.012 |
| SNU-3230 | 1.680 | 1.663 | 0.828 | 2.289 | 1.965 | 0.530 |
| SNU-3351 | 2.076 | 2.173 | 1.499 | 2.088 | 2.101 | 1.481 |
| SNU-3380 | 1.677 | 1.786 | 1.197 | 1.995 | 1.708 | 0.841 |
| SNU-3393 | 1.637 | 1.798 | 1.136 | 2.001 | 1.550 | 0.929 |
| SNU-3698A | 1.934 | 1.827 | 1.388 | 2.161 | 1.883 | 0.889 |
| SNU-3698B | 1.823 | 1.916 | 1.425 | 2.232 | 1.913 | 1.322 |
| SNU-3698C | 2.094 | 2.090 | 1.694 | 2.138 | 1.904 | 1.607 |
| SNU-3705 | 2.027 | 1.824 | 1.335 | 2.068 | 1.749 | 0.874 |
| SNU-3716 | 1.932 | 1.739 | 1.406 | 1.971 | 1.703 | 0.654 |
| SNU-3730 | 1.929 | 1.740 | 1.124 | 1.819 | 1.753 | 0.615 |
| SNU-4842 | 1.392 | 1.625 | 1.229 | 1.444 | 1.172 | 0.398 |
| SNU-5126 | 1.735 | 1.607 | 1.280 | 2.100 | 2.047 | 1.136 |
| SNU-5188 | 1.733 | 2.183 | 0.890 | 2.019 | 1.102 | 1.045 |
| SNU-5226B | 1.731 | 0.993 | 0.618 | 1.911 | 1.510 | 1.040 |
| SNU-5884B | 1.605 | 1.746 | 1.340 | 2.191 | 1.692 | 1.149 |
| SNU-4842-TO | 1.206 | 1.037 | 0.631 | 1.219 | 1.131 | 0.270 |
| SNU-4856-TO | 2.028 | 1.849 | 1.012 | 1.582 | 1.640 | 0.800 |
| SNU-5126-TO | 1.458 | 1.245 | 1.098 | 1.484 | 1.808 | 1.082 |

*Continued*

| SNU Name | Erlotinib | Gefitinib | Trametinib | Everolimus | Akt 1/2 kinase inhibitor | 5-FU |
|---|---|---|---|---|---|---|
| **SNU-2480** | 1.412 | 1.344 | 0.509 | 1.455 | 1.437 | 0.676 |
| **SNU-2532A** | 1.583 | 1.504 | 1.700 | 1.889 | 1.318 | 0.506 |
| **SNU-2924** | 1.565 | 1.398 | 1.795 | 1.879 | 1.724 | 0.660 |
| **SNU-3129** | 1.487 | 1.585 | 2.035 | 1.905 | 1.440 | 1.041 |
| **SNU-3171** | 1.505 | 1.778 | 1.752 | 1.783 | 1.528 | 1.238 |
| **SNU-3196** | 1.488 | 1.642 | 1.630 | 1.739 | 1.654 | 1.138 |
| **SNU-3223** | 1.255 | 1.232 | 1.606 | 1.655 | 1.436 | 0.531 |
| **SNU-3224** | 1.205 | 1.003 | 1.668 | 1.444 | 1.408 | 0.171 |
| **SNU-3230** | 0.587 | 0.607 | 1.595 | 1.190 | 1.093 | 1.107 |
| **SNU-3351** | 1.699 | 1.784 | 1.665 | 1.602 | 1.239 | 0.671 |
| **SNU-3380** | 1.251 | 0.945 | 1.502 | 1.339 | 1.313 | 0.678 |
| **SNU-3393** | 1.413 | 1.121 | 1.587 | 1.557 | 1.374 | 0.794 |
| **SNU-3698A** | 1.364 | 1.326 | 1.700 | 1.571 | 1.287 | 1.718 |
| **SNU-3698B** | 1.308 | 1.701 | 1.837 | 1.613 | 1.479 | 1.678 |
| **SNU-3698C** | 1.301 | 1.723 | 1.928 | 1.832 | 1.641 | 1.610 |
| **SNU-3705** | 1.589 | 1.624 | 1.597 | 1.698 | 1.385 | 1.233 |
| **SNU-3716** | 1.653 | 1.437 | 1.853 | 1.820 | 1.377 | 1.745 |
| **SNU-3730** | 1.381 | 1.125 | 1.766 | 1.505 | 0.994 | 1.749 |
| **SNU-4842** | 0.829 | 0.860 | 1.324 | 1.109 | 0.929 | 1.144 |
| **SNU-5126** | 1.572 | 1.648 | 1.617 | 1.570 | 1.364 | 0.987 |
| **SNU-5188** | 1.256 | 1.183 | 1.439 | 1.253 | 1.284 | 1.075 |
| **SNU-5226B** | 0.875 | 1.043 | 1.448 | 1.086 | 1.395 | 0.257 |
| **SNU-5884B** | 1.194 | 1.271 | 1.009 | 1.226 | 1.499 | 0.544 |
| **SNU-4842-TO** | 0.804 | 0.668 | 1.315 | 1.014 | 1.106 | 0.844 |
| **SNU-4856-TO** | 1.143 | 1.050 | 1.374 | 1.291 | 1.314 | 0.587 |
| **SNU-5126-TO** | 0.954 | 1.037 | 1.055 | 1.280 | 1.347 | 0.825 |

*Continued*

| SNU Name | Pemetrexed | Gemcitabine | Cisplatin | Docetaxel | paclitaxel | Irinotecan |
|---|---|---|---|---|---|---|
| SNU-2480 | 1.936 | 1.088 | 1.582 | 0.448 | 0.462 | 1.503 |
| SNU-2532A | 1.986 | 1.674 | 1.426 | 1.322 | 1.387 | 1.662 |
| SNU-2924 | 2.015 | 1.829 | 1.978 | 2.106 | 2.122 | 1.960 |
| SNU-3129 | 2.220 | 1.798 | 1.671 | 1.723 | 1.721 | 1.841 |
| SNU-3171 | 2.215 | 2.215 | 1.696 | 1.883 | 1.769 | 2.082 |
| SNU-3196 | 1.744 | 1.824 | 1.608 | 1.700 | 1.859 | 1.904 |
| SNU-3223 | 1.924 | 1.613 | 1.267 | 0.867 | 0.857 | 1.544 |
| SNU-3224 | 2.183 | 1.755 | 0.847 | 0.913 | 0.850 | 1.865 |
| SNU-3230 | 2.070 | 1.992 | 1.293 | 1.494 | 1.524 | 1.905 |
| SNU-3351 | 1.966 | 1.987 | 1.848 | 1.841 | 1.931 | 1.807 |
| SNU-3380 | 2.128 | 1.774 | 1.643 | 1.495 | 1.575 | 1.867 |
| SNU-3393 | 2.004 | 1.472 | 1.598 | 1.391 | 1.375 | 1.603 |
| SNU-3698A | 1.841 | 2.150 | 1.836 | 1.989 | 2.103 | 1.870 |
| SNU-3698B | 2.036 | 2.129 | 1.881 | 2.101 | 2.127 | 2.039 |
| SNU-3698C | 1.911 | 1.959 | 2.055 | 2.036 | 2.073 | 1.858 |
| SNU-3705 | 1.994 | 1.844 | 1.798 | 1.770 | 1.836 | 1.837 |
| SNU-3716 | 1.917 | 2.175 | 1.920 | 1.946 | 2.003 | 1.978 |
| SNU-3730 | 1.981 | 2.153 | 1.917 | 1.966 | 2.175 | 2.061 |
| SNU-4842 | 2.042 | 1.468 | 1.701 | 1.332 | 1.404 | 1.284 |
| SNU-5126 | 2.041 | 2.089 | 1.780 | 1.798 | 1.892 | 1.946 |
| SNU-5188 | 2.179 | 1.683 | 1.686 | 1.486 | 1.567 | 1.797 |
| SNU-5226B | 1.395 | 0.364 | 1.398 | 0.420 | 0.363 | 0.986 |
| SNU-5884B | 1.881 | 1.352 | 1.681 | 1.373 | 1.359 | 1.636 |
| SNU-4842-TO | 1.968 | 0.800 | 1.465 | 1.024 | 0.872 | 0.916 |
| SNU-4856-TO | 1.735 | 1.270 | 1.415 | 0.809 | 0.703 | 1.136 |
| SNU-5126-TO | 1.838 | 0.859 | 1.508 | 1.009 | 0.966 | 1.354 |

*Continued*

| SNU Name | Epirubicin | Doxorubicin |
|---|---|---|
| **SNU-2480** | 0.254 | 0.849 |
| **SNU-2532A** | 0.482 | 1.438 |
| **SNU-2924** | 0.594 | 1.432 |
| **SNU-3129** | 1.444 | 1.650 |
| **SNU-3171** | 1.145 | 2.100 |
| **SNU-3196** | 1.350 | 1.825 |
| **SNU-3223** | 0.591 | 0.887 |
| **SNU-3224** | 0.576 | 1.652 |
| **SNU-3230** | 0.797 | 1.790 |
| **SNU-3351** | 1.560 | 1.565 |
| **SNU-3380** | 1.095 | 0.914 |
| **SNU-3393** | 1.073 | 0.846 |
| **SNU-3698A** | 1.533 | 1.851 |
| **SNU-3698B** | 1.403 | 1.910 |
| **SNU-3698C** | 1.745 | 1.865 |
| **SNU-3705** | 1.115 | 1.744 |
| **SNU-3716** | 1.211 | 1.987 |
| **SNU-3730** | 1.208 | 1.835 |
| **SNU-4842** | 0.842 | 1.080 |
| **SNU-5126** | 1.201 | 1.912 |
| **SNU-5188** | 0.859 | 1.068 |
| **SNU-5226B** | 0.474 | 0.337 |
| **SNU-5884B** | 0.471 | 0.106 |
| **SNU-4842-TO** | 0.602 | 0.576 |
| **SNU-4856-TO** | 0.960 | 0.421 |
| **SNU-5126-TO** | 0.446 | 0.400 |

### 3.4. Fusion gene analysis

Among the fusion genes (FGs) found in the RNA sequencing results of cell lines corresponding to patient 1 (SNU-3223, SNU-3224, SNU-3230), 2 (SNU-3380, SNU-3393) and 3 (SNU-3698A, SNU-3698B, SNU-3698C, SNU-3705, SNU-3716, SNU-3730) sets, the FG reported in the database (FusionGDB: Fusion Gene annotation DataBase, https://ccsm.uth.edu/FusionGDB) are organized in a table 8. As a result, it can be seen that cell lines from the same patient have multiple different FGs. In the case of SNU-3223, it had much more known FG than SNU-3224 and SNU-3230. CSNK1D-SECTM1 and HER2-IKZP3 were identical in three cell lines, and FAM168A-RAB6A and ITGB4-SAP30BP were found only in SNU-3223 and SNU-3230. The FG thought to have SNU-3224 uniquely from the other two cell lines were AHNAK-NXF1, HER2-WSB1, GALNT16-RAD51B and TNRC18-RNF216. SNU-3223 also had HSP90B1-APP, MED1-CDK12, NPLOC4-PDE6G, SCARB1-UBC, TYMS-GNAS and WSB1-MED1. Patient 2 cell lines, SNU-3380 and SNU-3393 had few reported FGs. Both cell lines were found to have FARP1-IPO5 and PTK2-TRAPPC9 in common, and an additional GRB2-VMP1

was found in SNU-3380. patient 3 cell lines (SNU-3698A, SNU-3698B, SNU-3698C, SNU-3705, SNU-3716, SNU-3730) had relatively different FG profiles between them. First, all six cell lines had NEBL-DNAJC1. SDC1-PUM2 was observed only in SNU-3698A, SNU-3698B, SNU-3698C. Although these three cell lines have little diversity, they have similar FG profiles compared to the other three (SNU-3705, SNU-3716, SNU-3730). In SNU-3698A, SRCIN1-PSMB3 was additionally found, and SNU-3698C had FGFR3-TACC3. These two FGs were also found in other three cell lines. SRCIN1-PSMB3 was observed in all SNU-3705, SNU-3716 and SNU-3730. In addition, HER2-WSB1 and FGFR3-TACC3 were detected in SNU-3705 and ZNF207-RHOT1 was found in SNU-3730.

Table 8. Reported fusion genes of 11 established breast cancer cell lines

| Cell line | H−gene | T−gene | FusionGID (FusionGDB) | Fusion point for H−gene | Fusion point for T−gene | Related disease |
|---|---|---|---|---|---|---|
| SNU−3223 | CSNK1D | SECTM1 | 8637 | 17:80231043:− | 17:80280806:− | Bipolar disorder, Schizophrenia, Major depressive disorder |
| | ERBB2 | IKZF3 | 11907 | 17:37869522:+ | 17:37934020:− | Mammary/Ovarian/Stomach neoplasms, Rheumatoid arthritis |
| | ERBB2 | PPP1R1B | 11913 | 17:37863394:+ | 17:37785423:+ | Mammary/Ovarian/Stomach neoplasms, Neoplasm metastasis, Schizophrenia |
| | FAM168A | RAB6A | 12631 | 11:73308968:− | 11:73390765:− | Stomach neoplasms |
| | HSP90B1 | APP | 16845 | 12:104341160:+ | 21:27394330:− | Alzheimer's disease, Memory disorders, Nerve degeneration, Bipolar disorder |
| | ITGB4 | SAP30BP | 17876 | 17:73747192:+ | 17:73686751:+ | Epidermolysis bullosa with pyloric atresia, Adenoid cystic carcinoma |
| | ITGB4 | SAP30BP | | 17:73747192:+ | 17:73689520:+ | |
| | MED1 | CDK12 | 21502 | 17:37599803:− | 17:37671984:+ | Endometriosis, Liver carcinoma |
| | NPLOC4 | PDE6G | 24733 | 17:79589192:− | 17:79618715:− | not reported |
| | SCARB1 | UBC | 32788 | 12:125348141:− | 12:125398320:− | Renal cell carcinoma, Mammary neoplasms |
| | TYMS | GNAS | 40245 | 18:662320:+ | 20:57470667:+ | Pseudohypoparathyroidism, Colorectal/Stomach/Mammary neoplasms |
| | WSB1 | MED1 | 42083 | 17:25624334:+ | 17:37588269:− | Endometriosis, Liver carcinoma |
| SNU−3224 | AHNAK | NXF1 | 1230 | 11:62314132:− | 11:62571450:− | Renal cell carcinoma, Liver Cirrhosis, Chloracne |
| | CSNK1D | SECTM1 | 8637 | 17:80231043:− | 17:80280806:− | Bipolar disorder, Schizophrenia, Major depressive disorder |
| | ERBB2 | IKZF3 | 11907 | 17:37869522:+ | 17:37934020:− | Mammary/Ovarian/Stomach neoplasms, Rheumatoid arthritis |
| | ERBB2 | WSB1 | 11925 | 17:37850012:+ | 17:25640067:+ | Mammary/Ovarian/Stomach neoplasms, Neoplasm metastasis |
| | GALNT16 | RAD51B | 14307 | 14:69727184:+ | 14:68758601:+ | Substance−related disorders, Breast neoplasms |
| | TNRC18 | RNF216 | 38962 | 7:5352134:− | 7:5781275:− | Cerebellar ataxia and hypogonadotropic hypogonadism |
| | TNRC18 | RNF216 | | 7:5352134:− | 7:5781446:− | |
| SNU−3230 | CSNK1D | SECTM1 | 8637 | 17:80231042:− | 17:80280805:− | Bipolar disorder, Schizophrenia, Major depressive disorder |
| | ERBB2 | IKZF3 | 11907 | 17:37869522:+ | 17:37934020:− | Mammary/Ovarian/Stomach neoplasms, Rheumatoid arthritis |
| | FAM168A | RAB6A | 12631 | 11:73308968:− | 11:73390765:− | Stomach neoplasms |
| | FAM168A | RAB6A | | 11:73308968:− | 11:73390972:− | |
| | ITGB4 | SAP30BP | 17876 | 17:73747192:+ | 17:73686751:+ | Epidermolysis bullosa with pyloric atresia, Adenoid cystic carcinoma |
| | ITGB4 | SAP30BP | | 17:73747192:+ | 17:73689520:+ | |

* H−gene : Head−gene (5end−fusioned gene)  ** T−gene : Tail−gene (3end−fusioned gene)

*Continued*

| Cell line | H−gene | T−gene | FusionGID (FusionGDB) | Fusion point for H−gene | Fusion point for T−gene | Related disease |
|---|---|---|---|---|---|---|
| SNU−3380 | FARP1 | IPO5 | 12939 | 13:98795746:+ | 13:98673246:+ | Substance−related disorders, Schizophrenia |
| | GRB2 | VMP1 | 15370 | 17:73315681:− | 17:57866512:+ | Schizophrenia |
| | PTK2 | TRAPPC9 | 29357 | 8:142011224:− | 8:141321473:− | Squamous cell carcinoma, Neoplasm invasiveness, Rheumatoid arthritis, Mammary neoplasms |
| SNU−3393 | FARP1 | IPO5 | 12939 | 13:98795746:+ | 13:98673246:+ | Substance−related disorders, Schizophrenia |
| | PTK2 | TRAPPC9 | 29357 | 8:142011224:− | 8:141321473:− | Squamous cell carcinoma, Neoplasm invasiveness, Rheumatoid arthritis, Mammary neoplasms |

*Continued*

| Cell line | H−gene | T−gene | FusionGID (FusionGDB) | Fusion point for H−gene | Fusion point for T−gene | Related disease |
|---|---|---|---|---|---|---|
| SNU−3698A | NEBL | DNAJC1 | 23930 | 10:21461312:− | 10:22055238:− | Melanoma |
| | SDC1 | PUM2 | 32924 | 2:20401879:− | 2:20461310:− | Malignant mesothelioma |
| | SRCIN1 | PSMB3 | 35922 | 17:36734743:− | 17:36918664:+ | not reported |
| SNU−3698B | NEBL | DNAJC1 | 23930 | 10:21461312:− | 10:22055238:− | Melanoma |
| | SDC1 | PUM2 | 32924 | 2:20401879:− | 2:20461310:− | Malignant mesothelioma |
| SNU−3698C | FGFR3 | TACC3 | 13420 | 4:1808661:+ | 4:1739325:+ | Seborrheic keratosis, Achondroplasia, Hypochondroplasia, Carcinoma |
| | NEBL | DNAJC1 | 23930 | 10:21461312:− | 10:22055238:− | Melanoma |
| | SDC1 | PUM2 | 32924 | 2:20401879:− | 2:20461310:− | Malignant mesothelioma |
| SNU−3705 | ERBB2 | WSB1 | 11925 | 17:37850012:+ | 17:25640067:+ | Mammary/Ovarian/Stomach neoplasms, Neoplasm metastasis |
| | | | | 17:37870256:+ | 17:25640013:+ | |
| | FGFR3 | TACC3 | 13420 | 4:1808661:+ | 4:1739325:+ | Seborrheic keratosis, Achondroplasia, Hypochondroplasia, Carcinoma |
| | NEBL | DNAJC1 | 23930 | 10:21461312:− | 10:22055238:− | Melanoma |
| | SRCIN1 | PSMB3 | 35922 | 17:36734743:− | 17:36918664:+ | not reported |
| | | | | 17:36734743:− | 17:36912136:+ | |
| | | | | 17:36734743:− | 17:36916684:+ | |
| SNU−3716 | NEBL | DNAJC1 | 23930 | 10:21461312:− | 10:22055238:− | Melanoma |
| | SRCIN1 | PSMB3 | 35922 | 17:36734743:− | 17:36918664:+ | not reported |
| | | | | 17:36734743:− | 17:36912136:+ | |
| | | | | 17:36734743:− | 17:36916684:+ | |
| SNU−3730 | NEBL | DNAJC1 | 23930 | 10:21461312:− | 10:22055238:− | Melanoma |
| | SRCIN1 | PSMB3 | 35922 | 17:36734743:− | 17:36912136:+ | not reported |
| | ZNF207 | RHOT1 | 43075 | 17:30678931:+ | 17:30551635:+ | not reported |

## 4. Discussion

The first breast cancer cell line was BT-20, established in 1958 [10]. Despite this early achievement, there are relatively few breast cancer cell lines established until recently because of the great difficulty in culturing a homogeneous population without not inconsiderable stromal contamination [8]. Indeed, relatively few breast cancer cell lines have been established and are being offered to researchers by several cell line banks or institutions. The incidence of breast and lung cancer is almost the same [1]. Considering this, it is in contrast to the lung cancer cell line that is used more than hundreds or more. Established cell lines and organoids that have undergone various characterizations such as NGS, RNA sequencing, and anticancer drug test in this experiment will be deposited with Korean Cell Line Bank (KCLB, Seoul, Korea). Therefore, we expect that our breast cancer research models will be distributed to the scientific community and contribute in various ways.

Intra-tumor heterogeneity refers to the coexistence of subpopulations of cancer cells that differ in genetic, phenotypic or other characteristics within a derived primary tumor, and between a given primary tumor and its metastasis. This variety

can be attributed to genetic or epigenetic factors. Non-hereditary effectiveness, such as adaptive responses and fluctuation in signal pathways, also have a significant impact [11, 12]. Therefore, intra-tumor heterogeneity is a tremendous challenge in the treatment and characterization of biomarkers [13]. Cell lines are used widely to identify predictors of response and resistance to drug treatment experiment because they are can growth unlimitedly. Due to these characteristics, it is relatively easy to handle after establishment and show amenability to high-throughput screening, and formation of xenograft models for in vivo testing [14, 15]. However, the established cell lines under in vitro culture conditions will be selected from specific tumor subsets. This makes cancer cell lines that do not show tumor heterogeneity more and more [16]. Patient-derived xenografts (PDXs) are an important preclinical cancer model that can overcome the limitations of cell lines. These PDXs grown in immunodeficient mice is known to preserve major molecular and biological variations in the patient [17, 18]. During the decades, numerous efforts have been made to establish a stable transplantable breast cancer xenograft directly from patients into immunocompromised mice, but unlike

a fairly high (~40–90%) initial intake rate (i.e., initial outgrowths directly from patients), a stable establishment (defined as PDXs, which is not official but can generally be maintained in passage 3 or higher in mice) has a relatively low success rate (in the 10% range overall) [19–31]. In recent years, through newly developed immunodeficient host mouse models and changes in transplantation conditions, the overall intake rate is stabilized, showing a success rate of 20% [32]. However, as the tumors are grown in vivo in mice, the inevitable inclusion of the mouse genome remains a concern in assays using the PDX models. Several filtering systems and analytical tools are being developed to explain this, but there is still no complete solution [33]. One of the other experimental models, organoids, is known to better recapitulate the heterogeneity and morphological features of origin patient's tumor by culturing in a three-dimensional structure [34]. It has the advantages of both in vitro and in vivo experiments, but the difficulty is high and has not been fully verified. Since each research platform has various characteristics and advantages and disadvantages, the more experimental models with various types of characteristics are

established, the more researchers can overcome heterogeneity and become the basis for developing effective treatments.

Our cell lines were established with 20 pleural effusion-derived, 1 ascites-derived from breast cancer patients. Thirteen of them are cell lines established by extracting cells from four different patients on the same day but treated separately or collecting on different days. Being able to extract cancer cells from the pleural effusion and ascites indicates that the primary tissue is no longer in normal condition. And since it is not derived directly from the primary tissue, but based on the cancer cells that have burst, it may differ from the patient's primary tumor characteristics. However, because malignant pleural effusions (MPE) is a random population of cells in a tumor, it offers a unique opportunity to shed various cancer cells from a single individual to describe and characterize the extent of heterogeneity in the tumor [35]. In the case of ascites, it is a major patient sample for establishing experimental models such as cell lines and organoids in a similar context and has been used for a long time [36, 37]. The three pairs of cell lines and organoids were established by processing PDX tissue from three individual patients. PDX has the advantage of preserving the

heterogeneity of the derived tumor better than the cell line, but has the disadvantage that the experiment is complex and difficult to maintain and passaging. However, if the establishment is successful, the tumor that mimics the characteristics of the patient' s tumor can be rapidly and excellently grown [9]. Therefore, if organoids are derived from PDX tumor tissues, it is easier to handle and can be high-throughput screening, and can create an experimental model in which the heterogeneity of primary carcinoma is preserved compared to cell lines.

Except for the three derived from PDX tissues, the rest of the cell lines did not originate from the primary tumor tissue but retained the characteristics of floating nature in only a few cell lines, although they were obtained from the malignant pleural fluid. This morphological feature may explain the mechanism of how cells invade the pleural cavity. SNU-3196, which has the longest doubling time, was not the slowest to observe directly during cultivation. However, since this cell line was grown as a colony and piled high in a tensely clumping pattern and spread slowly, the results may have been significantly different if other measurement methods were used. Cell lines of the same origin did not show a completely consistent growth pattern, but they

were almost similar when compared to other cell lines. Perhaps because the time interval between taking patient samples was short or there were no big events in between, and the detailed characteristics may be different due to the heterogeneity in the tumor, but the population composition is basically the same. Organoids have quite a variety of forms, such as densely packed cells, hollow type, and ball shape, which are thought to be indicators of heterogeneity.

Numerous studies have been conducted to uncover the correlation between the survival rate and the prognosis of breast cancer patients with germline BRCA1 or BRCA2 mutation. BRCA1/2 are crucial proteins in the process of homologous recombination repair of dsDNA breaks [38]. The biological background known for BRCA1/2 and different pathological characters of BRCA1-associated tumors suggest the hypothesis that patients with a BRCA1 and/or BRCA2 mutation have a worse prognosis for breast cancer than non-carrier patients. Germline mutations in BRCA1 and/or BRCA2 confer lifetime risks of breast cancer of up to 80%. Of course, research results were inconsistent, possibly due to the unrelated conditions such as the size of the study, the difference between study populations, and

differences in the method of conducting study. However, for more optimized treatment, independent of other breast tumor characteristics, more study into the BRCA1/2 carrier breast cancer situation is needed. Indeed, in patients with the BRCA1 and/or BRCA2 mutation, breast cancer specific and overall cancer survival rates tend to be relatively low. In addition, the BRCA1/2 mutation was found to be a little more common in patients with family history. About 25–30% of familial breast cancers were found, and about 3% of all breast cancers [39–41].

Among the overall WES results, 92 genes known for many mutations in breast cancer were analyzed. However, only 52 genes among our established cell lines and organoids had a significant impact on cancer characteristics in the above database (Clinvar). Although most of the genes were excluded for this reason, BRCA1 and/or BRCA2, the important genetic variation indicators in breast cancer, were considered when conducting characterization, even mutations known to have non-pathogenic effects.

In the confocal results, SNU-4856-TO and SNU-5126-TO barely showed any signals because EHER2 expression was scant,

but SNU-4842-TO observed accurate and strong signal. As shown in the western blot results, the cell line and organoid of set 1 (SNU-4842, SNU-4842-TO) seemed HER2 overexpression. In addition, this set was found to have a HER2 kinase domain mutation, V777L, it was confirmed that the origin patient of the derived PDX tissue had that aberration. HER2 V777L mutation can contribute to resistance to Trastuzumab and is also used as a predictive marker [3]. The status of HER2 is often used as a key parameter in diagnosing the condition of breast cancer, determining prognosis, and planning treatment [7].

Trastuzumab, widely known as a HER2 targeted drug, was also used in screening tests for anticancer drugs, but it was found that all cell lines and organoids had strong resistance because the max concentration of Trastuzumab could not be increased under experimental conditions. Also, due to the experimental design, the drug treatment time was not optimally set. Therefore, the results were not considered in the overall analysis. Instead, attention was paid to the results of dual EGFR/HER2 inhibitors afatinib and lapatinib. Also, the results of EGFR targeted drugs gefitinib and erlotinib, AKT 1/2 kinase inhibitor, everolimus targeting mTOR, and doxorubicin, were noted. This was because

drugs targeting the PI3K-AKT-mTOR pathway in HER2+(positive) breast cancers have already been shown to be effective [42]. In the HER2+ cell lines and organoids, more than half were partially responsive to these drugs. In the case of SNU-3351, it is explained that it has a PI3KCA mutation, so it has resistance to drugs acting downstream of the corresponding signaling pathway and has noticeably result different from other cell lines. HER2 V777L mutation might be a target for pan-HER inhibitor. It was also seen through our results that the hypothesis might actually work well. Since it is a greatly rare mutation, it is highly meaningful that two experimental models have been established. Patient 3 cell lines (SNU-3698A, SNU-3698B, SNU-3698C, SNU-3705, SNU-3716, SNU-3730) and SNU-2532A also had upstream gene PI3KCA mutation like SNU-3351, so it was inferred that they were mostly unresponsive to the above drugs, and partial reactivity was due to slightly different features as can be observed from the blot results. In particular, the patient originating from the patient 3 set (SNU-3698A, SNU-3698B, SNU-3698C, SNU-3705, SNU-3716) has that mutations, but since 4 out of 6 established cell lines showed some reactivity, they may have had some anti-cancer effect.

HR+HER2−breast cancer is also known to be of great importance for the PI3K/AKT/mTOR and CDK4/6/RB pathway. . ER+ breast cancer tends to be resistant to several endocrine treatments, such as selective estrogen receptor modulators (SERMs) including tamoxifen and the selective ER downregulator (SERD) fulvestrant [43] because these cell line were established from patients who failed standard endocrine treatments including tamoxifen, aromatase inhibitor and fulvestrant. In particular, if ER+HER2− breast cancer is resistant to these endocrine therapies, PI3K pathway and CDK signaling pathway target drugs like afatinib, erlotinib, gefitinib and abemaciclib are known to be useful in addition to endocrine therapy. Although there are some differences in degree, established ER+HER2− cell lines and organoid of us were relatively responsive to these drugs.

Except for SNU−4856−TO, ER−HER2− cell lines were shown to be largely resistant to all screened drugs. Since the positive or negative of PR is not accurately identified, it cannot be concluded that the cell line and organoid are triple negative. However, if they are TNBCs (Triple Negative Breast Cancer, HR−HER2−), the responsiveness to many commonly used

breast anticancer drugs will be explained [44]. Therefore, additional testing and verification experiments are needed.

Although the three organoids had almost the same genetic profiles as cell line derived from the same patient or had additional variations, the overall drug reactivity was much better on average. This might be because organoids and cell lines differ from tissue primary treat methods, and drug testing procedures are the same in principle, but they are overall different. Organoids are structures derived from PSCs or ASCs and grown in vitro in 3D [45]. That is, if the niche is properly matched, cells that maintain stemness can survive. On the other hand, in order to be established as a cell line, anoikis resistance is required to have aggressive and significantly rapid growth characteristics and metastatic spread patterns [46]. Even in these establishment features, even if the mutation profile is the same, the cell line is generally more resistant to the drug than the organoid. As can be seen from the previous results, whether there is a mutation resistant to the drug acts as a higher determinant than whether there is a target for the treated drugs. In addition, since the mutation status found through WES is an all or none result without considering variant allele frequency (VAF), the drug

results that the variation affects may be different. VAF is the percentage of total sequence reads for particular variations. If the difference the corresponding parts is considered through further study, the distinction in drug reactivity results between the cell line and the organoid may be relatively less significant. Indeed, preliminary data obtained from the established organoids were confirmed to reflect the major histological, molecular and genetic characteristics of the primary tumor origin [47-49]. Therefore, if compared with the results of actual patients derived, the results of organoids are more likely to resemble clinical data.

RNA sequencing was performed by extracting RNA from patient 1 (SNU-3223, SNU-3224, SNU-3230), 2 (SNU-3380, SNU-3393) and 3 (SNU-3698A, SNU-3698B, SNU-3698C, SNU-3705, SNU-3716, SNU-3730) set cell lines, and based on the raw data results, fusion genes possessed by each cell line were found. Gene fusion created by chromosomal rearrangement after DNA double-strand breakage is one of the hall marks of the cancer genome [50]. In the early stages of tumor formations, these phenomenon has an critical impact [51]. Numerous fusion genes (FGs) in a variety of cancers have been used as indicators of tumor characterization or treatment planning. As in the case

of WES analysis, the mutation profile was derived based on the database, and when constructing the fusion gene (FG) profile, the analysis was conducted by selecting the FG reported in the database called FusionGDB. Comparing all the results, it was found that different cell lines derived from the same patient sample had various degrees, but all had more than one different FG. Of course, like the other observation and/or analysis results, the differences within the set were relatively more consistent than when comparing different patient sets cell lines. In particular, there were quite a few FG profile differences in the patient 3 cell lines (SNU-3698A, SNU-3698B, SNU-3698C, SNU-3705, SNU-3716, SNU-3730) with the largest number of cell lines established at six. In addition, SNU-3698A, SNU-3698B and SNU-3698C were found to have variability despite being the pleural effusion of the same patient taken on the same day. On the other hand, only one FG difference was found in the patient 2 cell lines (SNU-3380, SNU-3393). However, these small differences can also be significant. Combining these results, it can be concluded that the pleural effusion sample is a good enough patient sample to represent tumor heterogeneity, so that the more cell lines or other research models established, the

richer the data for tumor heterogeneity will accumulate. It has also been demonstrated that our cell lines can help to provide a variety of approaches to future studies.

As a result, cell lines and organoids with various characteristics were established, and experimental results using them were consistent with previously published breast cancer research data. This show the possibility that our newly added cell lines and organoids will be useful tools for future breast cancer research. In addition, not only increasing the number of experimental models of various characteristic factors, but also securing diverse types such as cell lines, organoids and PDX may be a method of making research data more accurate and meaningful.

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## 국문 초록

유방암은 여성에게서 가장 흔하게 발생하는 암 질환이며, 암으로 인한 사망률이 높다. 유방암은 호르몬 수용체와 성장인자 수용체에 따라 3개의 대표적인 아형으로 구분이 되며, 이에 따라 치료를 하며, 표적 치료에 대한 내성이 생기며, 종양내 이종성이 다양하고 연관된 인자가 많기 때문에 환자 개개인에게 적절한 치료법을 찾는 것이 중요하다. 본 연구에서는 20 개의 악성 흉수 유래 유방암 세포주와 1 개의 복막 전이와 동반된 복수 유래 유방암 세포주를 새롭게 수립하였다. 또한 유방암 환자 유래 이종 이식 모델의 종양 조직에서 3쌍의 세포주-오가노이드를 확립하였다. 총 24개의 새로운 세포주와 3개의 오가노이드의 세포 및 분자생물학적 특성을 분석하였다. *TP53, PIK3CA, PTEN, HER2, RB1*, *MAP3K, BRCA* 등 유방암에서 변이가 많이 발견되는 돌연변이 유전자들에 대한 스크리닝을 통해 유전적 특징을 밝혔다. 한편, 유방암의 치료에서 주요하게 여겨지는 판단 척도인 ER과 HER2의 상태를 확인하고, 전체 세포주와 오가노이드를 그룹화하여 나누었다. 또한 유방암 연구와 임상에서 널리 쓰이는 다양한 약물들에 대한 감수성을 측정하였다. 이미 밝혀진 약물의 표적의 발현 여부나, 반응성에 영향을 미치는 것으로 알려진 표적 돌연변이의 유무 상태와 일치하는 결과를 보였다. 같은 환자에게서 유래되었더라도 각 세포주들은 일부 차이 나는 결과를 보였고, 2쌍의 세포주-오가노이드는 각각 서로 유사한 돌연변이 패

턴을 가짐에도 불구하고 눈에 띄는 반응성 정도의 차이를 보여주었다. 데이터 베이스를 토대로 한 유전자 스크리닝 결과는 유방암의 종양 이종성에 관련된 실험 모델을 제작하는 것을 추가적으로 고려할 가능성을 보여준다. 또한 유래 환자 간의 차이 혹은 같은 환자 유래 세포주들 간의 유사성과 다양성은 실제 환자 치료의 토대가 될 수 있다. 이러한 결과들은, 유방암 연구 모델의 다채로운 형태와 종류의 누적이 실험과 임상 정보 데이터베이스의 정확도와 적합성을 높이는 데에 기여를 할 가능성이 있음을 시사해준다.

**주요어 : 흉수 유래 유방암 세포주, 복수 유래 유방암 세포주, 유방암 오가노이드, 환자 유래 이종이식모델(PDX), 특성 분석, 약제 감수성, 융합 유전자**

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