Human Cell (2022) 35:756–766
https://doi.org/10.1007/s13577-022-00671-y

CELL LINE

Establishment and characterization of NCC-UPS4-C1: a novel cell line of undifferentiated pleomorphic sarcoma from a patient with Li–Fraumeni syndrome

Takuya Ono[1,2] · Yuki Yoshimatsu[1] · Rei Noguchi[1] · Yooksil Sin[1] · Ryuto Tsuchiya[1] · Taro Akiyama[1] · Jun Sugaya[3] · Suguru Fukushima[3] · Naoki Kojima[4] · Akihiko Yoshida[4] · Akira Kawai[3] · Tadashi Kondo[1]

Received: 30 October 2021 / Accepted: 5 January 2022 / Published online: 3 February 2022

Abstract

Li–Fraumeni syndrome (LFS) is a cancer predisposition syndrome caused by a germline mutation of the *TP53*. The lifetime risk of cancer in individuals with LFS is ≥ 70% for men and ≥ 90% for women. Undifferentiated pleomorphic sarcoma (UPS) is one of the core cancers associated with LFS. UPS is a subtype of undifferentiated soft tissue sarcoma that shows no identifiable line of differentiation. The standard curative treatment for UPS is complete surgical resection. However, local recurrence and distant metastasis to the lung can usually be found after resection of the UPS. Therefore, a novel treatment strategy for patients with UPS is required. Although well characterized, patient-derived tumor cell lines facilitate the high-throughput screening of a large number of drugs, and no sarcoma cell lines derived from a patient with LFS have been registered in public cell banks. Thus, this study aimed to establish a novel, well-characterized UPS cell line from a patient with LFS. From surgically resected UPS tumor tissues, we established the first UPS cell line from a patient with LFS and named it NCC-UPS4-C1. NCC-UPS4-C1 harbored copy number alterations and had the *TP53* tumor suppressor gene mutation. The cells exhibited constant cell growth and invasive ability. This well-characterized NCC-UPS4-C1 cell line was then utilized for high-throughput screening of 214 anti-cancer drugs, and two effective drugs were identified. One of the two drugs, romidepsin, was commonly effective for the NCC-UPS1-C1, NCC-UPS2-C1, and NCC-UPS3-C1 cell lines that we previously reported; a potential drug for the treatment of UPS was suggested using well-characterized UPS cell lines. These data indicate that NCC-UPS4-C1, which is the first sarcoma cell line established from a patient with LFS, enables researchers to conduct vigorous preclinical research on UPS.

Keywords Soft tissue sarcoma · Li–Fraumeni syndrome · Undifferentiated pleomorphic sarcoma · Patient-derived cell line · Anti-cancer drug screening

Introduction

Li–Fraumeni syndrome (LFS) is a cancer predisposition syndrome caused by a germline mutation of the *TP53* tumor suppressor gene on chromosome 17p13.1 [1]. LFS associates with an early onset of a broad spectrum of malignancies and a high lifetime cancer risk [2]. The risk of cancer imparted by *TP53* mutations is evident at an early age, with women in LFS families who carry such mutations having a cumulative 49% risk of developing cancer by the age of 30 years, whereas men with *TP53* mutations have a 21% cancer risk at the same age [3, 4]. For the patients with LFS, the lifetime risk of cancer in individuals is ≥ 70% for men and ≥ 90% for women [5]. Thus, cancer risk management is required for the patient with LFS through a whole-body magnetic resonance

✉ Tadashi Kondo
takondo@ncc.go.jp; proteomebioinformatics@gmail.com

1 Division of Rare Cancer Research, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

2 Graduate School of Biomedical Sciences, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan

3 Division of Musculoskeletal Oncology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

4 Department of Diagnosis Pathology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

imaging (MRI) or positron emission tomography–computed tomography (PET–CT) [4]. Notably, soft tissue sarcoma accounts for the majority of LFS-associated tumors along with adrenocortical carcinomas, breast cancer, central nervous system tumors, and bone sarcomas [6, 7].

Undifferentiated pleomorphic sarcoma (UPS) is one of the most common soft tissue sarcomas. According to the 5th edition of WHO classification of soft tissue tumors, UPS is defined as a subtype of undifferentiated soft tissue sarcoma which shows no identifiable line of differentiation [8, 9]. The UPS harbors vast numbers of copy number alterations (CNAs) and does not contain a defining fusion product or gene mutation [8, 10]. In patients with UPS, surgical resection remains the principal treatment [11]. After the resection of UPS, local recurrence occurs in 13–42% of patients, and distant metastasis to the lung can be found in 31–35% of patients despite surgery [12–14]. According to previous reports, chemotherapy might be palliative, although it has shown some benefits. The 5- and 10-year overall survival rates for the patients with UPS were 60% and 48%, respectively [15]. Thus, there is a need to develop novel therapeutic strategies for UPS.

Preclinical models have been a useful tool in the development of therapies for various diseases, including malignancies [16–18]. Patient-derived tumor cell lines represent a mainstay of tumor biology and drug discovery through facile experimental manipulation, global and detailed mechanistic studies, and various high-throughput applications [19–22]. In particular, well-characterized cell lines are useful for evaluating the effects of anti-cancer drugs. In the Cellosaurus database [23], 54 UPS cell lines have been reported (Supplementary Table 1). However, UPS cell lines established from the patients with LFS have not bee registered [23] and, to our best knowledge, sarcoma cell lines from patients with LFS not established.

In this study, we aimed to establish a novel well-characterized UPS cell line from a patient with LFS. To accomplish this goal, surgically resected UPS tumor tissues were collected from a patient with LFS. The established cell line was characterized with respect to proliferation, spheroid formation, and invasion. Moreover, we examined the antiproliferative effects of these drugs on the cell line.

Materials and methods

Patient data

The patient was a 32-year-old woman with a history of osteosarcoma in the right humerus, endometrial polyps, and uterine fibroids. She perceived a strange sensation at the medial side of her right knee. MRI showed a soft tissue tumor, and she was referred to the National Cancer Center Hospital (Chuo-ku, Tokyo, Japan) for further inspection (Fig. 1a, b). The needle biopsy indicated UPS. Breast cancer, angiomyolipoma in the liver, and leiomyosarcoma in the right kidney were detected during the inspection of PET–CT data. The patient received chemotherapy consisting of etoposide and ifosfamide; however, the tumor was progressive. The patient underwent wide resection for the UPS. According to the results of genetic analysis, she was diagnosed with LFS. A part of the resected tumor at the operation was used to establish the cell line described in this study. A year has passed since the wide resection of UPS and the patient is still alive with no recurrence in the right knee.

Pathologically, the tumor consisted of pleomorphic spindle to polygonal cells without a clear line of differentiation in a fibrous background. Approximately, 30% of the tumor cells remained viable after chemotherapy, whereas the rest were necrotic or hyalinized (Fig. 1c). Immunoexpression of α-smooth muscle actin (α-SMA) was negative in needle biopsy (Fig. 1d) and p53 was diffuse positive in surgically resected UPS specimen (Fig. 1f). The ethical committee of the National Cancer Center approved the use of clinical materials for this study, and written informed consent was obtained from the donor patient.

Hematoxylin and eosin staining of tumor tissue

To perform a histological examination, 4-μm-thick sections from a representative paraffin-embedded tumor sample were prepared. After deparaffinization with xylene and ethanol, the tissue sections were stained with hematoxylin and eosin (HE). To observe the stained cell morphology, we used BZ-X710 (KEYENCE Corp., Osaka, Japan).

Immunohistochemical staining of tumor tissue

Immunohistochemistry was also performed using the deparaffinized tumor sample. Endogenous peroxidase activity was inhibited by exposing the sections to 3% hydrogen peroxide.

The heat-induced epitope retrieval was performed to reverse the loss of antigenicity that occurs with some epitopes in formalin-fixed, paraffin-embedded tissues. Primary antibodies against α-SMA (1A4, prediluted; Dako, Glostrup, Denmark) and p53 (DO-7, prediluted; Dako) were used for immunohistochemical analysis of the tissues. EnVision™ FLEX System (Dako) was used to detect immunoreactivity. We used hematoxylin, a nuclear stain, as a counterstain.

Primary cell isolation and culture

Primary tumor cells of the UPS were collected from surgically resected tumor tissues that were dissected into small pieces with scissors and digested with 1 mg/mL

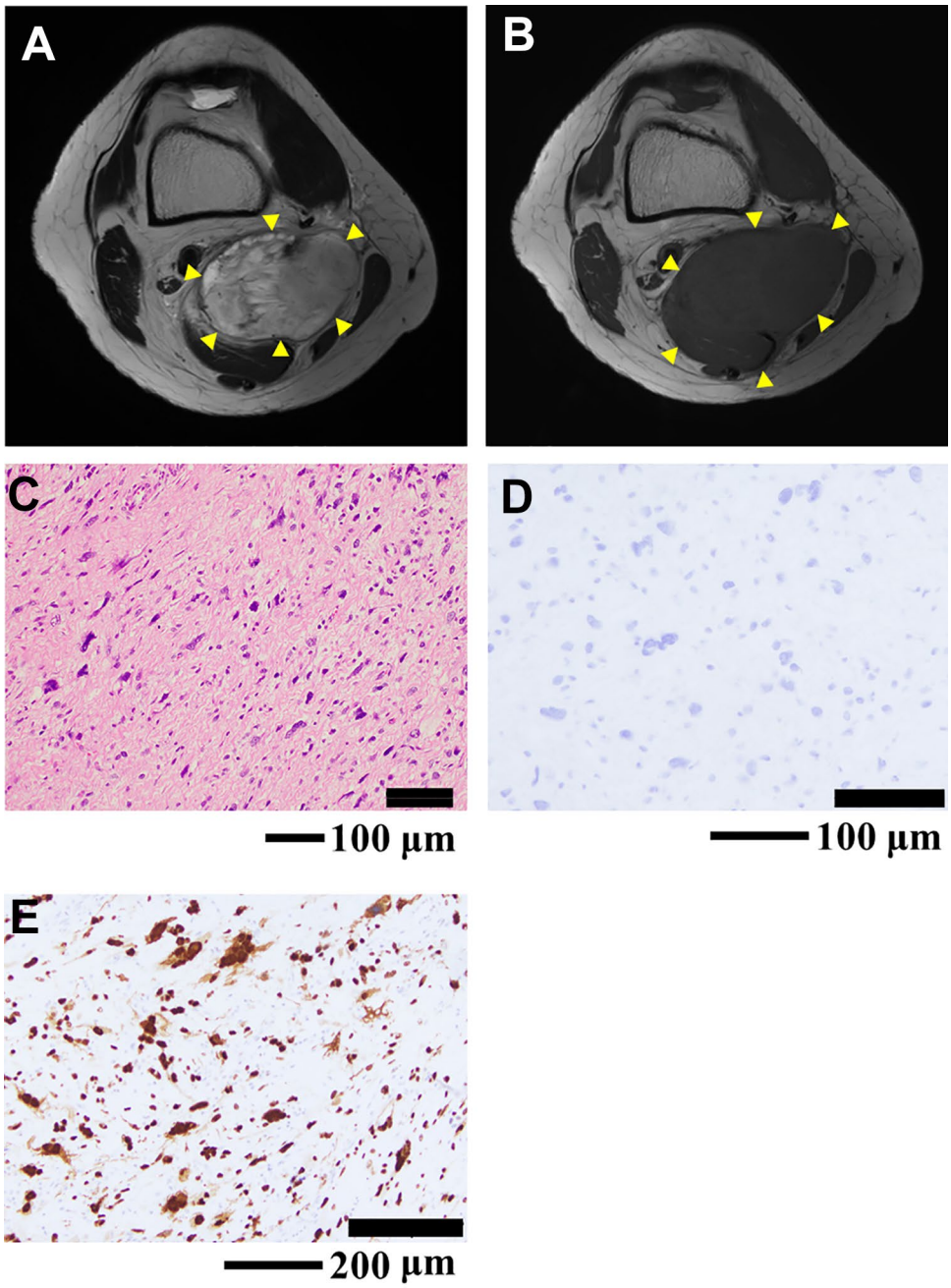

Fig. 1 Clinical and pathological data of a patient with NCC-UPS4-C1. Magnetic resonance imaging showing a mass. **A** T2-weighted image showing high intensity and heterogenicity. **B** T1-weighted image showing a homogeneously low intensity. **C** HE staining showing spindle-to-polygonal cell proliferation with focal necrosis. **D** Immunohistochemical staining showing diffuse pattern of p53 expression. **E** Immunohistochemical staining showing negative expression of α-smooth muscle actin

collagenase type II (Worthington Biochemical Corp., Lakewood, NJ, USA) for 30 min at 37 °C. The cells were seeded on a collagen type I-coated culture plate (Sumitomo Bakelite Co. Ltd., Tokyo, Japan). To maintain the cells, we used DMEM/F12 supplemented with GlutaMAX (Thermo Fisher Scientific Inc., Waltham, MA, USA), 5% heat-inactivated fetal bovine serum (FBS) (Thermo Fisher Scientific Inc.), 10 µM Y-27632 (ROCK inhibitor; Selleck Chemicals, Houston, TX, USA), 10 ng/mL bFGF (Sigma-Aldrich Co. LLC, St. Louis, MO, USA), 5 ng/mL EGF (Sigma-Aldrich Co. LLC), 5 µg/mL insulin (Sigma-Aldrich Co. LLC), 0.4 µg/mL hydrocortisone (Sigma-Aldrich Co. LLC), 100 µg/mL penicillin, and 100 µg/mL streptomycin (Nacalai Tesque Inc., Kyoto, Japan). The change of culture medium was conducted every 2–3 days. Microscopic observations (Carl Zeiss AG, Land Baden-Württemberg, Germany) were performed to observe the cell status, and when the cultured cells reached sub-confluency, they were washed with PBS (−) (Nacalai Tesque Inc.), which was followed by dissociation with Trypsin–EDTA solution (Nacalai Tesque Inc.); they were then transferred to another tissue culture plate. The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

Authentication and quality control

Authentication and quality control of established cell lines were conducted. In brief, the Qiagen DNeasy Blood and Tissue Kit (QIAGEN N.V., North Rhine-Westphalia, Germany) was used to extract DNA from the tumor tissues and establish a cell line, and the DNA concentration was measured with a Nano Drop 8000 (Thermo Fisher Scientific Inc.). Cell authentication was conducted with short tandem repeat (STR) analysis for ten loci using the GenePrint 10 system (Promega Co., Madison, WI, USA) and a 3500 × L Genetic Analyzer (Thermo Fisher Scientific Inc.). The STR profiles of the tumor tissues and the cells were analyzed using GeneMapper software (Thermo Fisher Scientific Inc.), and the results were compared to the established cell lines in public cell banks using the Cellosaurus 38.0 STR similarity search tool, CLASTR 1.4.4 [7, 23] with a standard match threshold of 80% [24]. To check the quality of the established cell line, a mycoplasma contamination test using the cell extracts, the DNA fragmentation of mycoplasma was examined using the e-Myco Mycoplasma PCR Detection Kit (iNtRON Biotechnology Inc., Gyeonggi-do, Korea). DNA fragments amplified with GeneAmp PCR System 9700 (Thermo Fisher Scientific Inc.) were separated and stained with SYBR Safe DNA gel stain (Invitrogen Corp., Waltham, MA, USA) using agarose gel electrophoresis. LAS-3000 Imager (FUJIFILM Corp., Tokyo, Japan) was used to detect the DNA bands.

Single nucleotide polymorphism array

Single nucleotide polymorphism (SNP) array genotyping was performed to identify CNAs using an Infinium OmniExpressExome-8 v. 1.4 BeadChip (Illumina Inc., San Diego, CA, USA). Using R studio ver. 1.4 (R studio, Boston, MA, USA), we analyzed SNP array data except for chromosomes X and Y, and abnormal copy number regions were detected using the R package 'DNAcopy' version 1.64.0 from Bioconductor (https://bioconductor.org/). Amplifications were defined as regions where the copy number was more than three and deletions were defined as regions where the copy number was less than one copy in the tumor cells. We annotated the genes with CNAs using the biomaRt package version 2.46.0 (Bioconductor) and "Cancer Gene Census" in the Catalogue Of Somatic Mutations In Cancer (COSMIC) database (GRCh 37 v91) [25].

Next-generation sequencing with NCC oncopanel test

NCC Oncopanel test, a hybridization capture-based next-generating sequencing assay [26], was conducted to detect the alterations including mutations, amplifications, and homozygous deletions in the entire coding region of 114 genes of clinical or preclinical relevance, along with rearrangements of 12 oncogenes (Supplementary Table 2). Genomic DNA extracted from the cell line was prepared for the sequencing libraries (ranging from 50 to 800 ng) using the SureSelect XT reagent (Agilent Technologies Inc., Santa Clara, CA, USA) and the KAPA Hyper Prep Kit (KAPA Biosystems, Wilmington, MA, USA). Then, genomic sequencing was performed on the Illumina MiSeq or NextSeq (Illumina Inc.) with 150-base pair paired-end reads.

Spheroid formation assay

The spheroid formation capability of the established cells was confirmed by seeding the cells in a 96-well Clear Round Bottom Ultra Low Attachment Microplate (Corning Inc., Corning, NY, USA). The density of the seeding cells was 1×10^5 cells/well. After 3 days of culture, spheroid formation was confirmed by microscopic observation (KEYENCE Co.), and the spheroids were transferred from the plate to a 1.5 ml Eppendorf tube (Eppendorf, Hamburg, Germany). The spheroids were covered with gel (iPGell; GenoStaff Co. Ltd., Tokyo, Japan) and fixed with 10% formalin neutral buffer solution. Before HE staining, the gel-covered spheroids were embedded in paraffin and sliced into 4-μm-thick paraffin sections. After HE staining, the sections underwent microscopic observation (KEYENCE Co.).

Tumor cell proliferation assay

To assess the potential for proliferation, the established cells were seeded in a 24-well culture plate (Corning Inc.). The seeding density of the cells was 2.5×10^4 cells/well. The number of cells was measured at four time points over 96 h using a CCK-8 reagent (Dojindo Molecular Technologies Inc., Kumamoto, Japan) and the doubling time was calculated based on the growth curve. All experiments were performed in triplicate.

Tumor cell invasion assay using real time cell analyzer

The invasive potential of the cells was examined using a real-time cell analyzer, xCELLigence (Agilent Technologies Inc.). The MG63 osteosarcoma cell line (Japanese Collection of Research Bioresources Cell Bank, Osaka, Japan) [27] was used as control. Subsequently, on the membrane in the upper chamber, Matrigel basement membrane matrix (9.3 mg/mL) (Corning Inc.) was layered. The seeding density of the cells on the Matrigel layer was 2.5×10^4 cells/well. DMEM/F12 supplemented with GlutaMAX, 5% FBS, 10 μM Y-27632, 10 ng/mL bFGF, 5 ng/mL EGF, 5 μg/mL insulin, 0.4 μg/mL hydrocortisone, 100 μg/mL penicillin, and 100 μg/mL streptomycin was added to the lower chamber. The upper

chamber was filled with DMEM/F12 without FBS. The cells in the upper chamber invaded the bottom chamber through a Matrigel-coated membrane and adhered to the electronic sensors on the underside of the membrane. The attached cells influenced the electrical impedance of the electronic sensors. Based on the positive correlation between impedance and the number of cells, we observed the cell invasion ability. Using a real-time cell analyzer, the impedance was monitored every 15 min for 120 h and plotted as a function of time after seeding.

Tumorigenicity assay in nude mice

To assess the potential for tumorigenesis in nude mice, 1×10^6 cells suspended in 50 µL of PBS (−) were mixed with an equal volume of Matrigel (21.2 mg/ml). The cell suspension was subcutaneously injected into BALB/c nude mice (CLEA Japan Inc., Tokyo, Japan) using a 5 ml syringe (Terumo Corp., Tokyo, Japan) and a 26 G needle (Terumo Corp.). The tumor size was measured weekly. The animal experiments were conducted in compliance with the guidelines of the Institute for Laboratory Animal Research, National Cancer Center Research Institute.

Screening for anti-cancer drugs

Drug screening was conducted using an established cell line with 214 anti-cancer drugs, as previously described [28]. Using a Bravo automated liquid handling platform (Agilent Technologies Inc.), the cells were seeded in a 384-well plate (Thermo Fisher Scientific Inc.) at a seeding density of 5×10^3 cells/well. The cells were maintained in DMEM/F12 supplemented with GlutaMAX, 5% heat-inactivated FBS, 10 µM Y-27632, 10 ng/mL bFGF, 5 ng/mL EGF, 5 µg/mL insulin, and 0.4 µg/mL hydrocortisone and incubated at 37 °C in a humidified atmosphere containing 5% CO_2. The day after cell seeding, using the Bravo automated liquid handling platform, a drug library that included 214 anti-cancer drugs (Selleck Chemicals) (Supplementary Table 3) was applied at a concentration of 10 µM to the cells and incubated for 72 h. After the incubation, cell viability was measured using the CCK-8 reagent (Dojindo Molecular Technologies Inc.) following the manufacturer's protocol. In terms of % relative growth inhibition, the response readout was calculated relative to the DMSO-treated control.

IC_{50} values, which are the concentrations that inhibit cell growth by 50% compared to the growth of control cells, were calculated from growth curves fabricated by plotting cell viability (%) versus drug concentration (µM). Using the Bravo automated liquid handling platform, cell suspensions (25 µL) were dispensed into 384-well plates. The seeding density of the cells was 5×10^3 cells/well. Subsequently, 19 drugs were selected according to the results of 214 anti-cancer drug screening. Additionally, we selected five drugs (eribulin mesylate, doxorubicin, gemcitabine, pazopanib HCl, and trabectedin) that were utilized for the treatment of sarcomas. A total of 24 drugs were added to the 384-well plates at a serial dilution of 0.1–100,000 nM. The cells were maintained for 72 h and cell viability was assessed using the CCK-8 assay. The readout was plotted against the concentrations of drugs and examined with GraphPad Prism 9.1.1 software (GraphPad Software Inc., San Diego, CA, USA). This anti-cancer drug screening test was conducted in duplicate.

Results

Establishment and authentication of NCC-UPS4-C1

We successfully established a cell line named NCC-UPS4-C1. The cell line was maintained for more than 25 passages. To authenticate this cell line, STR analysis was performed, which showed that all examined STRs were identical among NCC-UPS4-C1 and the original tumor tissues (Table 1, Supplementary Fig. 1). Moreover, the STR similarity search tool showed that the STR patterns of NCC-UPS4-C1 did not match the cell lines registered in the public cell banks. The mycoplasma test did not identify mycoplasma DNA fragments (data not shown).

Characterization of NCC-UPS4-C1

SNP analysis showed that the NCC-UPS4-C1 had CNAs. Partial allelic amplification was not detected, whereas losses were detected in chromosomes 1p, 8p, and 12q in NCC-UPS4-C1 and the original tissue (Fig. 2, Supplementary Table 4). No loss or gain of cancer-related genes registered in COSMIC database was identified in NCC-UPS4-C1 and the original tissue. Using NCC Oncopanel test, we found the pathogenic

Table 1 Results of short tandem repeat analysis of NCC-UPS4-C1 and original tumor tissue

Microsatellite (choromosome)	NCC-UPS4-C1	Tumor tissue
Amelogenin (X Y)	X	X
TH01 (3)	7, 8	7, 8
D21S11 (21)	29, 32.2	29, 32.2
D5S818 (5)	10, 11	10, 11
D13S317 (13)	9, 13	9, 13
D7S820 (7)	11	11
D16S539 (16)	12	12
CSF1PO (5)	11, 12	11, 12
vWA (12)	14, 19	14, 19
TPOX (2)	8, 9	8, 9

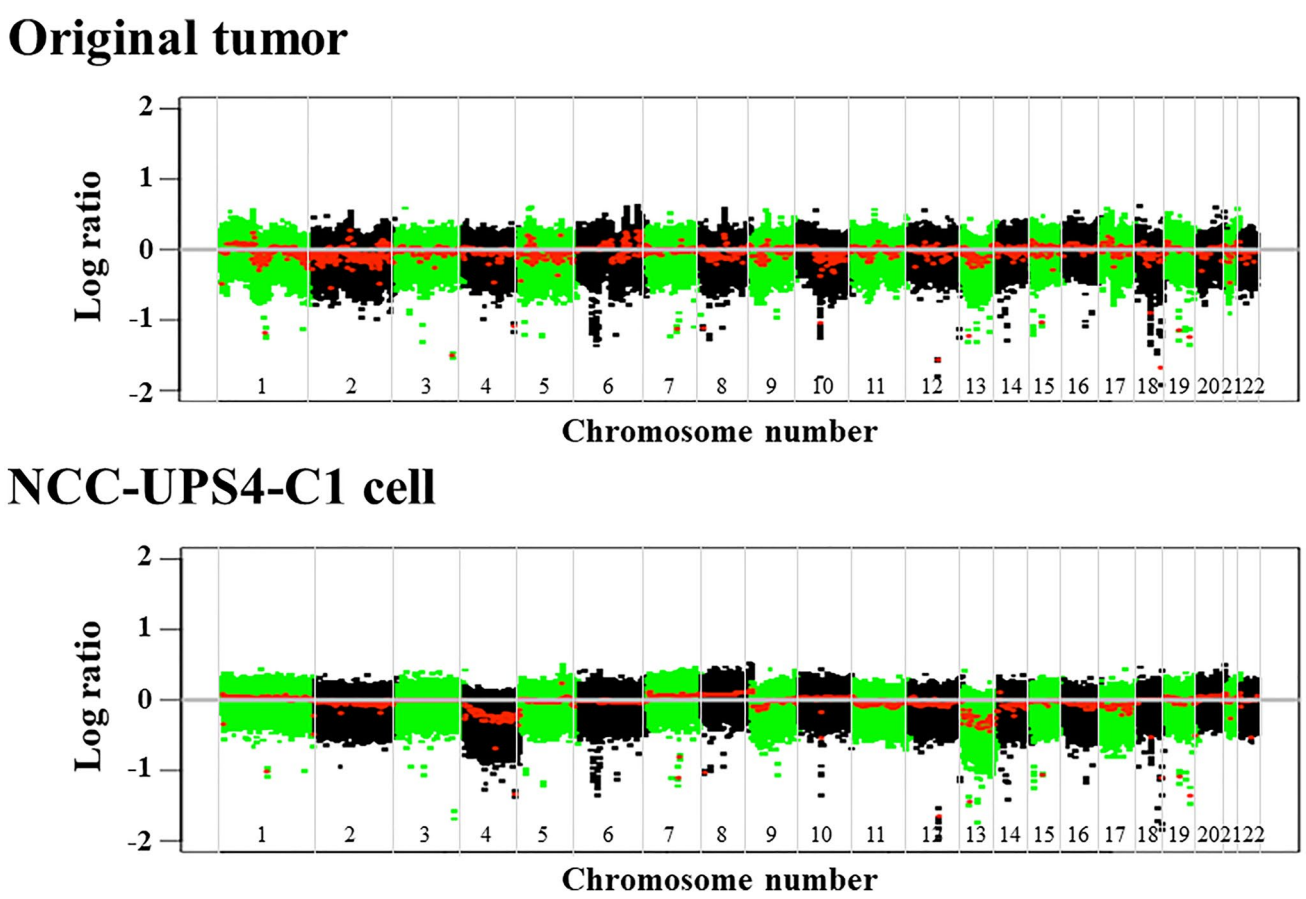

Fig. 2 Analysis of the single nucleotide polymorphism (SNP) array. Allele-specific copy number analysis revealed DNA copy number alterations in **A** original tumor tissue of NCC-UPS4-C1 and **B** NCC-UPS4-C1 (passage 19). The X- and Y-axes indicate the chromosome number and the log ratios of copy, respectively

mutation of *TP53* in NCC-UPS4-C1 (Table 2, Supplementary Table 5–7).

With respect to cell morphology, NCC-UPS4-C1 exhibited adherent characteristics with a spindle-like and polygonal appearance (Fig. 3a, b). The cells demonstrated the capability to form spheroids when they were seeded on low-attachment plates. HE-stained spheroid sections showed pleomorphic morphology of the cells (Fig. 3c, d). The population doubling time was 76 h based on the growth curve (Fig. 3e). The NCC-UPS4-C1 demonstrated more constant invasion than the MG63 osteosarcoma cell line which is used as control cells (Fig. 3f). After subcutaneous injection of NCC-UPS4-C1, tumorigenesis in nude mice was not observed in this study for a month (data not shown).

Anti-proliferation effect of anti-cancer drugs

The cell viability after treatment with the 214 anti-cancer drugs is summarized in Supplementary Table 8. We identified 19 anti-cancer drugs that showed notable inhibitory effects on the growth of NCC-UPS4-C1. The IC_{50} values of these 19 drugs and the 5 additional drugs which are standard for chemotherapies for soft tissue sarcomas are shown in Supplementary Table 9. The IC_{50} values for bortezomib and romidepsin in NCC-UPS4-C1 were less than 100 nM (Table 3). The growth inhibition curves of bortezomib and romidepsin are shown in Fig. 4.

Table 2 Mutations in the NCC-UPS4-C1 detected with NCC oncopanel

Gene	Mutation	Amino acid change	Type of mutation	Classification
TP53	c.G856A	p.E286K	Nonsynonymous SNV	Pathogenic
TP53	c.C637T	p.R213X	Stopgain SNV	Pathogenic

SNV single nucleotide variants

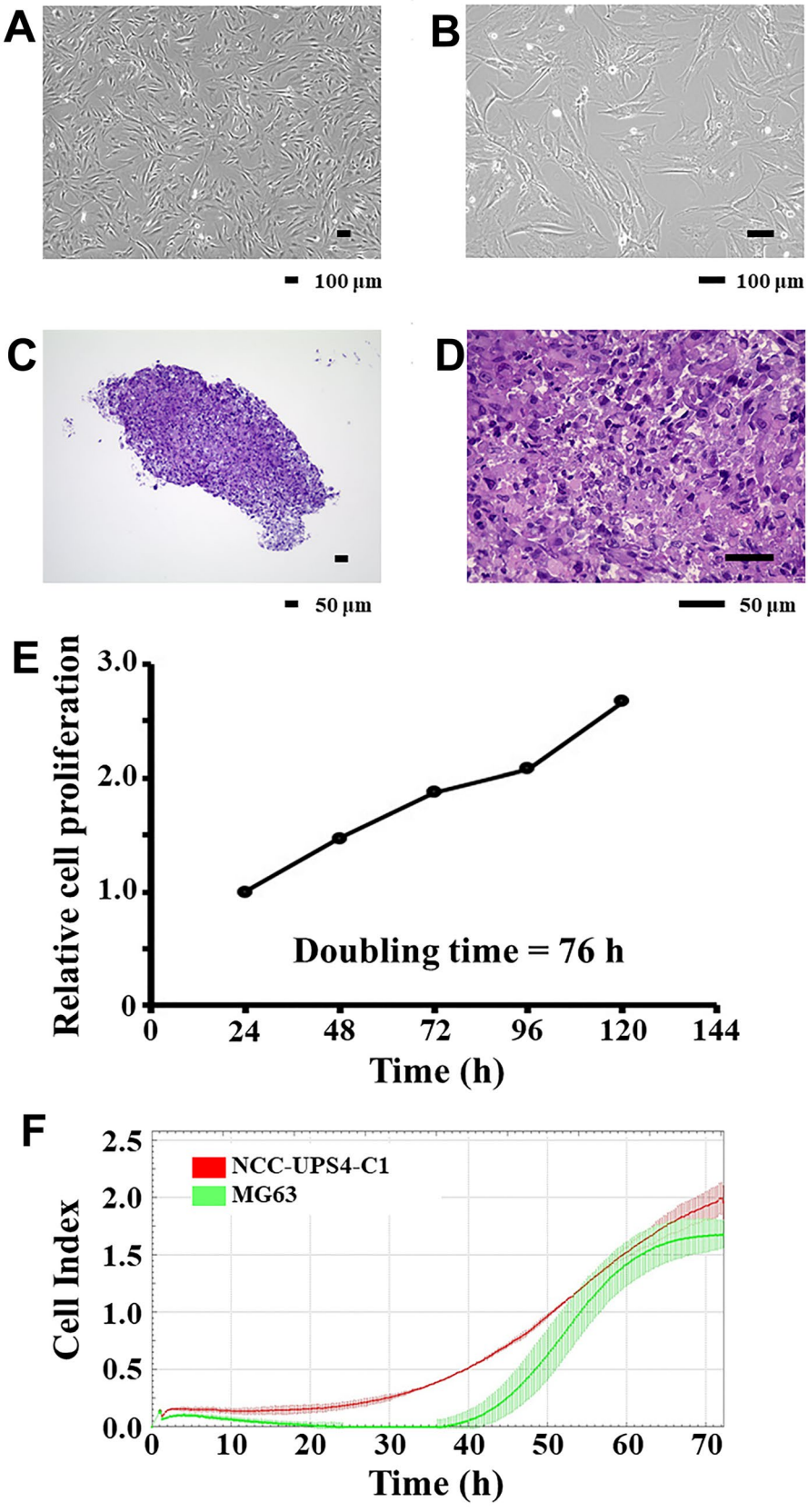

Fig. 3 Characterization of NCC-UPS4-C1. **A**, **B** NCC-UPS4-C1 (passage 20) had a spindle like and pleomorphic appearance under 2D culture conditions. **C**, **D** The HE-stained spheroids section of NCC-UPS4-C1 (passage 26) fabricated in 96-well low attachment round bottom plates. **E** Growth curve of NCC-UPS4-C1 (passage 23). Each point represents the mean ± standard deviation ($n = 3$). **F** Invasion capability of the NCC-UPS4-C1 (passage 28) was observed using Real Time Cell Analyzer. MG63 osteosarcoma cell line was used as control cells

Table 3 List of drugs with the lowest IC_{50} values (< 100 nM)

Name of drugs	IC_{50} (nM)
Bortezomib (PS-341)	11.08
Romidepsin (FK228, depsipeptide)	78.73

Discussion

We established and characterized a novel cell line, NCC-UPS4-C1, from a patient with LFS. Despite adequate loco-regional treatment, up to 40% of patients with UPS develop postoperative metastatic disease, with the shortest progression-free survival in the palliative stage among all histological sarcoma subtypes [29]. Therefore, novel treatments for patients with UPS are required. The effectiveness of conventional chemotherapy has not been proven in UPS because of the difficulty of large-scale randomized clinical trials. In recent years, multiple large-scale drug screening analyses have been conducted for major tumors using several hundreds of cell lines [22, 30, 31]. We believe that NCC-UPS4-C1 will be useful for in vitro preclinical evaluation.

LFS is a cancer predisposition syndrome caused by a germline mutation of the *TP53* tumor suppressor gene on chromosome 17p13.1 [1]. LFS associates with high risks for a diverse spectrum of childhood- and young adult-onset malignancies [2]. Five cancer types account for the majority of LFS tumors: adrenocortical carcinomas, breast cancer, central nervous system tumors, and soft tissue and bone sarcomas [6]. It is intriguing how sarcomas occur in the molecular backgrounds of LFS. However, according to the cell line database, there were no sarcoma cell lines established from the patients with LFS [23]. In this study, we established the NCC-UPS4-C1, a cell line of UPS which is one of the most common soft tissue sarcomas. The NCC-UPS4-C1 cells had *TP53* gene mutation according to the examination with NCC Oncopanel test, which was matched with the criteria of LFS [7]. According to the ClinVar which is a public archive of reports of the relationships among human variations and phenotypes with supporting evidence, the detected *TP53* mutation (c. C637T (p.R213X) is reported in patients with LFS syndrome. Thus, this paper is the first report about the establishment of sarcoma cell line from patients with LFS. Considering the worldwide low incidence of LFS (1 in 5000 to 20,000 peoples) [32] and soft tissue sarcomas (between 1.8 and 5.0 cases per 100,000 per year) [33], NCC-UPS4-C1 was established from a patient who had extremely rare backgrounds.

The NCC-UPS4-C1 cell line was established from a 32-year-old female patient with LFS. According to the 20-year clinical research using data from 266 patients with UPS, the median age of patients was 63.8 years [15]. In addition, without 7 cell lines that did not specify the patients' age of sampling, only 5 of 47 UPS cell lines in public cell banks are from patients aged < 40 years (Supplementary Table 1). Thus, UPS cell line derived from 32-year-old patients is rare. Having a variety of patient ages in UPS cell lines enables researchers to examine the differences in prognosis between young and elderly patients [15].

CNAs lead to altered gene expression and eventually contribute to the development of sarcoma [34] including UPS [10]. We found the NCC-UPS4-C1 apparently preserved the CNAs of its original tumor. In NCC-UPS4-C1, copy number losses were observed in chromosomal arms 1p13.3, 8p23.2, and 12q15 which harbor the *AKNAD1*, *CSMD1*, and *CNOT2* genes, respectively. Notably, previous TCGA sarcoma analysis using 44 UPS samples was conducted and the deletion of *CSMD1* detected [35]. The *CSMD1* which acts as a putative tumor suppressor gene has previously been reported [36]. Using NCC-UPS4-C1, we may address the significance of *CSMD1* in UPS.

We found that the NCC-UPS4-C1 consisted of spindle- and polygonal-shaped cells in 2D culture. HE staining revealed that pleomorphic cells were included in the spheroids, which were fabricated by the 3D culture of NCC-UPS4-C1. 3D culture such as spheroids can reproduce mechanical and biochemical cues that are crucial for

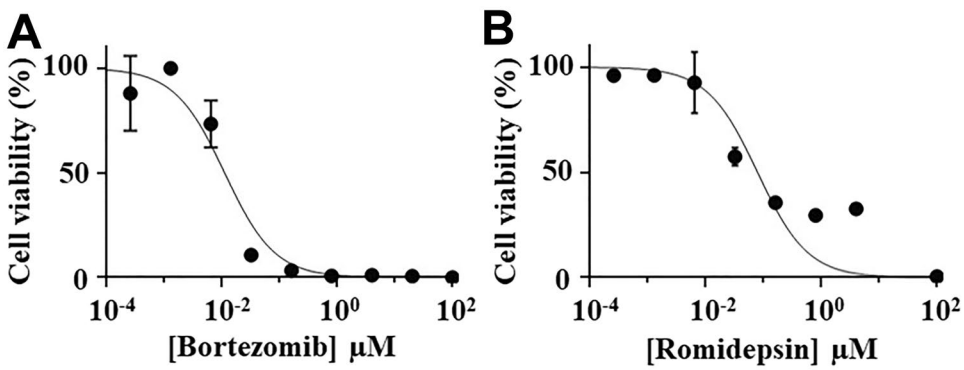

Fig. 4 Growth curves of NCC-UPS4-C1 exposed to bortezomib and romidepsin. The growth-suppressive effects of the anti-cancer drugs on NCC-UPS4-C1 (passage 25) were assessed via drug screening. Based on the growth curves of **A** bortezomib and **B** romidepsin, IC_{50} values were calculated for each drug

tumor development, such as cell–cell/cell–extracellular matrix interactions, tissue stiffness, and specific gradients [37]. The natural cell shape and polarization were preserved when the cells were grown in 3D culture [38]. These reports may explain the differences in morphology between the 2D and 3D cultures of NCC-UPS4-C1. A constant growth and invasion capability of NCC-UPS4-C1 was observed. Therefore, NCC-UPS4-C1 are suitable for in vitro studies, such as drug screening. In contrast, NCC-UPS4-C1 may not be suitable for xenograft experiments, because tumorigenesis in nude mice injected with NCC-UPS4-C1 was not observed in this study. To reveal the interaction between UPS cells and tumor microenvironments, which include stromal cells and the extracellular matrix, xenografts using UPS cells may be required. Therefore, additional UPS cell lines are needed for in vivo research on UPS.

Before surgical resection, the donor of NCC-UPS4-C1 received chemotherapy consisting of etoposide and ifosfamide; however, the tumor was progressive. In drug screening using 214 anti-cancer drugs at a high concentration (10 μM), NCC-UPS4-C1 showed high cell viability against both etoposide (81.8%) and ifosfamide (86.2%) (Supplementary Table 8). Therefore, the results of drug screening with NCC-UPS4-C1 may reflect the clinical results. Moreover, bortezomib and romidepsin showed the lowest IC_{50} values. NCC-UPS1-C1 [39, 40], NCC-UPS2-C1 [41], and NCC-UPS3-C1 [42], UPS cell lines established in our previous studies, also exhibited high sensitivity to romidepsin, a histone deacetylase (HDAC) inhibitor, which was approved by the US Food and Drug Administration for the treatment of cutaneous T-cell lymphoma [43]. The IC_{50} values of romidepsin to NCC-UPS1-C1 [39, 40], NCC-UPS2-C1 [41], NCC-UPS3-C1 [42], and NCC-UPS4-C1 were 14.58 nM, 67.87 nM, 1.96 nM, and 78.73 nM respectively. According to the clinical trial database ClinicalTrials.gov [44], 76 clinical studies of MFH and UPS were performed. However, no studies have used HDAC inhibitors for the treatment of patients with UPS. Using UPS cell lines, we identified potential drugs for the treatment of UPS. Considering the diversity of UPS, more UPS cell lines will be required to obtain conclusive results.

We found the difference in drug sensitivity among NCC-UPS1-C1, NCC-UPS2-C1, NCC-UPS3-C1, and NCC-UPS4-C1. In the drug screening using 214 anti-cancer drugs, romidepsin was identified as the potential drug in NCC-UPS1-C1, NCC-UPS2-C1, NCC-UPS3-C1, and NCC-UPS4-C1. However, comparing the IC_{50} values of romidepsin among these four cell lines, NCC-UPS2-C1 (67.87 nM) and NCC-UPS4-C1 (78.73 nM) showed higher values than NCC-UPS1-C1 (14.58 nM) and NCC-UPS3-C1 (1.96 nM). According to the NCC Oncopanel test performed in NCC-UPS1-C1, NCC-UPS2-C1, and NCC-UPS4-C1, *TP53* mutation was found in NCC-UPS2-C1 [c.C430T (p.Q144X)] and NCC-UPS4-C1 (Table 2). Our results supported the previous reports of the connection between *TP53* mutation and drug resistance [45]. The SNP array showed that NCC-UPS3-C1 did not have CNA in the *TP53* gene. The presence of *TP53* mutation in NCC-UPS3-C1 will be investigated with NCC Oncopanel test.

In conclusion, we established a novel UPS cell line, NCC-UPS4-C1, which was the first sarcoma cell line derived from a patient with LFS. This cell line showed continuous cell proliferation, spheroid formation, and aggressive invasiveness. Genetic analysis revealed CNAs and the *TP53* gene mutation in NCC-UPS4-C1. The established UPS cell lines in our laboratory provided valuable information that the anticancer effects of romidepsin have potential for the treatment of patients with UPS. This study demonstrated the utility of NCC-UPS4-C1 in accelerating preclinical research on UPS.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13577-022-00671-y.

Acknowledgements We thank Drs. E. Kobayashi, F. Nakatani, S. Iwata, T. Komatsubara (Department of Musculoskeletal Oncology), Drs. C. Sato, H. Tanaka, T. Shibayama (Department of Diagnostic Pathology) and the National Cancer Center Hospital for sampling tumor tissue specimens from surgically resected materials. We also appreciate the technical assistance provided by Mrs. Y. Kuwata (Division of Rare Cancer Research), and the technical support provided by Mrs. Y. Shiotani, Mr. N. Uchiya, and Dr. T. Imai (Central Animal Division, National Cancer Center Research Institute). We would like to thank Editage (www.editage.jp) for providing English language editing services and for their constructive comments on the manuscript. This research was technically assisted by the Fundamental Innovative Oncology Core in the National Cancer Center.

Funding This research was supported by the Japan Agency for Medical Research and Development (grant number 20ck0106537h0002).

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval The ethical committee of the National Cancer Center approved the use of clinical materials for this study (approval number 2004-050). The animal experiments were conducted in compliance with the guidelines of the Institute for Laboratory Animal Research, National Cancer Center Research Institute.

Informed consent Written informed consent was provided by the patient.

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