ANTICANCER RESEARCH *40*: 759-766 (2020)
doi:10.21873/anticanres.14007

Establishment and Characterization of a Novel Primitive Yolk Sac Tumour Cell Line, TC587

TAKESHI IWASAKI[1,2], KENICHI KOHASHI[1], MASASUKE OHNO[3], TOMOAKI TAGUCHI[4] and YOSHINAO ODA[1]

[1]*Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan;*
[2]*Department of Diagnostic Pathology, Japan Community Healthcare Organization, Kyushu Hospital, Fukuoka, Japan;*
[3]*Department of Neurosurgery, National Hospital Organization Nagoya Medical Center, Nagoya, Japan;*
[4]*Department of Pediatric Surgery, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan*

Abstract. *Background/Aim: Yolk sac tumour (YST) is a rare malignant ovarian germ cell tumour that often occurs in young women or adolescents and exhibits an unfavourable outcome. To evaluate the biological behavior of carcinomas in vitro, permanent tumour cell lines are required. However, previously, only a few human YST cell lines have been established. Therefore, we aimed to establish a novel YST cell line. Materials and Methods: We established a novel YST cell line, TC587, from an adolescent patient with ovarian YST. Results: The cell line expressed AFP and SALL4, the characteristics of YST. In addition, we evaluated somatic mutations using next-generation sequencing and revealed some pathogenic variants, including mutations in the NRAS, KIT, KMT2C, RSF1, and TP53 genes. Conclusion: The newly established TC587 cell line may represent an effective tool for developing treatments and conducting molecular analyses for YST.*

Ovarian germ cell tumour is an extremely rare tumour comprising 2%-5% of all ovarian malignant tumours (1). Yolk sac tumour (YST) is the second most frequent histological subtype after ovarian dysgerminoma, it accounts for 20% of all ovarian germ cell tumours and predominantly occurs in women aged <35 years (1). Among all subtypes of malignant ovarian germ cell tumour, YST is a highly malignant germ cell tumour characterized by the expression of α-fetoprotein (AFP) and unfavourable outcomes, with a 5-year survival rate of 66.6% (2). Additionally, YST has 5-year survival rates of 92.5%, 75%, 30%, and 25% in patients with stage I, II, III, and IV disease, respectively (3). Therefore, to improve the aggressive course and unfavourable prognosis of YST long-term, new treatments, including molecular-targeted drugs based on molecular and genetic characteristics of YST are required. To evaluate the biological behavior of carcinomas *in vitro*, permanent tumour cell lines are required. However, only a few human YST cell lines have been established. Molecular, genetic, and biological characterization of YST *in vitro* and *in vivo* models is desired. In this study, we established and characterized a new YST cell line, TC587, derived from a recurrent tumour in a patient with YST.

Correspondence to: Yoshinao Oda, MD, Ph.D., Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Japan, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. Tel: +81 926426061, Fax: +81 926425968, e-mail: oda@surgpath.med.kyushu-u.ac.jp

Key Words: Yolk sac tumour, primitive germ cell tumour, AFP, stem cell.

Materials and Methods

Patient's clinical history. A 12-year-old girl was admitted to the Department of Pediatric Surgery, Kyushu University Hospital for increasing abdominal girth and vomiting. Her serum AFP and CA 125 levels were 32,421 ng/ml and 286 U/ml, respectively, whereas her human chorionic gonadotropin (HCG) level (<0.5 mIU/ml) was within the normal range. CT revealed a 10×8 cm^2-sized pelvic tumour (Figure 1A). After a series of clinical and laboratory examinations, YST in the ovaries was diagnosed, and preoperative chemotherapy (ifosfamide, carboplatin, and etoposide) was initiated. Next, surgery (left oophorectomy and omentectomy) was performed. The primary resected sections were typified by the proliferation of oval to polygonal cells having prominent nucleoli and eosinophilic cytoplasm arranged in endodermal sinus, hepatoid, and glandular patterns (Figure 1B, C). Immunohistochemically, the tumour cells were positive for AFP, Cytokeratin AE1/AE3, and CAM5.2 but negative for EMA and HCG (Figure 1D-F). Despite postoperative adjuvant chemotherapy, repeat surgery (simple hysterectomy, bilateral salpingo-oophorectomy, and colostomy) was required because of local recurrence. The 20 specimens from the recurrent tumour exhibited similar histological and immunohistochemical features (data not shown) and were diagnosed as pure YST.

Chemosensitivity to 20 chemotherapeutic agents was evaluated using the succinate dehydrogenase inhibition (SDI) method as described previously (4). We performed chemotherapy using Actinomycin D, Vincristine and Adriamycin (regimen DD-4A) based

Table I. *Chemosensitivity to 20 chemotherapeutic agents.*

Anticancer drugs	Chemosensitivity rates (%)	Drug sensitivity
Docetaxel	8.2	-
Irinotecan (SN-38)	32.2	+/-
Etoposide	24.0	-
Cisplatin	45.6	+/-
Endoxane	46.6	+/-
Ifosfamide	2.5	-
Carboplatin	18.6	-
Pirarubicin	48.8	+/-
Vinblastine	76.7	+
Vincristine	77.0	+
Vindesine	79.3	+
Paclitaxel	61.2	+
Dactinomycin	84.7	+
Gemcitabine	58.1	+
Adriamycin	84.8	+
Epirubicin	80.0	+
IFNα	10.7	-
IFNβ	18.6	-
Methotrexate	0.0	-
Fluorouracil	46.1	+/-

IFN: Interferon; +: drug sensitive; -: drug resistant; +/-: indeterminate.

on the results of the chemosensitivity test (Table I). Further, the tumour exhibited an aggressive clinical course, and the patient died 1 year after the initiation of therapy. No autopsy was performed. This study was conducted in accordance with the principles embodied in the Declaration of Helsinki.

Immunohistochemical analysis. Immunohistochemical analysis was performed for original tumours (first and second surgeries). The formalin fixed, paraffin-embedded sections were cut into 4 μm, dewaxed in xylen and rehydrated in a grade series of ethanol. Endogenous peroxidase was blocked with 3% hydrogen peroxide in methanol for 30 min, and washed twice with PBS. These sections were treated with trypsin for 30 min at room temperature for CAM5.2, and heated with microwave in 0.01 M citrate buffer (pH6) for 20 min at 99˚C for Cytokeratin AE1/AE3. The endogenous biotin-avidin was blocked using an endogenous biotin-avidin blocking kit (Nichirei Corp., Tokyo, Japan), and washed twice with PBS. The primary monoclonal antibodies and antigen retrieval methods used in this study are summarized in Table II. Overnight primary antibody incubation at 4˚C was performed, and then the immune complex was detected with Nichirei Histofine SAB-PO kit following the manufacturer's protocols (Nichirei Corp, Tokyo, Japan). The sections were developed with diaminobenzidine (DAB) and counterstained with Mayer's hematoxylin and mounted.

Establishment of the TC587 cell line. Tumour tissue obtained during the second operation was minced with scissors and seeded in a 25-cm^2 tissue culture flask (Corning Costar, Tokyo, Japan) at 37˚C in a moist atmosphere containing 5% CO_2. The culture medium was Ham's F12 containing 10% fetal bovine serum. When subconfluent layers were obtained, the cells were dispersed with phosphate-buffered saline (PBS) containing 0.1% trypsin and 1 mM EDTA and

Table II. *List of primary antibodies.*

Antibodies	Clone	Source	Dilution	Host	type
CK5/6	D5/16 B4	DAKO	1:100(IHC)	Mouse	Monoclonal
Alpha-1-Fetoprotein	A0008	DAKO	1:400(IHC), 1:1000(WB)	Rabbit	Polyclonal
hCG	A581	DAKO	1:500(IHC)	Rabbit	Polyclonal
Oct3/4	H134	Santa Cruz Biotechnology	1:1000(WB)	Rabbit	Polyclonal
HSP 90α/β	H-114	Santa Cruz Biotechnology	1:1000(WB)	Rabbit	Polyclonal
SALL4	6E3	Abnova	1:1000(WB) 1:200 (IF)	Mouse	Monoclonal
Nanog	RCAB001P	Repro Cell	1:1000(WB)	Rabbit	Polyclonal
Oct3/4	H134	Santa Cruz Biotechnology	1:1000(WB)	Rabbit	Polyclonal

IHC: Immunohistochemistry; WB: western blotting; IF: immunofluorescence.

divided among several dishes. After 100 passages, we named this cell line "TC587" and then we carried out the assays described below.

Cells. TC587, Nec8, Hela and Hep3B were grown in Ham's F-12 (TC587), RPMI 1640 (NEC8), DMEM/F12 (Hep3B) and DMEM (Hela) supplemented with 10% fetal bovine serum.

Immunoblotting. Cells were washed twice with PBS, re-suspended in 2×SDS sample buffer, and denatured at 97˚C for 3 min. The samples were separated *via* SDS-PAGE (Any kD™ Mini-PROTEAN® TGX™, Bio-Rad, Tokyo, JAPAN) and electrotransferred to a polyvinylidene fluoride membrane using the Trans-Blot Turbo Transfer System (Bio-Rad Laboratories, Hercules, CA; 2.5 A, 25 V, 7 min). The membrane was blocked for 15 min in Blocking One-P (Nacalai Tesque) and incubated with primary antibodies in Hikari Solution A (Nacalai Tesque), followed by incubation with secondary antibodies and detection using Chemi-Lumi One Ultra (Nacalai Tesque). The primary antibodies used for immunoblotting are summarized in Table II.

Immunocytochemistry. Cells were plated on coverslips, washed twice with PBS, fixed in 4% paraformaldehyde in PBS for 10 min, permeabilized in 0.5% Triton X-100 in PBS for 5 min, and washed twice with PBS. A 10-min incubation in Blocking One-P was followed by overnight incubation at 4˚C with anti-SALL4 and anti-AFP (Table II). The coverslips were then washed three times with TBS-T and incubated for 30 min at room temperature in Alexa 488 plus labelled goat anti-mouse or anti-rabbit antibody (1:1000, Thermo Fisher). Coverslips were again washed three times in PBS and mounted in ProLong Gold Diamond Antifade Reagent with DAPI (Life Technologies). Images were visualized using a fluorescence microscope (BZ-X700, Keyence, Osaka, Japan).

DNA extraction, Next-generation sequencing and bioinformatics. Total DNA from TC587 cells was isolated using a QIAmp DNA Micro kit (Qiagen, Hilden, Germany). Next, DNA quality was tested using the Genomic DNA ScreenTape system (Agilent). An amplicon library of the target exons was prepared using an Ion AmpliSeq

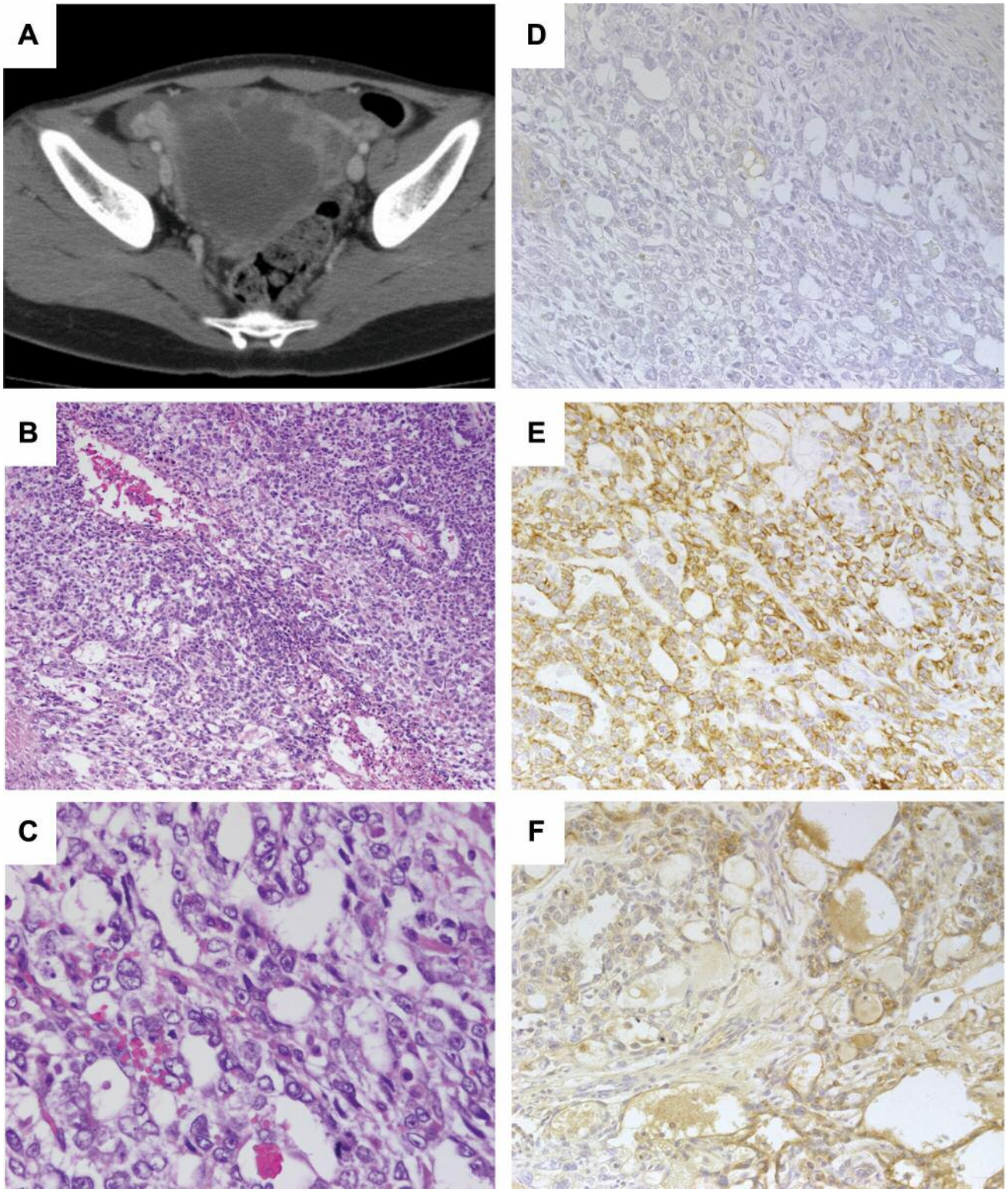

Figure 1. *Characteristics of the primary tumor used in this study. A. Contrast-enhanced CT revealed a 10×8 cm^2 pelvic tumour. B-C. The primary resected sections featured the proliferation of oval to polygonal cells having prominent nucleoli and eosinophilic cytoplasm arranged in endodermal sinus, hepatoid, and glandular patterns. D-F. Immunohistochemically, the tumour cells were negative for human chorionic gonadotropin (HCG) (D) and positive for CAM5.2 (E) and alpha-fetoprotein (AFP) (F).*

Table III. *List of genes analyzed by the custom Cancer Panel.*

ABL1	*CHD2*	*ILK*	*POU2F2*	*TFF1*
ACTB	*CHD3*	*INO80*	*PSG6*	*TFPT*
ACTL6A	*CHD4*	*INO80B*	*PTEN*	*TGFB1*
ACTL6B	*CHD5*	*INO80C*	*PTPN11*	*TGFBR1*
ACTL7B	*CHD6*	*INO80D*	*PREX2*	*TGFBR2*
ACTL8	*CHD7*	*INO80E*	*RB1*	*TLE4*
ACTR5	*CHD8*	*JAK2*	*RBBP4*	*TP53*
ACTR8	*CHD9*	*JAK3*	*RBBP7*	*TP53BP2*
ACVR1B	*CHRAC1*	*JAG1*	*RBM10*	*TPO*
ACVR2A	*CIC*	*KDM6A*	*RET*	*TTN*
ADAMTS12	*CNTN5*	*KDR*	*RNF43*	*TWIST1*
ADAMTS16	*CR1*	*KIT*	*ROBO1*	*TWIST2*
AKT1	*CSF1R*	*KLF4*	*ROBO2*	*U2AF1*
ALK	*CSF1R*	*KMT2D*	*RPA1*	*UCHL5*
APC	*CTNNB1*	*KRAS*	*RSF1*	*USP28*
ARID1A	*DPF1*	*MAPK1*	*RUVBL1*	*VHL*
ARID1B	*DPF2*	*MAP2K4*	*RUVBL2*	*YY1*
ARID2	*DPF3*	*MAPK4*	*RYR1*	*ZEB1*
ARID3A	*DYRK1A*	*MBD2*	*RYR2*	*ZEB2*
ARID3B	*DYRK1B*	*MBD3*	*SALL4*	
ARID4A	*EGFR*	*MCRS1*	*SDK2*	
ARID4B	*EIF1AX*	*MET*	*SETD2*	
ARID5A	*EPAS1*	*MLH1*	*SF3B1*	
ARID5B	*ERBB2*	*MLL2*	*SLIT2*	
ATF2	*ERBB4*	*MLL3*	*SMAD2*	
ATM	*EZH2*	*MPL*	*SMAD3*	
ATRX	*FAT1*	*MSH2*	*SMAD4*	
AXIN1	*FAT3*	*MSH6*	*SMAD7*	
BAZ1A	*FBXW7*	*MTA1*	*SMARCA1*	
BAZ2A	*FGFR1*	*MTA2*	*SMARCA2*	
BAZ1B	*FGFR2*	*MTA3*	*SMARCA4*	
BMI1	*FGFR3*	*MYC*	*SMARCA5*	
BCORL1	*FLT3*	*MYCBP2*	*SMARCAD1*	
BPTF	*FOXM1*	*NANOG*	*SMARCAL1*	
BRAF	*FOXO1*	*NF2*	*SMARCB1*	
BRCA1	*GATAD2A*	*NFRKB*	*SMARCC1*	
BRCA2	*GATAD2B*	*NOTCH1*	*SMARCC2*	
BRD1	*GATA6*	*NOV*	*SMARCD1*	
BRD2	*GLI3*	*NPM1*	*SMARCD2*	
BRD3	*GNA11*	*NRAS*	*SMARCD3*	
BRD4	*GNAS*	*NSD1*	*SMARCE1*	
BRD7	*GNAQ*	*PALB2*	*SMO*	
BRD8	*GRID2*	*PBRM1*	*SNAI1*	
BRD9	*HDAC1*	*PDGFRA*	*SNAI2*	
CDH1	*HDAC2*	*PHF10*	*SOX2*	
CDK6	*HNF1A*	*PIK3CA*	*SOX9*	
CDKN1B	*HRAS*	*PIK3R3*	*SRC*	
CDKN2A	*IDH1*	*PMS2*	*ST6GAL2*	
CECR2	*IDH2*	*POLE3*	*STK11*	
CHD1	*IGF1R*	*POU5F1*	*TERT*	

Custom Panel (Applied Biosystems, Life Technologies) and designed using Ion AmpliSeq Designer (http://ampliseq.com) for 219 genes, including the Ion AmpliSeq Cancer Hotspot Panel v2 targeted genes and chromatin remodelling factor-related genes according to the manufacturer's instructions (Table III). The NGS library construction and analysis were performed by Cell Innovator (Fukuoka, Japan).

Cytogenetic analysis. A metaphase cytogenetic analysis was performed using the 20th passage of TC587 cells. The preparation was obtained using a standard trypsin-Giemsa banding technique. The karyotype was analyzed according to the rules of the International System for Human Cytogenetic Nomenclature (ISCN1995).

Results

Cell line establishment. We established a novel human yolk sac cell line (TC587) from the resected recurrent tumour (Figure 2A). Cultured cells comprised small, polygonal cells. The doubling time was approximately 36 h.

Immunocytochemical and immunoblotting results. Immunoblotting revealed that the cell line was positive for AFP and SALL4 and negative for Nanog, OCT3/4, and SOX2 (Figure 2B). Immunocytochemical staining also confirmed the expression of SALL4 and AFP (Figure 2C).

Cytogenetic findings. Cytogenetic analysis of 10 samples of high-quality metaphase TC587 cells revealed an extremely complex karyotype, with chromosome numbers varying from 79 to 83. The cells featured the following composite chromosomal complements: 79~83<4n>, X, -X[10], add(X)(p11.2)[10], der(X)t(X;12)(p11.2;q12)[10], add(1)(p31)[10], del(1)(p34p36.1)[10], -2[10], add(2)(q11.2)[10], -3[9], add(3)(q11.2)[10], add(3)(q11.2)[3], -4[10], der(4)t(4;5)(q23;q13)ins(4;?)(q23;?)[10], -5[10], -5[10], add(5)(p13)[9], -6[10], -6[4], add(6)(p21)[2], add(7)(p13)[10], add(7)(p11.2)[9], del(7)(q11.2q21)[10], -8[10], -9[10], add(9)(p13)[10], -10[10], -10[10], add(10)(p11.2)[9], -12[10], -13[9], -14[10], -14[9], -15[10], -15[9], add(15)(p11.2)[9], -16[10], -17[10], add(17)(q21)[7], -18[10], -18[7], del(18)(q12)[9], +19[2], add(19)(q13.3)[10], -20[10], -20[3], add(20)(q13.1)[3], -21[10], -22[10], -22[3], +der(?)t(?;2)(?;q21)[7], +11~16 mar (Figure 2D).

Next-generation sequence analysis. Next-generation sequencing identified 42 somatic variants, including pathogenic variants of the *NRAS, KIT, KMT2C, RSF1*, and *TP53* genes (Table IV). SNVs identified in these genes were missense variants, and all were associated with damaging effects on the coding product. One SNV identified in the *SMARCA2* gene was a previously unknown stop-gained variant.

Discussion

The biology of YST is poorly understood. Permanent tumour cell lines serve as preclinical research tools for better understanding several tumour-related pathological aspects and exploring novel therapeutic targets. In this study, we established the novel YST cell line that exhibited the same

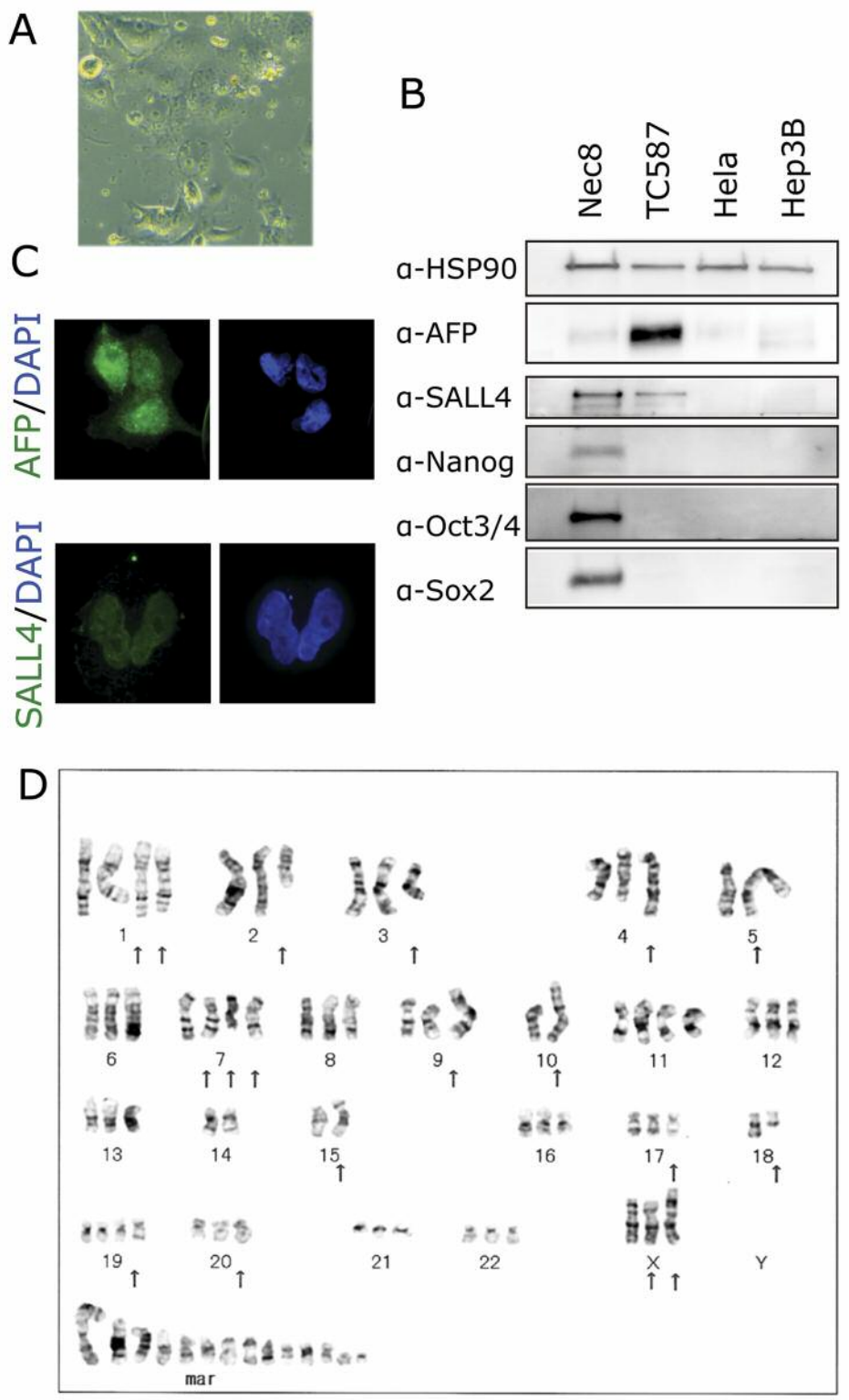

Figure 2. *Characteristics of the TC587 cell line. A. Light microscopic findings of TC587 cells in vitro. The tumor cells were small and polygonal cells. B. Immunoblotting of extracts from testicular germ cell tumour (Nec8), Yolk sac tumour (TC587), endocervical carcinoma (HeLa), and hepatocellular carcinoma cells (Hep3B). TC587 cells expressed AFP and SALL4. C. The tumour cells were positive for AFP and SALL4. D. Chromosome analysis. The G-banding analysis for the TC587 cell line revealed that the number of chromosomes ranged between 79 and 83, with a modal chromosome number of 82.*

Table IV. *Clinical characteristics and bioinformatic analysis of TC587 cell line variants*.

GENE	Coding DNA change	Protein change	EFFECT	dbSNP	COSMIC	ClinVar	FATHMM	Polyphen-2	SIFT
NRAS	c.38G>A	p.Gly13Asp	Missense	rs121434596	COSM573	Likely pathogenic	Pathogenic	B	D
CR1	c.4029A>G	p.Gly1343Gly	Synonymous	rs41274762					
	c.4033G>A	p.Ala1345Thr	Missense	rs202021797				B	T
ALK	c.3516-278A>C		Intron						
MTA3	c.1160G>A	p.Arg387His	Missense	rs76558479				D	D
ACTR8	c.779-11dupT		Intron	rs74580677					
PDGFRA	c.628+1G>T		Splice_donor&intron						T
KIT	c.2446G>T	p.Asp816Tyr	Missense	rs121913506	COSM1310	Likely pathogenic	Pathogenic	D	D
SMARCA5	c.870T>C	p.Leu290Leu	Synonymous						
FBXW7	c.1236+6T>A		Splice_region&intron	rs529891909					
BRD9	c.1190T>C	p.Val397Ala	Missense	rs145107515				D	T
	c.1165G>A	p.Ala389Thr	Missense	rs779060319				B	T
CHD1	c.682G>T	p.Asp228Tyr	Missense					P	T
EGFR	c.2370G>A	p.Thr790Thr	Synonymous	rs376452156					
KMT2C	c.3414C>G	p.Pro1138Pro	Synonymous	rs150537569					
	c.2769+10G>C		Intron	rs200539997					
	c.2653-233A>G		Intron	rs75646957					
	c.2645_2646delTCinsCA	p.Ile882Thr	Missense						
	c.2538T>C	p.Ala846Ala	Synonymous	rs766200477					
	c.1038G>A	p.Val346Val	Synonymous	rs770240292	COSM4768617				
	c.1013C>T	p.Ser338Leu	Missense &splice_region	rs763762478	COSM6056930		Pathogenic	D	T
	c.1013-2A>G		Splice_acceptor&intron	rs751158858					
	c.162-7delT		Splice_region&intron	rs560528049					
	c.162-6C>G		Splice_region&intron						
PREX2	c.1818_1819delATinsGC	p.GlyLeu606GlyLeu	Synonymous	rs368406603	COSM3763378		Neutral		
SMARCA2	c.1765C>T	p.Gln589*	Stop_gained						
GNAQ	c.105C>T	p.Asp35Asp	Synonymous	rs751518366	COSM3670056		Neutral		
RET	c.1880-8A>C		Splice_region&intron						
DPF2	c.330G>T	p.Gly110Gly	Synonymous						
RSF1	c.3398C>T	p.Pro1133Leu	Missense	rs200271333	COSM4036875		Pathogenic	P	D
NFRKB	c.1751C>T	p.Ser584Leu	Missense	rs751435932				D	D
KMT2D	c.1799T>C	p.Phe600Ser	Missense					B	T
HNF1A	c.572G>A	p.Gly191Asp	Missense					B	T
FLT3	c.2013C>T	p.His671His	Synonymous	rs149216274					
MYCBP2	c.1648-11dupT		Intron	rs11436373 rs398117587 rs549452634					
CHD8	c.1601+7A>G		Splice_region&intron						
CHD2	c.3540C>T	p.Ser1180Ser	Synonymous	rs76621355		Benign			
TP53	c.1040C>A	p.Ala347Asp	Missense	rs397516434	COSM6959640	Conflicting interpretations of pathogenicity	Pathogenic	D	D
CHD3	c.1797T>G	p.Arg599Arg	Synonymous						
RNF43	c.1825C>T	p.Arg609Trp	Missense	rs139405076	COSM3195618			P	D
MAPK1	c.108C>T	p.Tyr36Tyr	Synonymous	rs201495639	COSM7220760		Neutral		

B: Benign: P: probably damaging; D: damaging; T: tolerated.

protein expression patterns as YST. Only two YST cell lines had been previously reported (NOY1 and NOY2) (5). In addition, these two cell lines were established from the same patient, and it is difficult to investigate individual differences among patients with YST.

We also performed sequencing analysis using TC587 cells and identified various SNVs, including some pathogenic variants in the genes *TP53, NRAS, KIT, KMT2C*, and *RSF1*. The *TP53* gene is a major tumor suppressor gene and the variants identified have also been reported in YST (COSIMC data) (6). TC587 cells carried the *TP53* c.1040C>A variant, which has not been reported in YST. Variants in *NRAS* codons 12 and 61 have been reported in seminoma (16% and 59%, respectively) and non-seminoma (15% and 78%, respectively) (7, 8). In the COSMIC data, 1/27 patients with YST exhibited a c.34G>A mutation. TC587 cells carried the *NRAS* c.38G>A mutation, which has been reported in leukemia, malignant melanoma, and colorectal adenocarcinoma (8).

KIT mutations are common pathogenic mutations in gastrointestinal stromal tumour (GIST) that result in ligand-independent activation of tyrosine kinases. The most frequent mutations occur in *KIT* exon 11 (60%-70%), followed by a mutation in *KIT* exon 9. TC587 cells carried the *KIT* p.D816Y (exon 17) mutation, which encodes the second tyrosine kinase domain (9). In the COSMIC database, the *KIT* p.816Y mutation was found in GIST, acute myeloid leukemia (AML), and some germ cell tumours, including dysgerminoma, seminoma, and mixed germ cell tumour. AML featuring the D816Y mutation is highly resistant to imatinib mesylate (10).

The *KMT2C* gene encodes a histone methyltransferase that regulates gene transcription by modifying the chromatin structure. The *KMT2C* mutation identified in this study has been reported in various carcinomas, including endometrial carcinoma gastrointestinal carcinoma and urothelial carcinoma; however, this mutation has not been previously described in YST.

SMARCA2 (also called BRM) is a key component of the SWI/SNF chromatin-remodelling complex. TC587 cells carried the *SMARCA2* p.Gln589 mutation, which created a stop codon. This mutation is not annotated in the ClinVar or COSMIC database, but is predicted to cause truncation of the SMARCA2 protein at the SNF ATPase and BRM domains.

The *RSF1* gene encodes a chromatin-remodelling factor that has been linked to DNA damage response and DNA repair (10). No *RSF1* mutations have been previously reported in germ cell tumours. TC587 cells featured the p.Pro1133Leu mutation, and the same variant has been reported in gastric cancer (COSMIC database). This pathogenic mutation might be a candidate clinical therapy target. Further investigation using the TC587 cell line is needed.

In summary, we have established and characterized a novel YST cell line. This cell line has some pathogenic mutations and expresses AFP and SALL4. Using this cell line, we hope to contribute to the individualization of YST treatment.

Conflicts of Interest

The Authors declare no conflicts of interest regarding this study.

Authors' Contributions

TI, KK and YO designed this study and wrote the manuscript. TI, KK and MO performed the experiments. TI, KK and YO performed histological re-evaluation of samples and confirmed the diagnosis. TT and YO supervised the experiments.

Acknowledgements

The Authors appreciate the technical assistance from Cell Innovator and the English language review from Enago (www.enago.jp).

References

1 Boussios S, Attygalle A, Hazell S, Moschetta M, McLachlan J, Okines A and Banerjee S: Malignant ovarian germ cell tumors in postmenopausal patients: The royal marsden experience and literature review. Anticancer Res *35(12)*: 6713-6722, 2015. PMID: 26637887.

2 Umezu T, Kajiyama H, Terauchi M, Shibata K, Ino K, Nawa A and Kikkawa F: Long-term outcome and prognostic factors for yolk sac tumor of the ovary. Nagoya J Med Sci *70(1-2)*: 29-34, 2008. PMID: 18807293.

3 Nawa A, Obata N, Kikkawa F, Kawai M, Nagasaka T, Goto S, Nishimori K and Nakashima N: Prognostic factors of patients with yolk sac tumors of the ovary. Am J Obstet Gynecol *184(6)*: 1182-1188, 2001. PMID: 11349186. DOI: 10.1067/mob.2001.113323

4 Takeshita M, Koga T, Takayama K, Yano T, Maehara Y, Nakanishi Y and Sueishi K: Alternative efficacy-predicting markers for paclitaxel instead of chfr in non-small-cell lung cancer. Cancer Biol Ther *10(9)*: 933-941, 2010. PMID: 20855974. DOI: 10.4161/cbt.10.9.13320

5 Shibata K, Kajiyama H, Yamamoto E, Terauchi M, Ino K, Nawa A and Kikkawa F: Establishment and characterization of an ovarian yolk sac tumor cell line reveals possible involvement of nkx2.5 in tumor development. Oncology *74(1-2)*: 104-111, 2008. PMID: 18547965. DOI: 10.1159/000139138

6 Zehir A, Benayed R, Shah RH, Syed A, Middha S, Kim HR, Srinivasan P, Gao J, Chakravarty D, Devlin SM, Hellmann MD, Barron DA, Schram AM, Hameed M, Dogan S, Ross DS, Hechtman JF, DeLair DF, Yao J, Mandelker DL, Cheng DT, Chandramohan R, Mohanty AS, Ptashkin RN, Jayakumaran G, Prasad M, Syed MH, Rema AB, Liu ZY, Nafa K, Borsu L, Sadowska J, Casanova J, Bacares R, Kiecka IJ, Razumova A, Son JB, Stewart L, Baldi T, Mullaney KA, Al-Ahmadie H, Vakiani E, Abeshouse AA, Penson AV, Jonsson P, Camacho N, Chang MT, Won HH, Gross BE, Kundra R, Heins ZJ, Chen HW, Phillips S,

Zhang H, Wang J, Ochoa A, Wills J, Eubank M, Thomas SB, Gardos SM, Reales DN, Galle J, Durany R, Cambria R, Abida W, Cercek A, Feldman DR, Gounder MM, Hakimi AA, Harding JJ, Iyer G, Janjigian YY, Jordan EJ, Kelly CM, Lowery MA, Morris LGT, Omuro AM, Raj N, Razavi P, Shoushtari AN, Shukla N, Soumerai TE, Varghese AM, Yaeger R, Coleman J, Bochner B, Riely GJ, Saltz LB, Scher HI, Sabbatini PJ, Robson ME, Klimstra DS, Taylor BS, Baselga J, Schultz N, Hyman DM, Arcila ME, Solit DB, Ladanyi M and Berger MF: Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med *23(6)*: 703-713, 2017. PMID: 28481359. DOI: 10.1038/nm.4333

7 Ganguly S, Murty VV, Samaniego F, Reuter VE, Bosl GJ and Chaganti RS: Detection of preferential nras mutations in human male germ cell tumors by the polymerase chain reaction. Genes Chromosomes Cancer *1(3)*: 228-232, 1990. PMID: 1964583.

8 Bamford S, Dawson E, Forbes S, Clements J, Pettett R, Dogan A, Flanagan A, Teague J, Futreal PA, Stratton MR and Wooster R: The cosmic (catalogue of somatic mutations in cancer) database and website. Br J Cancer *91(2)*: 355-358, 2004. PMID: 15188009. DOI: 10.1038/sj.bjc.6601894

9 Growney JD, Clark JJ, Adelsperger J, Stone R, Fabbro D, Griffin JD and Gilliland DG: Activation mutations of human c-kit resistant to imatinib mesylate are sensitive to the tyrosine kinase inhibitor pkc412. Blood *106(2)*: 721-724, 2005. PMID: 15790786. DOI: 10.1182/blood-2004-12-4617

10 Min S, Kim K, Kim SG, Cho H and Lee Y: Chromatin-remodeling factor, rsf1, controls p53-mediated transcription in apoptosis upon DNA strand breaks. Cell Death Dis *9(11)*: 1079, 2018. PMID: 30348983. DOI: 10.1038/s41419-018-1128-2

Received December 13, 2019
Revised December 22, 2019
Accepted December 23, 2019