



Supplementary Materials

Modeling the diversity of epithelial ovarian cancer through ten novel well characterized cell lines covering multiple subtypes of the disease

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Figure S1. Patient progression and therapy graphs. Graphical representation of CA-125 levels over time. Treatment regimens are represented by highlighted zones overlapping CA-125 curves, and by color-coded symbols at the top of each graph as per graph legends. Arrowheads on the CA-125 curve indicate surgeries. The red symbol on each curve represents the sample that resulted in the patient's corresponding cell line, either arrowheads for surgeries, or diamond shapes for ascites collection. The vertical blue line on the x-axis indicates 6 months after the end of the patient's first treatment regimen.

0-

0











Figure S2. Additional IHC stainings. Shown are supplementary IHC staining of the tumor of origin from which each cell line was derived, separated by subtype. Each tumor was tested for relevant biomarkers for its respective subtype.

TOV3291G



Figure S3. H&E and IHC stainings of ovarian tumors from patients 2978 and 3291, and corresponding WB of the TOV2978 and TOV3291 cell lines. Data is reproduced, with modifications, from Figure 4 of our previous publication [1] with permission from the publisher.



Figure S4. Whole Western blots of protein expression of markers in tumor cell lines. (**a**) Detection of characteristic subtype-specific EOC markers (p53, CK7, CK8, CK18, CK19, WT1, PAX8 and ER) in whole cell lysates of each cell line, with β -Actin as a control. All blots were loaded with the same sample order, indicated by lane numbers on the top left panel. Correspondence of lane numbers and cell lines is indicated in (**b**). (**b**) Intensity ratio of biomarkers for each cell line, normalized with β -Actin.

0.67

0.13

0.80

0.11

0.71

0.68

0.00

0.00

0.01

0.43

0.01

0.01

TOV2414

TOV3392D

Lane 9 Lane 10 0.03

0.05

0.47

0.40



Figure 5. Gene expression of *TP53* (**a**), *CDKN1A* (**b**) and *CDKN2A* (**c**). Left panels are gene expression in the 10 novel EOC cell lines described in this work, whereas right panels are gene expression in the matched ascites and tumor cell lines derived from patients 2978 and 3291. The image on top (**a**) (left) shows protein bands of p53 from the WB of Figures 4 and S4, for ease of comparison. Red fonts denote cell lines with frameshift or splicing mutations.



Figure S6. Characterization of WT1 expression in the OV3291 cell line. (**a**) WT1 mRNA and (**b**) WT1 protein expression was analyzed in OV2085 (as a positive control), TOV3291G and OV3291 cells. Correlation between mRNA and protein expression of WT1 was observed in OV2085 and TOV3291G, but not in OV3291. WT1 protein was also analyzed in OV3291 treated for 2 h with MG132. MG132 is a proteasome inhibitor that reduces the degradation of ubiquitin-conjugated proteins. Intensity ratio of WT1 protein for each cell line was normalized with β -Actin.



Figure S7. Confluence-based proliferation curves by live cell imaging fitted into a single graph. Cell proliferation of each cell line was determined by measuring confluence every 2 h. Initial values of confluence were between 5 and 10%, and cells were left to proliferate until confluence reached approximately 100%. Curves were fitted to a single graph for easier comparison between cell lines. Refer to Figure 4 for the SEM of each curve.



Figure S8. Supplementary in vivo growth characteristics. (a) Evolution of tumor volume after SC injection in NRG mice, for cell lines that did not induce observable tumor growth (n = 5). Points represent average ± SEM, and curves were plotted until end-points were attained per group, when the first animal was sacrificed. (b) Kaplan-Meyer survival curves of NRG mice after SC injection with each of the cell lines (n = 5). Cell lines were separated for clarity. Censored data points represent mice that had reached end-points.

Table S1. Additional notes on patients from whom tumors were collected.

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3392 Treated with FEC Protocol (2004-2005), radiotherapy (2005) and Trastuzumab (2005-2006) for breast cancer; Ovarian metastases at peritoneum; Presence of ascites		Received bilateral salpingo-oophorectomy, total abdominal hysterectomy and omentectomy
Ovarian metastases at peritoneum; Presence of ascites	3392	Treated with FEC Protocol (2004-2005), radiotherapy (2005) and Trastuzumab (2005-2006) for breast cancer;
Presence of ascites		Ovarian metastases at peritoneum;
		Presence of ascites

	Table S2	2. Additional observations in in vivo growth experiments (SC injections).
Cell line	Mouse	Observations
	1117	-
	1118	_
OV2085	1125	thymic lymphoma; prostration; respiratory distress (exclusion due to early onset of thymic lymphoma)
	1127	-
	1128	-
	1208	-
	1209	respiratory distress; 25% body weight loss
TOV2835EP	1210	-
	1211	-
OV20851125thymic lymphoma; prostration; respiratory distress (exclus lymphoma)1127-1128-1209respiratory distress; 25% body weight 1210TOV2835EP12101211-1212-1212-1373-1373-1373-1380-1381-1482-1483-135-135-135-135-135-135-1373air in the intestines and fallopiar1174-1175air and fluid in fallopian tubes; pa1176-1176-0V329115650V329115661566-		-
	1372	
	1373	
TOV2881EP	1378	
	1380	
	1381	
	1480	-
	1481	
TOV2929D	1482	
1012)2)0	1/83	
	1484	
	1335	
	1226	
01/2078	1257	-
012978	1357	-
	1252	
	1332	
	11/2	
	11/3	air in the intestines and fallopian tubes
TOV3121EP	1174	
	1175	air and fluid in fallopian tubes; pale lungs
	1176	-
	1564	-
OV3291	1565	-
	1566	-
	1572	-
	1573	-
	1087	respiratory distress; weight loss; dehydration; white mucosae
	1088	-
OV3331	1089	-
	1090	respiratory distress; thymic lymphoma; enlarged spleen; filamentous mass below the heart
	1096	-
	1055	tumor ulceration
	1065	-

OV3331	1089	-
	1090	respiratory distress; thymic lymphoma; enlarged spleen; filamentous mass below the heart
	1096	-
	1055	tumor ulceration
TOV2414	1065	-
	1066	tumor ulceration; large intestinal tumor; small metastases; swollen abdomen and stomach
	1067	tumor ulceration
	1068	tumor ulceration
	1591	20% body weight loss
	1592	20% body weight loss
	1581	20% body weight loss
	1582	25% body weight loss; dark pink lungs
TOV3392D	1593	20% body weight loss
	2444	20% body weight loss
	2445	20% body weight loss; pale organs; very swollen stomach
	2446	20% body weight loss; large amount of feces in intestines
	2447	20% body weight loss; pale organs
	2448	20% body weight loss; swollen stomach

Cell line	Mouse	Observations
	1129	red ascites; metastases in fat near ovaries, bladder and above liver
OV2085	1137	2.5 mL red ascites; large ovarian tumors (R + L); metastases at mesentery and between liver and stomach; enlarged spleen with white spots; pale lungs
	1138	7 mL red ascites; large metastases at bladder, liver, spleen; small metastases at stomach and intestine;
012000	1139	5.5 mL red ascites; medium metastases at bladder, liver, stomach, spleen, intestines; small metastases
		near spleen, liver, stomach; tumors at ovaries; enlarged spleen
	1147	3 mL red ascites; inguinal and ovarian tumors; medium metastases at bladder and below stomach; small metastases near spleen, liver, stomach; enlarged spleen
	1218	weight loss; enlarged belly and spleen; prostration; hard yellow mesenteric tumor; pale lungs and liver
	1219	dyspnea; body weight loss; dizzy; air in stomach, intestines, caecum; pale lungs and liver
TOV2835EP	1220	swollen and liquid-filled red fallopian tubes
	1221	swollen and liquid-filled red fallopian tubes
	1222	small white spot on spleen
	1392	2 mL pinkish brown ascites; metastases at pancreas, liver, stomach, diaphragm; small nodules on kidnevs; very pale liver
	1393	2 mL red ascites; small metastases at mesentery and fallopian tubes; enlarged spleen; pale lungs
TOV2881EP	1394	5 mL red ascites; small metastases at liver, stomach; enlarged spleen; pale liver and lungs
	1395	5 mL red ascites; small metastases at liver, stomach; enlarged spleen; pale liver and lungs
	1396	enlarged intestine
		2 mL ascites; enlarged intestine and spleen; medium metastasis between liver and stomach; small
	1499	metastases at mesentery and stomach; swollen fallopian tubes
	1500	7 mL dark red ascites: small metastases at stomach, spleen, liver; enlarged spleen; light brown liver
TOV2929D	1501	5 mL dark red ascites: small metastases at bladder, liver, spleen and stomach
		6.5 mL dark red ascites; medium metastases at stomach; small metastases at stomach and pancreas;
	1502	enlarged spleen; pale liver
	1503	body weight loss: prostration: air in intestine and fallopian tubes: fluid in ovary (L)
	1341	small metastases below stomach/liver; fat at ovaries and fallopian tubes; small pockets in fallopian tubes
	1342	enlarged red fallopian tubes and intestine
OV2978	1343	lots of fat at ovaries and fallonian tubes: enlarged red fallonian tubes: speckled liver
012/10	1344	lots of fat at ovaries and fallopian tubes: enlarged red fallopian tubes and intestine
	1345	dyspnea: clear ascites near organs: nale and speckled lungs: small metastasis near stomach
	1182	swollen abdomen: enlarged and fluid-filled gallbladder
	1183	lots of abdominal fat
TOV3121FP	1184	lots of abdominal fat
101012121	1185	vivid red and partly clotted ascites: lots of abdominal fat: pale kidneys and liver
	1186	large tymor at overy (R)
	1576	internal bleeding: small metastases at mesentery near stomach
	1577	small metastasis at nancreas
OV2201	1578	shian metastasis at paneteas
0,0201	1579	
	1580	
	1097	8.5 mL red assistes: stomach and liver metastasses: large metastatic tumor at overy and fallonian tube (L)
	1097	8.5 mL red ascites; motastassas at stomach liver overias fallonian tube; white lungs
OV3331	1106	6.5 mL red asches, metastases at stomach, nver, ovaries, fanoplan tubes, white fungs
	1100	6 mL red ascites; small metastases at ovaries, bladder, fallopian tubes, mesentery; swollen fallopian
		tubes and spleen; pale lungs
	1108	4 mL red ascites; small metastases at bladder, fallopian tubes, mesentery; pale and speckled lungs
TOV2414	1076	important body weight loss; prostration; small inguinal tumor; pale lungs; small stomach
	1078	large SC/IP tumor; 4 small mesenteric metastases near pancreas
	1081	large tumor at injection site; tumor ulceration; SC/IP tumor
	1082	medium tumor at injection site; tumor ulceration; SC/IP tumor
	1083	large SC/IP tumor; tumor ulceration; small mesenteric metastases
	1575	tumor at injection site and ovary (L); inguinal metastasis; small metastases at stomach and liver
	1587	enlarged fallopian tubes
TOV3392D	1588	SC/IP tumor at injection site; small stomach and spleen; reddish intestines
	1589	large and medium inguinal metastases; SC/IP tumor at injection site
	1590	2 inguinal metastases; small inguinal metastases

Table S3. Additional observations in in vivo growth experiments (IP injections).

Antibody	Antigen Retrieval	Antigen retrieval Incubation (min)	Antibody Incubation (min)	Ventana Kit	Ventana Type
ARID1A	cc1	60	60	iVIEW DAB Detection Kit	BenchMark XT
CDX2	cc1	52	24	UltraView Universal DAB Detection Kit	BenchMark ULTRA
CK18	cc1	30	40	UltraView Universal DAB Detection Kit	BenchMark XT
CK19	cc1	60	40	UltraView Universal DAB Detection Kit	BenchMark XT
CK20	cc1	60	44	UltraView Universal DAB Detection Kit	BenchMark XT
CK7	cc1	60	44	UltraView Universal DAB Detection Kit	BenchMark XT
CK8	cc1	60	60	UltraView Universal DAB Detection Kit	BenchMark XT
ER	cc1	60	60	UltraView Universal DAB Detection Kit	BenchMark XT
HER2	cc1	60	60	UltraView Universal DAB Detection Kit	BenchMark XT
MUC2	cc1	36	32	UltraView Universal DAB Detection Kit	BenchMark ULTRA
MUC5A	cc1	36	32	UltraView Universal DAB Detection Kit	BenchMark ULTRA
Napsin A	cc1	32	32	OptiView DAB Detection Kit	BenchMark ULTRA
p53	cc2	60	32	UltraView Universal DAB Detection Kit	BenchMark XT
PAX8	cc1	36	32	UltraView Universal DAB Detection Kit	BenchMark XT
PR	cc1	60	60	UltraView Universal DAB Detection Kit	BenchMark XT
SATB2	cc1	64	32	UltraView Universal DAB Detection Kit with amplification BenchMark U	
WT1	cc1	60	44	UltraView Universal DAB Detection Kit	BenchMark XT

Table S4. IHC conditions for staining whole ovarian tissue.

cc1/cc2: cell conditioning #1/#2.

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Antibody	Clone	Dilution (IHC)	Dilution (WB)	Catalog Number	Supplier		
ARID1A	polyclonal	1/50	-	HPA005456	MilliporeSigma	Burlington, MA, USA	
CDX2	EPR2764Y	Pre-diluted	-	235R-16	Cell Marque	Rocklin, CA, USA	
CK18	CD-10	1/1000	1/2000	sc-6259	Santa Cruz Biotechnology Inc.	Dallas, TX, USA	
CK19	Ks19.1	1/2000	1/500	MS-198-P	Thermo Fisher Scientific Inc.	Waltham, MA, USA	
CK20	SPM140	1/200	-	sc-56522	Santa Cruz Biotechnology Inc.	Dallas, TX, USA	
CK7	OV-TL 12/30	1/200	1/1000	MS-1352-P	Thermo Fisher Scientific Inc.	Waltham, MA, USA	
CK8	TS1	1/1000	1/500	MS-997-P	Thermo Fisher Scientific Inc.	Waltham, MA, USA	
ER	SP1	1/100	1/100	ab16660	Abcam	Cambridge, UK	
HER2	3B5	1/1000	1/1000	OP15L	MilliporeSigma	Burlington, MA, USA	
MUC2	MRQ-18	1/100	-	291M-16	Cell Marque	Rocklin, CA, USA	
MUC5A	MRQ-19	1/250	-	292M-96	Cell Marque	Rocklin, CA, USA	
Napsin A	IP64	1/100	-	NAPSINA-L-CE	Leica Biosystems	Wetzlar, Germany	
p53	DO-1	1/1000	1/200	sc-126	Santa Cruz Biotechnology Inc.	Dallas, TX, USA	
PAX8	polyclonal	1/300	1/5000	10336-1-AP	Proteintech	Rosemont, IL, USA	
PR	Y85	Pre-diluted	-	323R-17	Cell Marque	Rocklin, CA, USA	
SATB2	EPNCIR130A	1/100	-	ab92446	Abcam	Cambridge, UK	
WT1	6F-H2	Pre-diluted	1/250	05-753	MilliporeSigma Burlington, MA, USA		
β-Actin	AC-15		1/1000	ab6276	Abcam Cambridge, UK		
Gt x Ms	polyclonal (2°)		1/5000	AP124P	Sigma-Aldrich	St. Louis, MO, USA	
Gt x Rb	polyclonal (2°)		1/10000	AP156P	Sigma-Aldrich	St. Louis, MO, USA	

2°: secondary.

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Filters

Eccentricity min. (µm²)

Eccentricity max. (µm²)

			j		1		I. I.				
Parameters		HGS						М	CC		
		OV2085	TOV2835EP	TOV2881EP	TOV2929D	OV2978	TOV3121EP	OV3291	OV3331	TOV2414	TOV3392D
Segmen	ntation augmentation	0.5	0.5	2	1.4	1	2	1.8	1	0.7	0.1
Cleanum	Hole fill (µm²)	0	5×10^{3}	8×10^{3}	1×10^4	2×10^4	5×10^{3}	2×10^{3}	2×10^{3}	5×10^4	2×10^{3}
Cleanup —	Adjust size (pixels)	-2	8	0	-3	0	4	5	1	5	-2
	Area min. (µm²)	100	1500	600	300	200	1000	850	500	1000	100
T'14	Area max. (µm²)	-	-	-	-	-	-	-	-	-	-

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Table S6. IncuCyte ZOOM 2016B cell line-specific confluence mask parameters.

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References

1. Fleury, H., et al., Novel high-grade serous epithelial ovarian cancer cell lines that reflect the molecular diversity of both the sporadic and hereditary disease. Genes Cancer, **2015**, *6*, 378-398.



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