

Figure S1. Representative images of H-score assessment. Cytoplasmic and nuclear staining of TFPI-2 in OCCC tissues were evaluated, respectively. H-score was calculated as sum of 1 x (percentage of 1+ cells), 2 x (percentage of 2+ cells) and 3 x (percentage of 3+ cells). After enclosing tumor area (black line), Aperio's annotation software (Aperio Cytoplasm Algorithm) identified the cytoplasm and nucleus and classified the signal intensity into 4 levels from 0 (yellow) to 3+ (brown) for cytoplasmic intensity and 0 (cyan) to 3+ (dark blue) for nuclear intensity as shown at the bottom of the panels. Upper panels are IHC image and lower panels are images processed by the software. Left: A representative image of TFPI-2-negative staining (H-score: Cytoplasm 0, nucleus 0). Right: A representative image of TFPI-2-positive staining (H-score: Cytoplasm 57, nucleus 116). TFPI-2, tissue factor pathway inhibitor-2; OCCC, ovarian clear cell carcinoma.

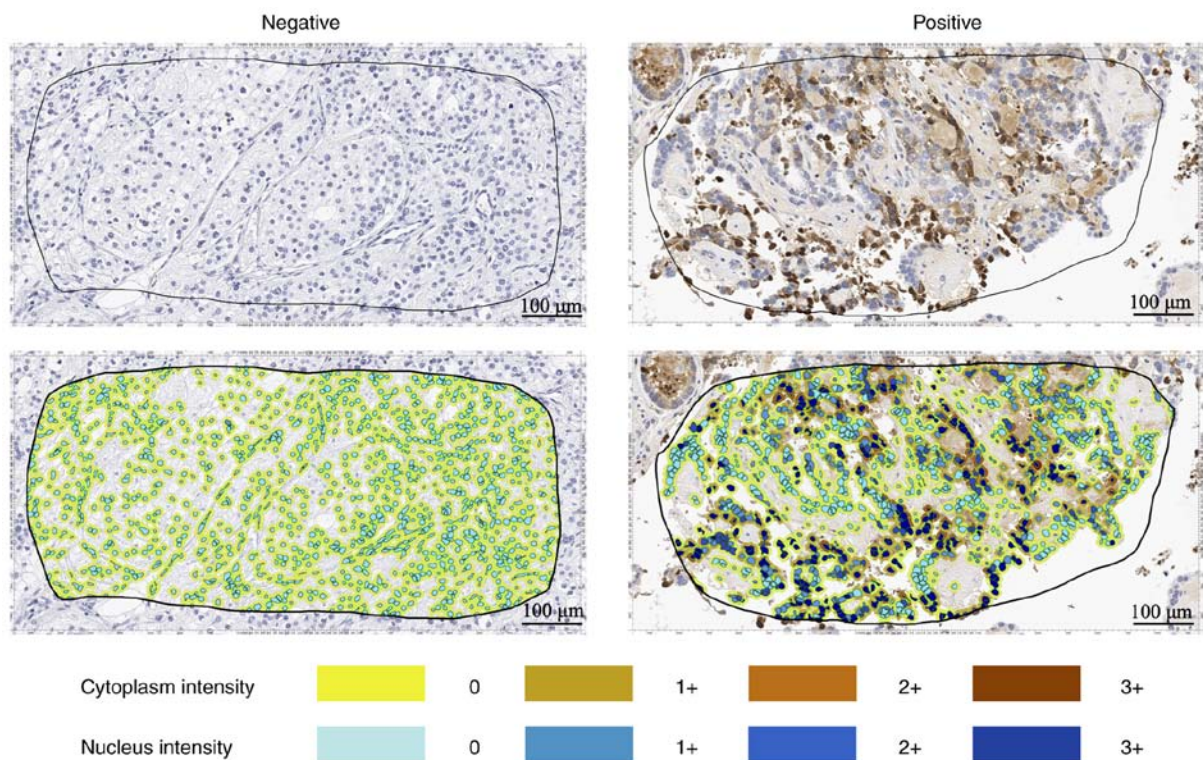


Figure S2. A Venn diagram of TFPI-2 staining pattern in the OCCC cases. Of all 77 OCC cases, 10 (12.9%) cases were nuclear positive (blue circle), 35 (45.5%) cases were cytoplasmic positive (yellow circle) and 35 (45.5%) cases were ECM positive (pink circle). Fifty-two cases (67.5%) were positive in any one of the 3 locations. Twenty-five cases were negative in any locations. TFPI-2, tissue factor pathway inhibitor-2; OCCC, ovarian clear cell carcinoma; ECM, extracellular matrix.

Negative (25)

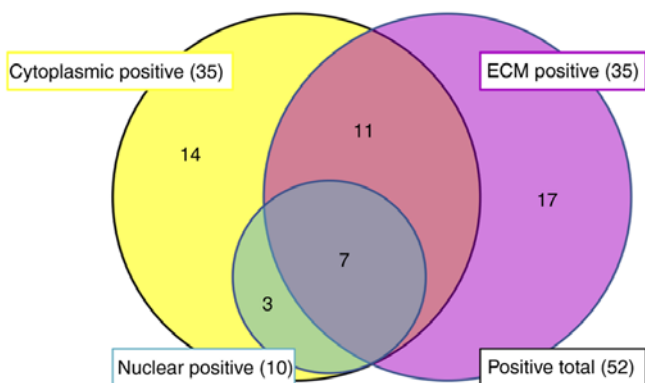


Figure S3. Relationship between cytoplasmic TFPI-2 level and 5-year overall survival evaluated by Kaplan-Meier analysis. TFPI-2 expression was evaluated in each fraction. (A) Cytoplasm. (B) Nucleus. (C) ECM. (D) Any fraction. TFPI-2, tissue factor pathway inhibitor-2; ECM, extracellular matrix.

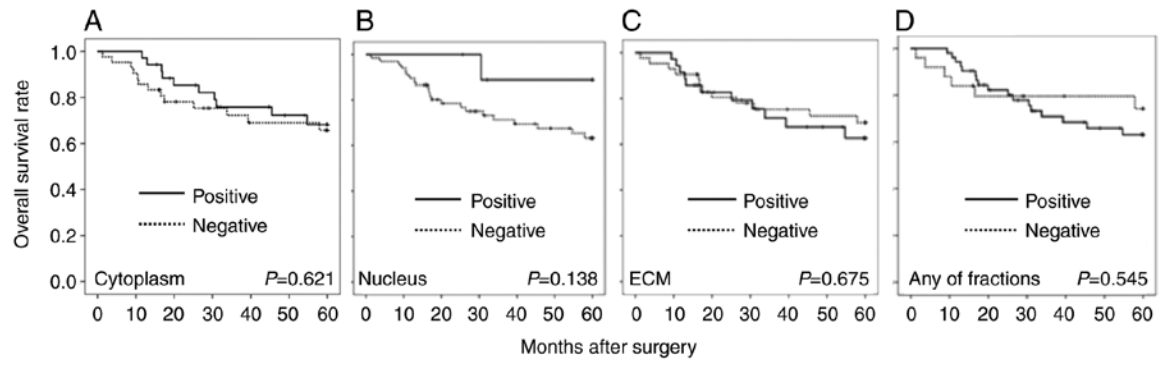


Table SI. Multivariate analysis of 5-year overall survival for cases grouped according to the subcellular localization of TFPI-2 expression in samples analyzed by IHC.

Localization	Variable	HR	95% CI	P-value
Cytoplasm	Age (year)	1.013	0.974 to 1.053	0.526
	Staging (FIGO I/II vs. III/IV)	6.268	2.688 to 14.613	<0.001
	TFPI-2 expression (negative vs. positive)	1.149	0.488 to 2.705	0.751
Nucleus	Age (year)	1.011	0.972 to 1.051	0.593
	Staging (FIGO I/II vs. III/IV)	5.488	2.358 to 12.771	<0.001
	TFPI-2 expression (negative vs. positive)	0.456	0.058 to 3.582	0.456
ECM	Age (year)	1.016	0.975 to 1.059	0.440
	Staging (FIGO I/II vs. III/IV)	6.251	2.728 to 14.325	<0.001
	TFPI-2 expression (negative vs. positive)	1.382	0.582 to 3.282	0.463
Any of the fractions	Age (year)	1.020	0.979 to 1.063	0.440
	Staging (FIGO I/II vs. III/IV)	6.904	2.947 to 16.176	<0.001
	TFPI-2 expression (negative vs. positive)	0.193	0.715 to 5.273	0.193

Cox regression analysis was used for multivariate analysis of 5-year overall survival for cases grouped into cytoplasm, nucleus, ECM or any location of TFPI-2 IHC positivity. Variables were age, FIGO staging and TFPI-2 expression. TFPI-2, tissue factor pathway inhibitor-2; IHC, immunohistochemistry; FIGO, Federation of Gynecology and Obstetrics; ECM, extracellular matrix; CI, confidence interval; HR, hazard ratio.

Table SII. Relationship between clinicopathological characteristics and TFPI-2 nuclear expression.

Characteristics	Negative (n=67)	Positive (n=10)	P-value
Age in years, median (range)	59 (36-75)	52.5 (42-72)	P=0.091
Parity (%)			
No (0)	31 (46.3)	5 (50.0)	P=0.99
Yes (≥ 1)	36 (53.7)	5 (50.0)	
Menopausal status			
Premenopause	15 (22.4)	3 (30.0)	P=0.691
Postmenopause	52 (77.6)	7 (70.0)	
CA125 (U/ml)			
<35	23 (34.3)	1 (10.0)	P=0.159
≥ 35	44 (65.7)	9 (90.0)	
FIGO			
I/II	51 (76.1)	10 (100)	P=0.110
III/IV	16 (23.9)	0 (0)	
pT			
pT1/2	52 (77.6)	10 (100)	P=0.195
pT3	15 (22.4)	0 (0)	
pN			
pN0	10 (14.9)	2 (20.0)	P=0.466
pN1	1 (1.5)	0 (0)	
pNx	56 (78.6)	31 (88.6)	
M			
M0	64 (95.3)	10 (100)	P=0.99
M1	3 (4.5)	0 (0)	

Mann-Whitney U test was used for continuous variables. Fisher's exact test was used for non-continuous variables. TFPI-2, tissue factor pathway inhibitor-2; CA125, cancer antigen 125; FIGO, Federation of Gynecology and Obstetrics.

Table SIII. Relationship between clinicopathological characteristics and TFPI-2 positivity in ECM.

Characteristics	Negative (n=42)	Positive (n=35)	P-value
Age in years, median (range)	58.5 (36-75)	57 (39-72)	P=0.496
Parity (%)			
No (0)	20 (47.6)	16 (45.7)	P=0.99
Yes (≥1)	22 (52.4)	19 (54.3)	
Menopausal status			
Premenopause	10 (23.8)	8 (22.9)	P=0.99
Postmenopause	32 (76.2)	27 (77.1)	
CA125 (U/ml)			
<35	14 (33.3)	10 (28.6)	P=0.805
≥35	28 (66.7)	25 (71.4)	
FIGO			
I/II	33 (78.6)	28 (80.0)	P=0.99
III/IV	9 (21.4)	7 (20.0)	
pT			
pT1/2	34 (81.0)	28 (80.0)	P=0.99
pT3	8 (19.0)	7 (20.0)	
pN			
pN0	5 (11.9)	7 (20.0)	P=0.53
pN1	1 (2.4)	0 (0)	
pNx	36 (85.7)	28 (80.0)	
M			
M0	41 (97.6)	33 (94.3)	P=0.588
M1	1 (2.4)	2 (5.7)	

Mann-Whitney U test was used for continuous variables. Fisher's exact test was used for non-continuous variables. TFPI-2, tissue factor pathway inhibitor-2; CA125, cancer antigen 125; FIGO, Federation of Gynecology and Obstetrics; ECM, extracellular matrix.

Table SIV. Relationship between the clinicopathological characteristics and TFPI-2 positivity in any subcellular fraction.

	Negative (n=25)	Positive (n=52)	P-value
Age in years, median (range)	60 (36-74)	57 (39-75)	P=0.105
Parity (%)			
No (0)	13 (52.0)	23 (44.2)	P=0.627
Yes (≥1)	12 (48.0)	29 (55.8)	
Menopausal status			
Premenopause	4 (16.0)	14 (26.9)	P=0.393
Postmenopause	21 (84.0)	38 (73.1)	
CA125 (U/ml)			
<35	9 (36.0)	15 (28.8)	P=0.603
≥35	16 (64.0)	37 (71.2)	
FIGO			
I/II	19 (76.0)	42 (80.8)	P=0.765
III/IV	6 (24.0)	10 (19.2)	
pT			
pT1/2	20 (80.0)	42 (80.8)	P=0.99
pT3	5 (20.0)	10 (19.2)	
pN			
pN0	4 (16.0)	8 (15.4)	P=0.538
pN1	1 (4.0)	0 (0)	
pNx	20 (80.0)	44 (84.6)	
M			
M0	24 (96.0)	50 (96.0)	P=0.99
M1	1 (4.0)	2 (3.8)	

Mann-Whitney U test was used for continuous variables. Fisher's exact test was used for non-continuous variables. TFPI-2, tissue factor pathway inhibitor-2; CA125, cancer antigen 125; FIGO, Federation of Gynecology and Obstetrics.