

Table S1. Summary of clinical information in malignant pleural effusion (MPE) and systemic disease with pleural effusion (SDPE)

Case ID	Age	Gender	Diagnosis	TTF-1	TNM	AJCC 8th	Pleural effusion	Serum
Adenocarcinoma with MPE								
1	71	F	Adenocarconoma	Positive	T2N0M1a	4A	A	A
2	65	M	Adenocarconoma	Positive	T3N1M1c	4B	A	A
3	43	F	Adenocarconoma	Positive	T1N3M1c	4B	A	A
4	54	F	Adenocarconoma	Positive	T4N2M1a	4A	A	A
5	68	M	Adenocarconoma	Positive	T3N1M1a	4A	A	A
6	67	F	Adenocarconoma	Positive	TxNxM1a	4A	A	A
7	58	M	Adenocarconoma	Positive	T4N3M1c	4B	A	A
8	66	F	Adenocarconoma	Positive	T4N2M1c	4B	A	A
9	81	M	Adenocarconoma	Positive	T4N3M1a	4A	A	A
10	65	M	Adenocarconoma	Positive	T2bN0M1a	4A	A	A
11	54	M	Adenocarconoma	Positive	T4N2M1b	4A	A	A
12	53	M	Adenocarconoma	Positive	T4N2M1b	4A	A	A
13	89	F	Adenocarconoma	Positive	TxNxM1a	4A	A	A
14	86	F	Adenocarconoma	Positive	TxNxM1a	4A	A	A
15	68	M	Adenocarconoma	Positive	T3N1M1a	4A	A	A
16	53	M	Adenocarconoma	Positive	T4N3M1b	4A	A	A
17	73	F	Adenocarconoma	Positive	T4N3M1a	4A	A	NA
18	62	F	Adenocarconoma	Positive	T1aN2M1a	4A	A	NA
19	90	M	Adenocarconoma	Positive	T2aN2M1b	4A	A	NA
20	92	M	Adenocarconoma	Positive	T4NxM1a	4A	A	NA
21	70	M	Adenocarconoma	Positive	T4NxM1a	4A	A	NA
22	95	M	Adenocarconoma	Positive	T4N0M1a	4A	A	NA
23	57	F	Adenocarconoma	Positive	T3N2M1b	4A	A	NA
24	63	M	Adenocarconoma	Positive	T4N3M1a	4A	A	NA
25	47	F	Adenocarconoma	Positive	T1N0M1a	4A	A	NA
26	46	F	Adenocarconoma	Positive	T1aN0M1a	4A	A	NA
27	66	M	Adenocarconoma	Positive	T3N3M1b	4A	A	NA
28	61	M	Adenocarconoma	Positive	T4N3M1a	4A	A	NA
29	83	M	Adenocarconoma	Positive	T4N3M1a	4A	A	NA
30	70	M	Adenocarconoma	Positive	T4N3M1b	4A	A	NA
31	53	F	Adenocarconoma	Positive	T4N3M1a	4A	A	NA
32	48	F	Adenocarconoma	Positive	T2aN0M1a	4A	A	NA
33	84	M	Adenocarconoma	Positive	T2bN2M1b	4A	A	NA
SDPE								
1	91	F	Acute heart failure	NA	NA	NA	A	A
2	89	M	Valvular heart disease	NA	NA	NA	A	A
3	89	F	Chronic obstructive pulmonary disease	NA	NA	NA	A	A

4	82	M	Congestive heart failure	NA	NA	NA	A	A
5	94	M	Chronic kidney disease	NA	NA	NA	A	A
6	70	M	Acute kidney failure	NA	NA	NA	A	A
7	91	F	Valvular heart disease	NA	NA	NA	A	A
8	83	F	Congestive heart failure	NA	NA	NA	A	A
9	93	F	Valve heart disease	NA	NA	NA	A	A
10	53	M?	Cellulitis related sepsis	NA	NA	NA	A	A
11	76	M	Coronary artery disease	NA	NA	NA	A	A
12	74	M	Chronic heart failure	NA	NA	NA	A	A
13	92	F	Pulmonary embolism	NA	NA	NA	A	A
14	52	M	Liiver cirrhosis	NA	NA	NA	A	A
15	76	M	Type 2 diabetes mellitus with hyperosmolar hyperglycemic state	NA	NA	NA	A	A
16	80	F	Coronary artery disease	NA	NA	NA	A	A
17	72	F	Coronary atery disease	NA	NA	NA	A	A
18	80	F	Liver cirrhosis	NA	NA	NA	A	A
19	48	M	Liver cirrhosis	NA	NA	NA	A	A
20	58	M	Liver cirrhosis	NA	NA	NA	A	A
21	80	F	Coronary artery disease	NA	NA	NA	A	A
22	89	M	Acute respiratory distress syndrome	NA	NA	NA	A	A
23	54	M	Liver cirrhosis	NA	NA	NA	A	A
24	43	M	Liver cirrhosis	NA	NA	NA	A	NA
25	76	M	Liver cirrhosis	NA	NA	NA	A	NA

Abbreviations: A: available, NA: not available

Table S2. Summary of clinical information in surgical specimens and cell blocks

Case ID	Age	Gender	Diagnosis	TTF-1*	TNM	AJCC 8 th when diagnosis	TD**	STAS	PL	Metastatic sites when diagnosis
1	M	66	Solid PADC	+	3A	T2bN2M0***	G3	1	0	Nil
2	F	34	Acinar PADC	+	3A	T1N2M0***	G3	1	0	Nil
3	F	53	Solid PADC	+	4A	T4N2M1a	G3	1	2	Pleura Lung
4	F	67	Papillary PADC	+	3A	T2aN3M0***	G3	1	0	Nil
5	M	67	Micropapillary PADC	+	4A	T3N0M1c	G3	1	1	Brain Pleura Pericardia Bone
6	F	54	Acinar PADC	+	4B	T3N0M1c	G3	1	1	Lung, Bone Brain
7	M	76	Micropapillary PADC	+	4A	T4N2M1b	G3	0	2	Lung, Bone
8	F	28	Solid PADC	+	4A	T2aN0M0***	G3	1	2	Nil
9	M	44	Micropapillary PADC	+	4A	T4N2M1a	G3	1	2	Pleura Lung Peritoneum
10	M	65	Solid PADC	+	3C	T3N3M0***	G3	1	0	Nil
11	F	57	Acinar PADC	+	3C	T3N3M0***	G2	0	2	Brain
12	M	77	Solid PADC	+	4A	T3N0M1a	G3	0	1	Pleura
13	F	64	Papillary PADC	+	4A	T4(m)N0M1a	G3	NA**	NA****	Lung
14	F	73	Cribriiform PADC	+	4b	T2bN0M1c	G3	NA**	NA****	Lung Bone Pleura
15	F	60	Acinar PADC	+	1A	T1aN0M0***	G2	0	0	Lung
16	F	68	Micropapillary PADC	+	3A	T1bN2M0**	G3	1	0	Nil
17	F	48	Acinar PADC	+	3A	T1aN2M0***	G2	0	0	Lung Pleura

* indicates that immunohistochemical staining for TTF-1 includes both surgical specimens and cell blocks.

**Indicates the tumor differentiation, with G2 representing poorly differentiated components including solid, micropapillary, and complex glandular structures less than 20%, whereas G3 indicates over 20%.

*** indicates the patient had no MPE at initial diagnosis; and the MPE occurred more than 3 months later.

**** indicates this is a lung metastasis surgical specimen; the status of the primary tumor including STAS and PL are not available

Abbreviations: SS: Surgical Specimen, CB: Cell Block, TD: tumor differentiation, TNM: Tumor, Node, Metastasis, AJCC: American Joint Committee on Cancer, and STAS: Spread Through Air Spaces.

Table S3. List of antibodies

Antibody	Antibody subtype	Dilution	Manufacturer
TTF-1	Mouse monoclonal	1:200	Dako
Nanog	Rabbit polyclone	1:50/1:1000	Abcam
OCT-4	Rabbit polyclone	1:250/1:000	Abcam
IL-8	Mouse monoclonal	1:50/1:1000	Santa Cruz
CXCR1	Rabbit polyclone	1:100/1:1000	Novus
CD133	Rabbit monoclonal	1:200/1:1500	Abcam
E cadherin	Rabbit polyclone	1:3000	Gene Tex
N cadherin	Rabbit polyclone	1:2000	Gene Tex
Vimentin	Mouse monoclonal	1:3000	Santa cruz
Snail	Rabbit monoclonal	1:1000	Abcam
Twist	Rabbit polyclone	1:1000	Cell Signalin
ABCG2	Rabbit monoclonal	1:1000	Abcam
MDR1	Rabbit monoclonal	1:1000	Abcam
Tubulin	Rabbit polyclone	1:5000	Cell Signalin

Table S4. A paired t-test on effusion samples (n=25) and serum samples (n=10). The statistical results show both p value less than 0.05 (p=0.001 and p=0.007).

Paired Sample T-Test

	Mean	N (Sample Size)	Standard Deviation	Standard Error of Mean
Paired 1 Pleural effusion (U/mL)	392.2243	25	379.03300	75.80660
Pleural effusion (U/mL)	80.450	25	58.9035	11.7807

Paired Sample Correlation

	N (Sample Size)	Correlation Coefficient	Significance (P-value)
Paired 1 Pleural effusion (U/mL) & Pleural effusion (U/mL)	25	-.130	.534

Paired Samples T-Test

	Paired difference					T-Value	Degrees of Freedom	Significance (Two-tailed P-value)
	Mean	Standard Deviation	Standard Error of the Mean	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound			
Paired 1 Pleural effusion (U/mL)- Pleural effusion (U/mL)	311.77465	391.10334	78.22067	150.33512	473.21417	3.986	24	.001

The IL-8 level of MPE and SDPE patients (n=25)

	Mean of paired differences	p-value
MPE_ Pleural effusion(U/mL)- SDPE_ Pleural effusion(U/mL)	311.77	0.001

Paired Sample T-Test

	Mean	N (Sample Size)	Standard Deviation	Standard Error of Mean
Paired 1 Serum (U/mL)	79.733	10	70.2125	22.2031
Serum (U/mL)	2.140	10	3.8018	1.2022

Paired Sample Correlation

	N (Sample Size)	Correlation Coefficient	Significance (P-value)
Paired 1 Serum (U/mL) & Serum (U/mL)	10	.027	.942

Paired Samples T-Test

	Paired difference					T-Value	Degrees of Freedom	Significance (Two-tailed P-value)
	Mean	Standard Deviation	Standard Error of the Mean	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound			
Paired 1 Serum (U/mL)- Serum (U/mL)	77.5922	70.2137	22.2035	27.3643	127.8200	3.495	9	.007

The IL-8 level of MPE and SDPE patients (n=10)

	Mean of paired differences	p-value
MPE_ Serum(U/mL)- SDPE_ Serum(U/mL)	77.59	0.007

Table S5. Wound healing assay raw data.

						Migration				AVG		
NCI-H1792		0	12h	24h		0	12h	24h		0	12h	24h
1st	IL-8(-)	801	738	663		1	1.085366	1.208145	NCI-H1792 IL-8 (-)	1	1.107514	1.223583
	IL-8(+)	805	500	480		1	1.61	1.677083	NCI-H1792 IL-8 (+)	1	1.579409	1.667749
2nd	IL-8(-)	808	723	657		1	1.117566	1.229833				
	IL-8(+)	800	518	476		1	1.544402	1.680672				
3rd	IL-8(-)	805	719	653		1	1.119611	1.232772				
	IL-8(+)	803	507	488		1	1.583826	1.645492				
									AVG			
P#2045		0	12h	24h						0	12h	24h
1st	IL-8(-)	803	700	655		1	1.147143	1.225954	P#2045 IL-8 (-)	1	1.143627	1.23366
	IL-8(+)	818	523	411		1	1.564054	1.990268	P#2045 IL-8 (+)	1	1.556045	1.964117
2nd	IL-8(-)	801	707	647		1	1.132956	1.238022				
	IL-8(+)	808	527	421		1	1.533207	1.91924				
3rd	IL-8(-)	809	703	654		1	1.150782	1.237003				
	IL-8(+)	809	515	408		1	1.570874	1.982843				
									AVG			
P#2045 invasion front		0	12h	24h						0	12h	24h
1st	IL-8(-)	803	700	655		1	1.147143	1.225954	P#2045 IL-8 (-)	1	1.143627	1.23366
	IL-8(+)	818	257	3		1	3.182879	272.6667	P#2045 IL-8 (+)	1	3.20763	169.204
2nd	IL-8(-)	801	707	647		1	1.132956	1.238022				
	IL-8(+)	807	255	5		1	3.164706	161.4				
3rd	IL-8(-)	809	703	654		1	1.150782	1.237003				
	IL-8(+)	809	247	11		1	3.275304	73.54545				

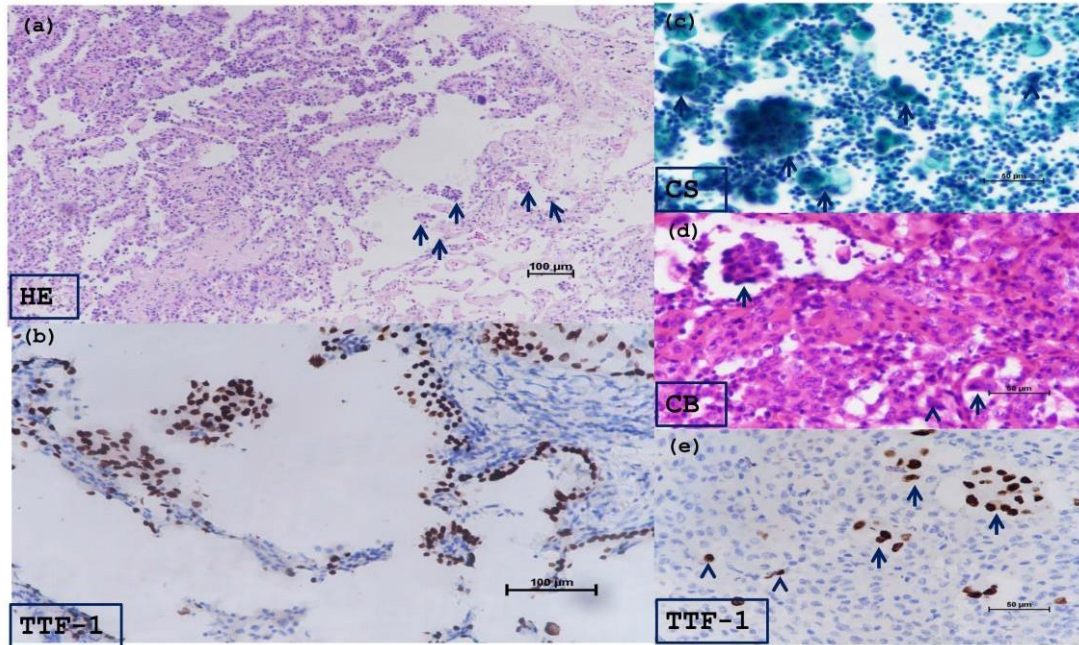


Figure S1. Re-confirmation of the PADC cell line, #P2045, by two pathologists.

(a) The tissue sections of representative cell line, #P2045, showed poorly differentiated PADC with micropapillary pattern and the spread through the air space, indicated by arrows (hematoxylin and eosin, magnification, ×100). (b) IHC confirmed the nuclear expression of TTF-1 indicative of micropapillary PADC (IHC, magnification, ×200). (c) The representative cell line was eventually chosen in our study owing to the grave outcome with transformation to MPE. The sections also showed similar micropapillary pattern with high N/C ratio, hyperchromatism, irregular membrane arranging in a 3-dimensional structure (smear after cytopsin, Papanicolaou stain, magnification, ×400). (D) Further representative cell line in the cell block also showed similar morphology (cell block, hematoxylin and eosin, magnification, ×200). (E) ICC reconfirmed the nuclear expression of TTF-1 in PADC with MPE (ICC, magnification, ×400)

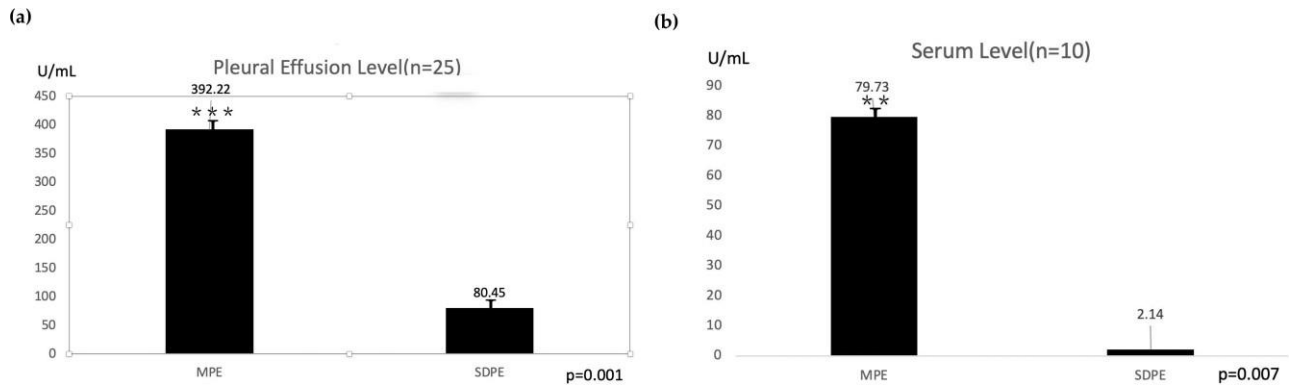


Figure S2. Comparative analysis of IL-8 levels in pleural effusion and serum, a paired t-test on effusion samples (n=25) and serum samples (n=10). (a) The IL-8 level in MPE is significantly elevated compared to SDPE, achieving statistical significance ($p=0.001$, paired t-test). (b) The IL-8 level in MPE is significantly higher than in SDPE, also reaching statistical significance ($p=0.007$, paired t-test) (Experiments were conducted in triplicate).

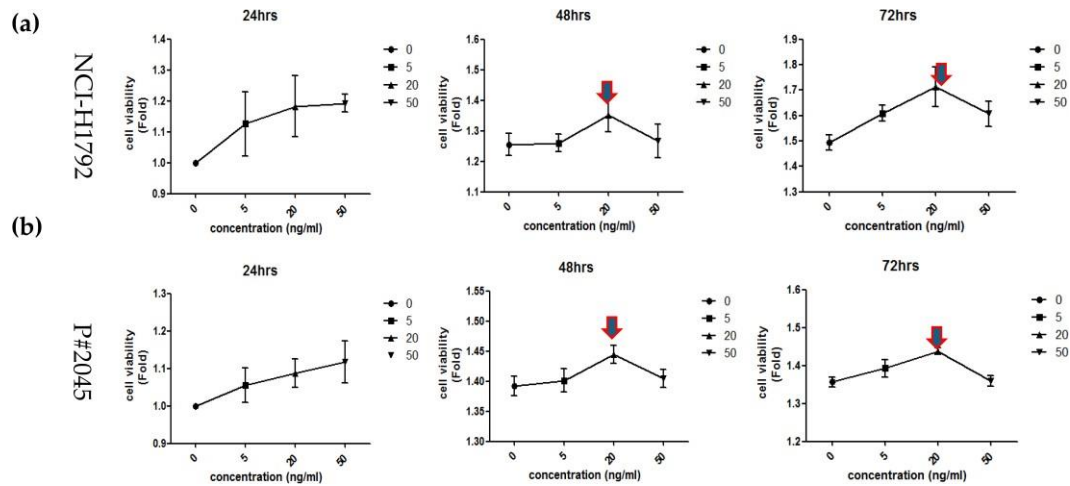


Figure S3. Testing of different dosages and time and determination of the most optimal rIL-8 concentration and reaction time. Experiments were conducted in triplicate. (a) NCI-H1792 was treated at various rIL-8 concentrations (0, 5, 20, and 50 μ M), and cell viability was observed at different times (24, 48, 72 hours). The concentration of 20 μ M and reaction time of 48 hours were chosen to validate whether IL-8 was able to increase the abilities of EMT and CSC properties. (b) The P#2045 was also performed in the same condition, which also showed similar results of the most optimal rIL-8 concentration and reaction time.