

MTAP-Deficiency Identifies a Myofibroblastic and Immune-Attenuated Tumor Microenvironment Subtype in Pancreatic Ductal Adenocarcinoma

Supplementary Information

Julian Karlbauer^{1†*}, Karl Knipper^{2†}, Tillmann Bedau¹, Hans Schloesser², Michael Thomas², Reinhard Buettner¹, Christiane Bruns², Felix C. Popp², Su Ir Lyu^{1#}, Alexander Quaas^{1#} on behalf of the PANCALYZE study group

¹ Institute of Pathology, Faculty of Medicine and University Hospital Cologne, Cologne, Germany

² Department of General, Visceral, Thoracic and Transplantation Surgery, Faculty of Medicine and University Hospital of Cologne, Cologne, Germany

†These authors contributed equally: Julian Karlbauer and Karl Knipper.

#These authors jointly supervised this work: Su Ir Lyu and Alexander Quaas.

*Corresponding author: Julian Karlbauer, julian.karlbauer@fau.de

Supplementary Table S1. Distribution of additional tumor microenvironment and tumor-cell markers within the MTAP-deficient subgroup, dichotomized at the median (low vs high).

Marker	Low, n (%)	High, n (%)	Evaluable cases, n
CD4	71 (49.7%)	72 (50.3%)	143
CD8	72 (50.0%)	72 (50.0%)	144
CD68	72 (49.7%)	73 (50.3%)	145
FOXP3	72 (50.0%)	72 (50.0%)	144
MUM1	72 (49.7%)	73 (50.3%)	145
ERG	72 (49.7%)	73 (50.3%)	145
MCT	72 (49.7%)	73 (50.3%)	145
Tenascin	70 (49.6%)	71 (50.4%)	141
BRM	48 (49.5%)	49 (50.5%)	97
BRG1	72 (49.7%)	73 (50.3%)	145

Markers were assessed only within the MTAP-deficient subgroup. None was significantly associated with overall survival (log-rank, all $p > 0.05$). Case numbers differ between markers due to marker-specific tissue availability on the tissue microarray. n, number of cases.

Supplementary Table S2. Antibodies, staining conditions and scoring criteria used for immunohistochemistry.

Marker	Control tissue	Antibody name	Manufacturer	Clone	Host	Catalogue no.	Dilution	Antigen retrieval	Scoring / cut-off
<i>Cell-type / immune markers (positive cells per sample, digital image analysis)</i>									
CD3	A/T	CD3	Quartett (IVDR)	QR004	Rabbit	C-C002-05	1:200	EDTA	Low <110 / high ≥110 cells per sample
CD20	A/T	CD20	Dako (CE)	L26	Mouse	M0755	1:1250	Citrate	Dichotomized at median
CD38	A/T	CD38	Novocastra (CE)	SPC32	Mouse	NCL-L-CD38-290	1:800	Citrate	Low <15 / high ≥15 cells per sample
CD56	A	CD56	Thermo Fisher (RUO)	123C3	Mouse	MA5-16445	1:500	EDTA	Dichotomized at median
CD66b	A/T	CD66b	Novus Biologicals	G10F5	Mouse	NB100-77808	1:200	EDTA	Low <158 / high ≥158 cells per sample
CD117	Sarcoma TMA	CD117	Biocare Medical (CE)	EP10	Rabbit	CME296C	1:50	Citrate	Dichotomized at median
CD163	A/T	CD163	Cell Marque (CE)	MRQ-26	Mouse	163M-16	1:100	EDTA	Dichotomized at median
<i>CAF / stromal markers (digital image analysis)</i>									
α-SMA	A	α-smooth muscle actin	Dako (CE)	1A4	Mouse	M0851	1:1000	EDTA	45th percentile
FAP	Female TMA	Fibroblast activation protein	Abcam (RUO)	EPR20021	Rabbit	ab207178	1:200	Citrate	Dichotomized at median
PDGFRβ	Male TMA	PDGFRβ	Abcam (RUO)	Y92	Rabbit	ab32570	1:300	EDTA	Dichotomized at median
Periostin	Colon	Periostin	Abcam	EPR19934	Rabbit	ab219056	1:2000	EDTA	Dichotomized at median
<i>Tumor-cell / molecular markers (scored manually by pathologists)</i>									
MTAP	A	MTAP	Cell Signaling Technology (RUO)	E5R1l	Rabbit	74683S	1:1000	EDTA	Binary: loss vs. retained
Claudin 18.2	Stomach	Claudin 18.2	Roche (CE)	43-14A	Mouse	8504148001	Ready-to-use	EDTA	Positive: ≥75% of cells at ≥2+ staining intensity
CK6	T	Cytokeratin 6	HUABIO	SN71-07	Rabbit	ET1611-70	1:200	EDTA	Three-tier: negative / low / high
CK5/6	T	Cytokeratin 5/6	Cell Marque	D5 & 16B4	Mouse	356M	1:50	EDTA	Three-tier: negative / low / high
γH2AX	Breast carcinoma	γH2AX	Abcam	EP854(2)Y	Rabbit	ab81299	1:200	EDTA	H-score #; high ≥120
XIAP		XIAP	Abcam	polyclonal	Rabbit	ab21278	1:1000	Citrate	Binary: negative vs. positive

Supplementary Table S2. Antibodies, staining conditions and scoring criteria used for immunohistochemistry.

Marker	Control tissue	Antibody name	Manufacturer	Clone	Host	Catalogue no.	Dilution	Antigen retrieval	Scoring / cut-off
HK2	Normal esophageal epithelium	HK2	Abcam	3D3	Mouse	ab104836	1:500	EDTA	H-score #; median <100 / ≥100
Extended panel — MTAP-deficient cases only (digital image analysis)									
CD4	A/T	CD4	Quartett (IVDR)	QR032	Rabbit	C-C012-10	1:100	EDTA	Dichotomized at median
CD8	A/T	CD8	Dako (CE)	C8/144B	Mouse	M7103	1:200	Citrate	Dichotomized at median
FOXP3	T	FOXP3	Abcam	36A/E7	Mouse	ab20034	1:100	EDTA	Dichotomized at median
CD68	T	CD68	Dako (CE)	PG-M1	Mouse	M0876	1:400	EDTA	Dichotomized at median
MUM1	A/T	MUM1	Dako (CE)	MUM1p	Mouse	M7259	1:300	Citrate	Dichotomized at median
ERG	A/T	ERG	Cell Marque (CE)	EP111	Rabbit	434R-15	1:300	EDTA	Dichotomized at median
MCT	A	MCT	Dako	AA1	Mouse	M7052	1:4000	Enzyme	Dichotomized at median
Tenascin C	Collagenous colitis	Tenascin C	Abcam (RUO)	EPR4219	Rabbit	ab108930	1:400	Citrate	Dichotomized at median
BRG1	Tumor with BRG1 loss	BRG1/SMARCA4	Abcam (RUO)	EPNCIR111 A	Rabbit	ab110641	1:300	EDTA	Dichotomized at median
BRM	Tumor with BRM loss	BRM	Cell Signaling Technology (RUO)	D9E8B	Rabbit	11966S	1:50	EDTA	Dichotomized at median

H-score computed as described by Mazières et al.[32]

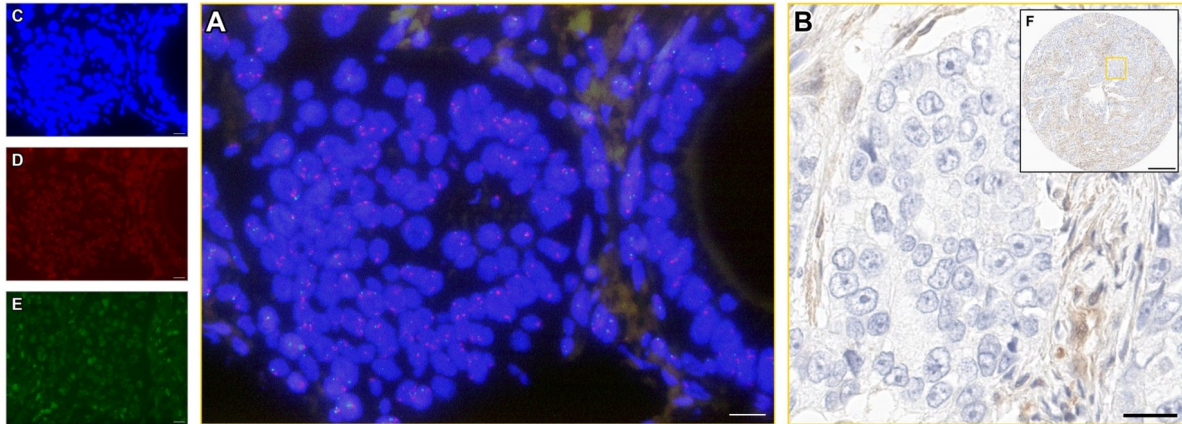
A: appendix vermiciformis; T: tonsilla palatina

Supplementary Table S3. Tumor microenvironment, CAF, and tumor subtype-associated markers stratified by MTAP status. Data corresponds to Forest Plot in Figure 2.

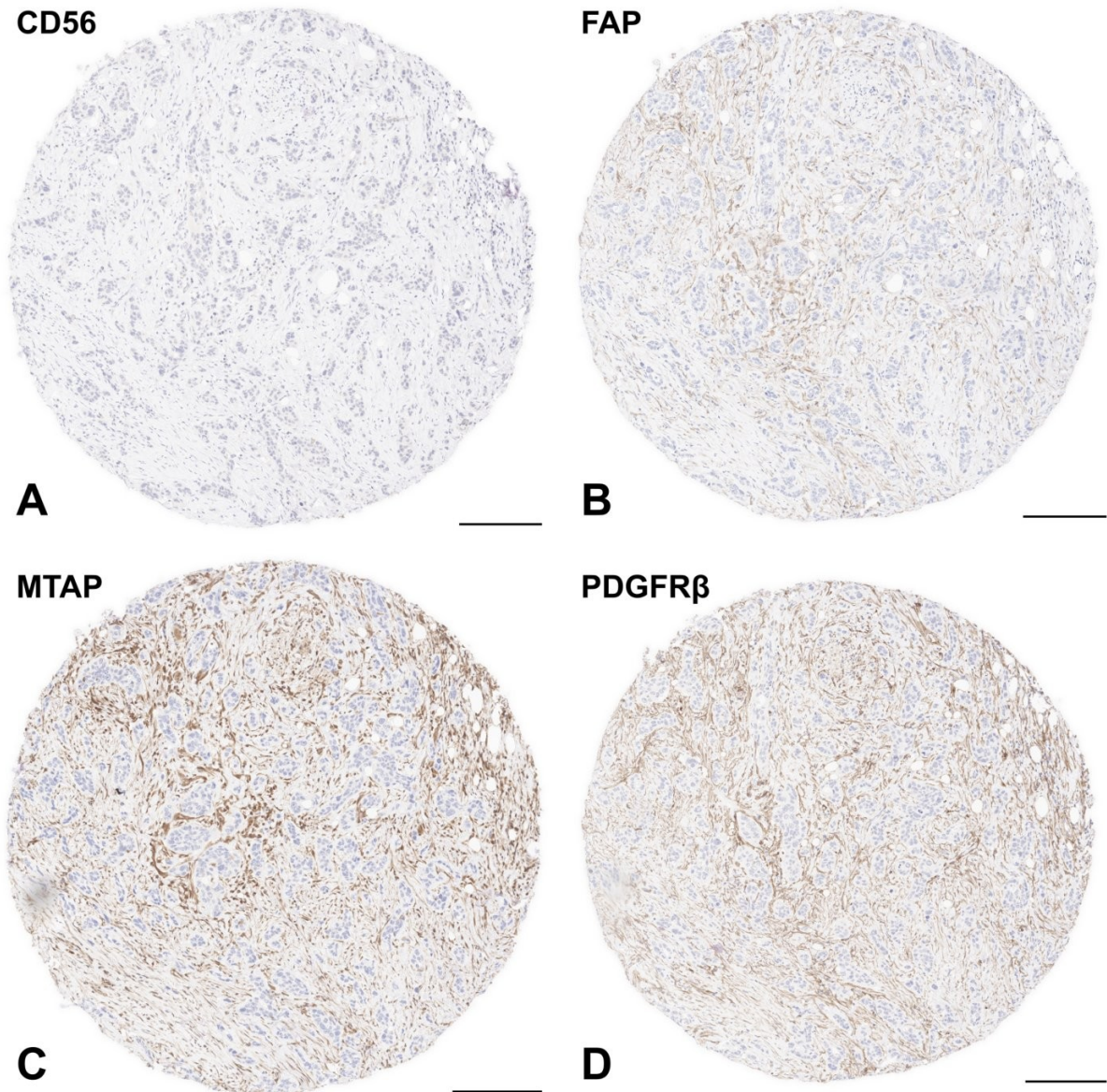
Marker	Compartment / interpretation	MTAP loss n/N (%)	MTAP retained n/N (%)	p
<i>Cancer-associated fibroblast markers</i>				
SMA	CAF / myCAF	55/94 (58.5)	104/224 (46.4)	0.049
FAP	CAF / myCAF	83/145 (57.2)	102/224 (45.5)	0.028
PDGFR β	CAF / stroma	57/145 (39.3)	128/224 (57.1)	<0.001
Periostin	CAF / stroma	73/145 (50.3)	112/224 (50.0)	0.948
<i>Immune cell markers</i>				
CD38	Plasma cells	39/94 (41.5)	120/224 (53.6)	0.049
CD56	NK cells	61/144 (42.4)	122/222 (55.0)	0.019
CD3	T cells	53/94 (56.4)	113/223 (50.7)	0.353
CD20	B cells	63/127 (49.6)	111/221 (50.2)	0.911
CD66b	Neutrophils	74/145 (51.0)	111/224 (49.6)	0.781
CD117	Mast cells	47/94 (50.0)	112/223 (50.2)	0.971
CD163	Macrophages	75/145 (51.7)	110/224 (49.1)	0.623
<i>Tumor subtype-associated and molecular markers</i>				
Claudin 18.2	Tumor subtype-associated marker	33/91 (36.3)	57/204 (27.9)	0.152
γ H2AX	DNA damage response	41/90 (45.6)	88/207 (42.5)	0.627
HK2	Tumor metabolism	40/88 (45.5)	113/214 (52.8)	0.246
CK6	Basal-like differentiation	25/94 (26.6)	59/220 (26.8)	0.652
CK5/6	Basal-like differentiation	18/94 (19.1)	43/220 (19.5)	0.722
XIAP	Apoptosis-related	79/94 (84.0)	179/221 (81.0)	0.835

Values are n/N (%) for the positive/high category shown; N = evaluable cases per marker. p-values are uncorrected Pearson χ^2 tests (original analysis); bold, $p < 0.05$. For the three ordinally scored markers the plotted proportion is dichotomized for display only — CK6/CK5/6, score 2; XIAP, scores 1–3 — while the p-value is the Pearson χ^2 of the full score distribution (0/1/2 or 0/1/2/3).

Supplementary Figure S1. MTAP loss corresponds to homozygous CDKN2A (9p21) deletion. Region-matched CDKN2A FISH and MTAP IHC on the same tumor core. CDKN2A FISH: (A) merged image, with the single channels shown separately — (C) DAPI (blue), (D) CEP9 (orange) and (E) CDKN2A/p16 (green). Tumor nuclei retain CEP9 (orange) but lack CDKN2A/p16 (green), indicating homozygous deletion of CDKN2A. (B) Corresponding MTAP IHC: MTAP loss in tumor glands with retained stromal expression. (F, inset) Low-magnification overview of the same core, with the boxed region shown magnified in (B). Scale bars: 10 μ m (A, C–E), 20 μ m (B) and 200 μ m (F).



Supplementary Figure S2. Significantly altered microenvironmental markers on a single MTAP-deficient tumor core, stained for (A) CD56, (B) FAP, (C) MTAP and (D) PDGFR β . Brown = positive, blue = negative. α -SMA and CD38 are not shown (no digital image of this core available). Scale bars: 200 μ m.



Supplementary Figure S3. MTAP immunohistochemistry in cases with ambiguous or heterogeneous loss. Representative cases in which MTAP IHC on the tissue microarray did not show unequivocally homogeneous loss and for which large-section (whole slide) staining was subsequently requested to clarify the expression pattern. Low magnification cores are shown with high-magnification insets. Several cases display regional or subclonal heterogeneity of MTAP expression. Brown = retained (positive) cytoplasmic MTAP; blue (hematoxylin counterstain only) = loss. Scale bars: 200 μ m (A, B, D, E, I, J), 100 μ m (F), 50 μ m (C, H, K) and 40 μ m (G).

