Respiratory Complex I Regulates Dendritic Cell Maturation in Explant Model of Human Tumor Immune Microenvironment

Supplementary material

Supplementary Fig. 1. Characterization of PDEC immune clusters and activity pathways a, clustering of single cell RNA data into major and **b,** minor immune cell subtypes based on known gene expression profiles for each cell type **c,** volcano plots of the genes used to compare basal activity of pathways including NK cell functions, T cell functions, B cell functions, cytokines and interleukins, TLR, senescence and macrophage functions of PDECs grown 72hrs compared to primary tumor material. The significance of individual genes is separated by horizontal lines of defined adjusted p-values.

Supplementary Fig. 2. Immune cell status of primary tumor material, and flow cytometry analysis of PDECs a, Primary tumor immune infiltration of three independent, randomly selected primary breast cancer samples (P832T, P837T, P838T) corresponding to PDECs from figure 2 showing CD45+ leukocytes (yellow) and tumor cells (purple). The scale is 50um. **b-d,** 3-7 technial replicates of fragments within the PDECs of donors P1560T, P1608T, and P1607T. The scale is 10um. Stainings are indicated on the images: Hoechst (blue), CD3 (green), CD45 (red), F-actin (yellow). **e,** flow cytometry gating guide to determine checkpoint marker expression of CD3+ T cells from PDECs. Debris, doublets, and non-viable cells were removed from the analysis. CD45+ leukocytes and CD3+ CD56- T cells were analyzed for figure 2k-o.

Supplementary Fig. 3. MYC protein staining of primary breast cancer tissue a, c-MYC staining of 10 biologically independent patient tumors used in Fig 3b. Samples were scored for MYC positivity by from 0-100% in increments of 0-20%, 21-40%, 41-60%, and 61-100%.

Supplementary Fig. 4. Modulation of APCs in response to paclitaxel, VeM, and metformin-alone a, volcano plot of gene-expression changes in response to paclitaxel + anti-PD-L1 **b,**

Turpin R, *et al. J Immunother Cancer* 2024; 12:e008053. doi: 10.1136/jitc-2023-008053

paclitaxel and **c,** Venetoclax +metformin + anti- PD-L1 through nanostring gene expression profiling **d,** RT-qPCR increase of LAMP-3 in 10 PDECs in response to metformin **e,** flow cytometry of absolute number of APCs in 4 PDECs with no significant change in cell numbers **f,** flow cytometry grating used to quantify **g,** the proportion of DC-like cells within the APCs and **h,** the proportion of mono-macs expressing high levels of CD163. Data are presented as mean values +/- SD. Statistical significance was tested with a one-way ANOVA with Fishers exact test**. i,** a statistically significant increase in CD86 expression in DC-like cells following metformin treatment using a student's t-test.

Supplementary Fig. 5. Ex vivo DC characterization and in vivo effects of metformin a, individual datapoints from Fig4e showing the baseline characterization of live CD45+ with varying levels of HLA-DR and CD11c expression **b,** spatial transcriptomics of WapMYC mouse tumor tissue showing decreased CD68 macrophage expression (n=1) following metformin treatment **c,d,** volcano plot of differentially expressed genes in metformin treated tumor tissue

Supplementary Fig 6. Characterization of tissue areas in spatial transcriptomics tumor tissue

a, Top expressed genes used to characterize 9 independent tissue type clusters from spatial transcriptomics data for comparison from WAPMYC mouse tumor tissue

Supplementary Fig 7. Gene expression chances in PBMC-derived DCs following metformin treatment a, 25 upregulated (red) and downregulated (blue) differentially expressed genes of PBMC-derived human DCs in response to 24hr treatment of 10mM metformin from bulk RNA sequencing.

Supplementary Figure 8. Metabolic changes in dendritic cells following metformin treatment, and effects on total T cell division. **a**, schematic representation of Seahorse extracellular flux assay **b,** OCR values of three donors following control (blue), 5mM (purple) or 10mM (pink) metformin treatment. c, ECAR values of three donors following control (blue), 5mM (purple) or 10mM (pink) metformin treatment **d,** schematic of timeline for DC, T cell co-culture assay used in Fig5j-k. **e,** flow cytometry analysis of CD4+ T cell division index

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indicating the proportion of the total sample that is dividing (G0-G4) with significant increase in overall CD4+ T cell division following 5mM metformin (p= 0.0050), and 30nM rotenone (p=0.0148) treatments, and a significance decrease in CD4+ T cell proliferation following 10nM A769662 treatment (p=0.0184). Statistical significance was tested with a one-way ANOVA with Fishers exact test.

Supplementary Fig. 9. Breast cancer patient molecular and histological subtypes a, molecular subtypes of patients from which PDECs were derived for this study. HER2+ = ER-, PR-, HER2+; TNBC = ER-, PR-, HER2-, Luminal = ER+, PR +/-, HER2 +/-. **b,** histological characterization of patients from which PDECs were obtained for this study.

Supplementary table 1: Flow cytometry antibodies

Target	Color	Species	Target	Clone	Catalog	Company
CD103	unconjugated	Rabbit		EPR22590-27	ab224202	abcam
CD11b	AlexaFluor488	Rat	Human	M1/70	557672	BD
CD11c	FITC		Human	3.9	301604	BioLegend
CD11c	PE	Mouse	Human	3.9	565910	BD
CD11c	unconjugated				PA5-90208	Invitrogen
CD11c	Pe-Cy5	Mouse	Human	3.9	301610	Biolegend
CD123	PE-Cy7	Mouse	Human	7G3	560826	BD
CD123	BV785	Mouse	Human	6H6	2130160	Sony
CD14	BV480	Mouse	Human	MøP9	566141	BD
CD14	BV510	Mouse	Human	MøP9	563079	BD
CD14	APC	Mouse	Human		555399	BD
CD14	Pacific blue	Mouse	Human	M5E2	301828	Biolegend
CD15	PerCP-Cy5.5	Mouse	Human	HI98	560828	BD
CD16	PE	Mouse	Human	B73.1	561313	BD
CD163	BV650	Mouse	Human	GHI/61	563888	BD
CD163	BV786	Mouse	Human	GHI/61	741003	BD
CD163	FITC	Mouse	Human	GHI/61	563697	BD
CD163	BV650	Mouse	Human	GHI/61	563888	BD
CD183	AlexaFluor488	Mouse	Human	1C6/CXCR3	558047	BD
CD184	APC	Mouse	Human	CXCR4	560936	BD
CD19	PE-CF594	Mouse	Human	HIB19	562321	BD
CD19	BV605	Mouse	Human	HIB19	302244	Biolegend

CD1c	PerCP-Cy5.5	Mouse	Human	F10/21A3	565423	BD
CD1c	APC					
CD206	APC-Cy7		Human	15-2	321120	BioLegend
CD206	PE	Mouse	Human		555954	BD
CD3	PerCP-Cy5.5	Mouse	Human	SK7	332771	BD
CD3	Pe-Cy5.5					
CD4	PE-Cy7	Mouse	Human	RPA-T4	560649	BD
CD45	APC-H7	Mouse	Human	2D1	560178	BD
CD45	Pacific orange	Mouse	Human	2D1	PO-160-T100	EXBIO
CD45 (NCL-L-LCA)	unconjugated	Mouse	Human		NCL-L-LCA	Novocastra
CD56	BV421	Mouse	Human	NCAM16.2	562751	BD
CD56	APC-Cy7		Human	HCD56	318332	BioLegend
CD56	APC-Cy7					
CD64	PE		Human	10.1	305008	BioLegend
CD64	PE					
CD66b	AlexaFluor647	Mouse	Human	G10F5	561645	BD
CD69	APC		Human	L78	340560	BD
CD8	BV510	Mouse	Human	SK1	563919	BD
CD80	BV510	Mouse	Human	L307.4	563084	BD
CD80	AlexaFluor647		Human	2D10	305216	BioLegend
CD86	PE-Cy7	Mouse	Human	2331	561128	BD
CD86	PE-Cy7		Human	IT2.2	305422	BioLegend
CD86	Pe-Cy7	Mouse	Human	IT2.2	305422	Biolegend
CD8a	unconjugated	Rabbit		EPR20305	ab209775	abcam
CD8a	Pe-Cy5		Human	HIT8a	300909	BioLegend
EpCAM	PerCP-Cy5.5		Human	EBA-1	347199	BD
Fc epsilon R1 alpha	FITC	Mouse	Human	CRA1	130-117-361	Miltenyi
FOXP3	PE	Mouse	Human	259D/C7	560046	BD
GZMB	PE-Cy7	Mouse	Human	GB11	561142	BD
HLA DR	AlexaFluor 594	Mouse	Human	L243	NB100-77855AF594	Novus Biologicals
HLA-DR	PE		Human	L243	307606	BioLegend
HLA-DR	APC	Mouse	Human		559866	BD
HLA-DR	APC-R700	Mouse	Human	G46-6	565127	BD
LAG-3	PE		Human	3DS223H	12-2239-42	eBioscience
Mannose Receptor	AlexaFluor 700	Mouse	Human	15 2	321132	Biolegend
PD1	FITC	Mouse	Human	MIH4	557860	BD
PDL1	PE-Cy7	Mouse	Human	MIH1	558017	BD

PDL1	PerCP-Cy5.5	Rat	Human	MIH5	NBP1-43262PECY55	NovusBio
Propidium Iodide					421301	Biolegend
PDL2 (CD273)	APC	Mouse	Human	MIH18	557926	BD
CD45	BV421	Mouse	Human	L161	331541	Biolegend
CD11c	FITC	Mouse	Human	3.9	565910	Biolegend
HLA-DR	PE	Mouse	Human	L243	307606	Biolegend
CD14	BV510	Mouse	Human	MφP9	563079	BD
CD86	PE-Cy7	Mouse	Human	IT2.2	305422	Biolegend
CD83	PerCP-Cy5.5	Mouse	Human	HB15e	305320	Biolegend
CD40	APC	Rat	Human	3/23	124611	Biolegend
CD1a	BV711	Mouse	Human	HI149	300139	Biolegend
CD1c	BV650	Mouse	Human	L161	331541	Biolegend

Supplementary table 2: IHC antibodies

Target	Fluorochrome	Species	Target	Dilution	Catalog	Company
FoxP3	TSA-488	Mouse	Human	1:200	ab20034	Abcam
CD3	TSA-555	Rabbit	Human	1:750	MA5-14482	Invitrogen
CD8	Alexa-647	Mouse	Human	1:300	M7103	DAKO
CD4	Alexa-750	Rabbit	Human	1:25	ab133616	Abcam
CD45	Alexa-647	Rabbit	Human	1:100	CST13917	Cell signaling technology
PanEpi Cocktail (PanCK, E-cadherin)	Alexa-750	Mouse	Human	1:150 1:100 1:200	ab7753, MA5-13156, 610182	Abcam Invitrogen BD
CD8a	Unconjugated	Rabbit	Mouse	1:2000	ab209775	Abcam
CD103	Unconjugated	Rabbit	Human/ Mouse	1:5000	ab224202	Abcam
c-MYC	Unconjugated	Rabbit	Human/ Mouse	1:200	ab32072	Abcam

Supplementary table 3: Primers

Name	Primer nucleotide sequence (5'-3'):
Human Arg1 Forward	GAAAGGCTGGTCTGCTTGAG
Human Arg1 Reverse	CACAGACCTTGGATTCTTCACA
Human iNOS Forward	AATCTCTGGTCAAGCTGGATG
Human iNOS Reverse	GCAAGATTTGGACCTGCAAG
Human Lamp3 Forward	TTGACCGTCTCAGATCCAGA
Human Lamp3 Reverse	CTCTGTTCACTCACGCACTT
Human CIITA Forward	TACTCAGAACCCGACACAGA
Human CIITA Reverse	CCGATCACTTCATCTGGTCC
Human IFNy Forward	TTTAATGCAGGTCATTCAGATG
Human IFNy Reverse	AGACAATTTGGCTCTGCATT
Human CD47 Forward	TAGATCCGGTGGTATGGATG

Human CD47 Reverse	ATATTCACCTGGGACGAAAG
Human PRF1 Forward	CGCCTACCTCAGGCTTATCTC
Human PRF1 Reverse	CCTCGACAGTCAGGCAGTC
Human GZMB Forward	CCCTGGGAAAACACTCACACA
Human GZMB Reverse	CACAACTCAATGGTACTGTCGT
Human GAPDH Forward	CTCTGCTCCTCCTGTTCGAC
Human GAPDH Reverse	GCCCAATACGACCAAATCC
Human ActB Forward	CTTCACCACCACGGC
Human ActB Reverse	CCATCTCTTGCTCGAAG
Human PUM1 Forward	GCCCCAGTCTTTGCAATTTA
Human PUM1 Reverse	AATCACTCGGCAGCCATAAG