Targeting VEGF-A induced lymphatic remodeling a new approach to boost immunity in PDAC

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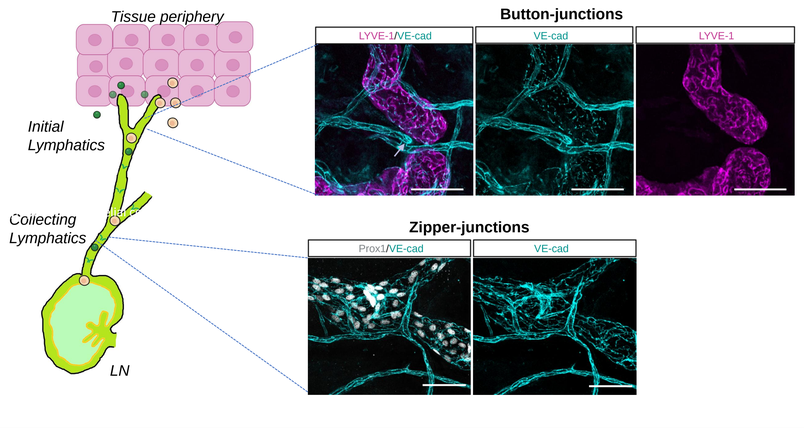
Abstract and Background

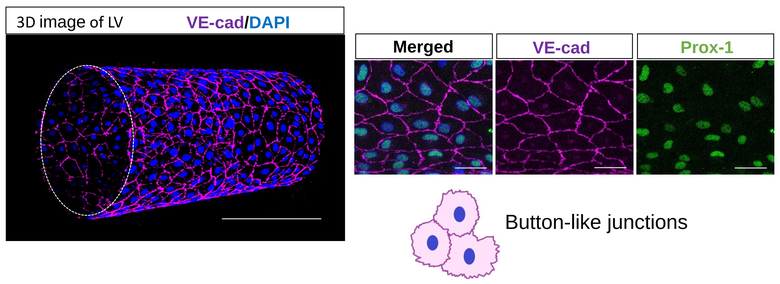
Pancreatic ductal adenocarcinoma (PDAC) remains one of the deadliest tumors in humans. PDAC has an immunosuppressive tumor microenvironment (TME), constituting poor immunotherapy outcomes [1]. Lymphatic vessels (LVs) traffic leukocytes and antigens from tumor peripheries to draining lymph nodes for effective activation of anti-cancer immune responses. Impairment of the lymphatic function may result in delayed or ineffective immune activation[2,3].

In this study we aim to investigate PDAC-induced LV remodeling and its consequences for immune cell egress from primary tumor through LVs. We use our innovative three-dimensional (3D) LV-on-chip technology to track phenotypic and functional changes in lymphatic endothelial cells (LECs) in co-culture with PDAC. Our studies discovered that PDAC progression tightens adherence junctions on LECs resembling “zipper-like” junctions, making them less permeable, compared to physiological LECs with jagged “button-like” junctions. This led us to hypothesize that altered LEC morphologies affect lymphatic fluid uptake and leukocyte transmigration through the LVs in PDAC. We demonstrated that VEGF-A secreted by PDAC cells activates tightening of endothelial junctions in a VEGFR2- dependent manner *in vitro*. Finally, we use the Kaede transgenic (Kd) mouse model to track the egression of leukocytes from the primary PDAC tumor site to the draining lymph nodes (dLNs). Kaede optogenetic model gives us power to label cells that recently were recruited to tissue (Kaede-green), cells that retained (Kaede-red) and cells that exited to dLNs (Kaede-red). We showed in our system that immune response progressively increases over time, while PDAC tumor environment may favor retention or exit of certain immune cell. Altogether, these data demonstrate the utility of the Kaede transgenic system for tracking multiple leukocyte lineages and support its use in mechanistic studies with VEGF-A/VEGFR-2 inhibitors

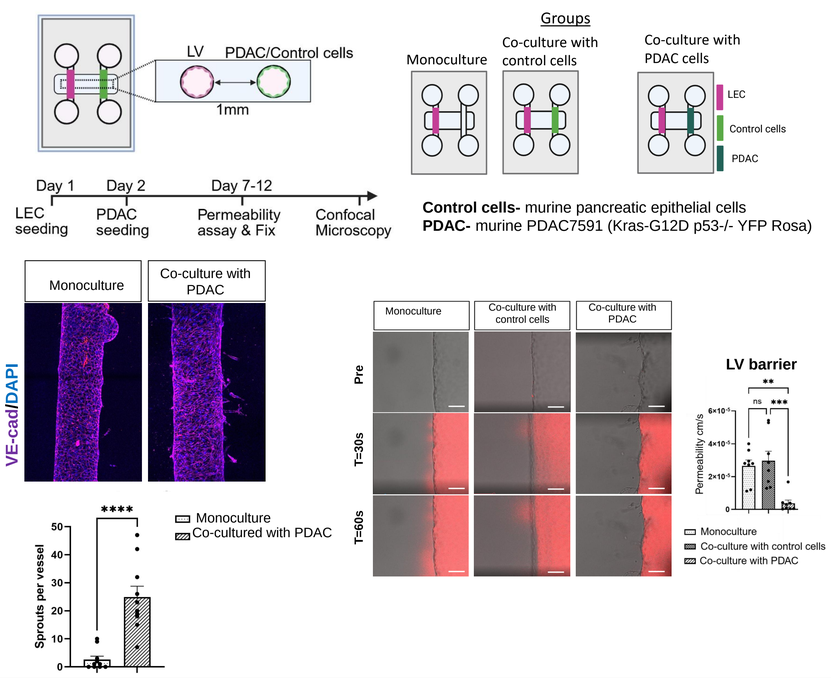
Structure of lymphatic vessels at steady state

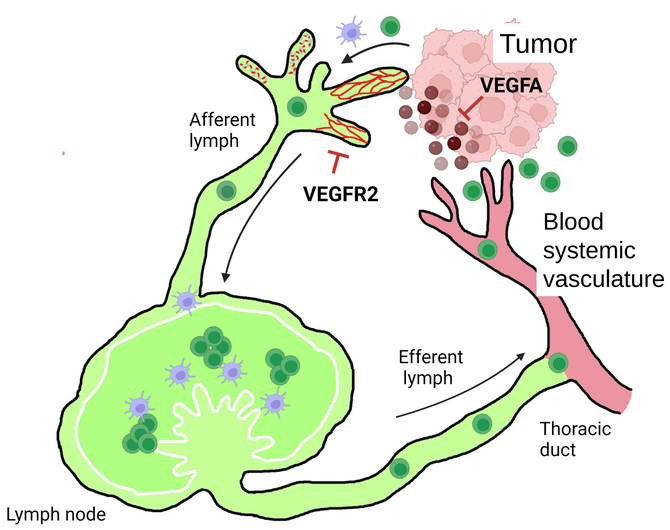


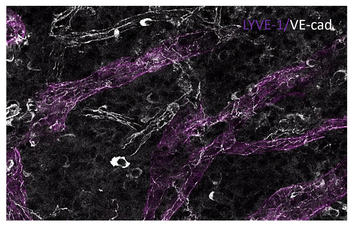
Bioengineered lymphatic vessel on-a-chip



PDAC- LVs co-culture model

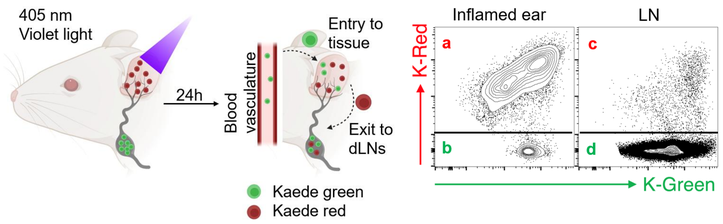






Left: Confocal image of LYVE1 positive lymphatic vessel surrounding PDAC tumor in pancreas. VE- cadherin stained in yellow shows zipper-like junctions. Cancer cells in Matrigel were injected into pancreas. Three weeks later tumor tissues were collected and subjected to IHC. Lyve-1 (Lymphatic vessel endothelial hyaluronan receptor) stains for primary lymphatic vessels, VE-cadherin stains junctions.

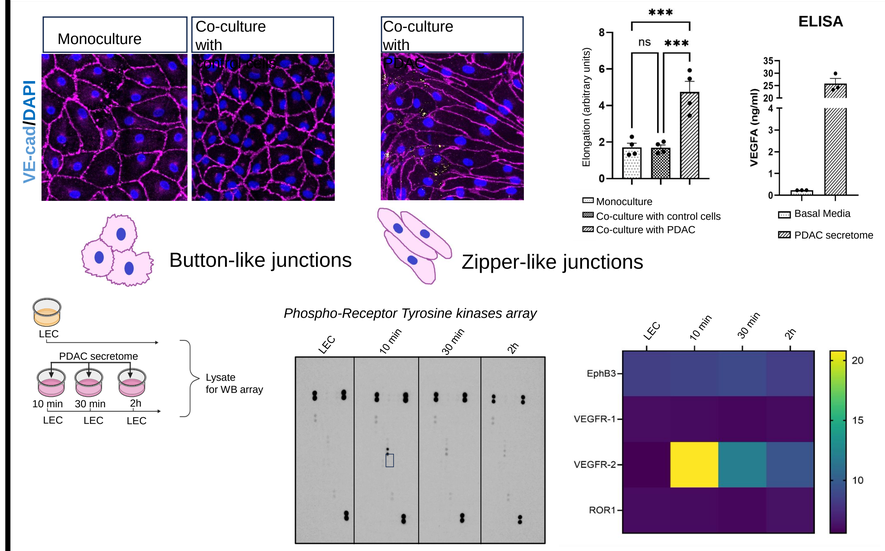
Methods: Kaede tg- a tool to track migration of leukocytes in time and space



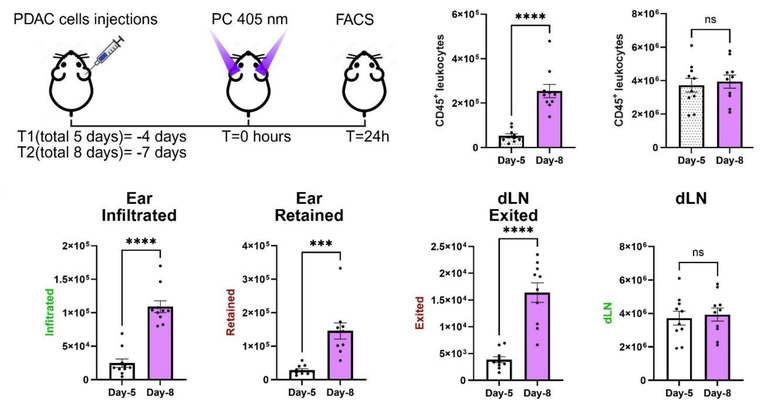
Groups post 24h PC

- **a** > 24h in tissue, K-red
- **b** < 24h entry to tissue, K-green
- **c** Exit to dLN, K-red
- **d** dLN, K green

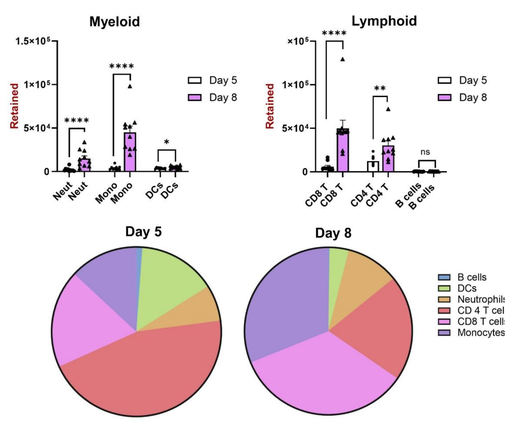
PDAC regulates junction zippering through VEGFR2/VEGFA signaling pathway



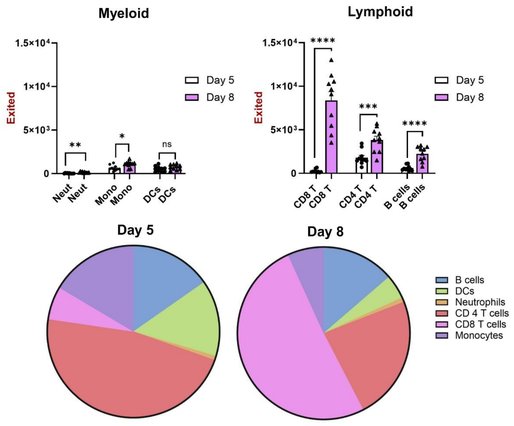
PDAC specific immune response progresses overtime



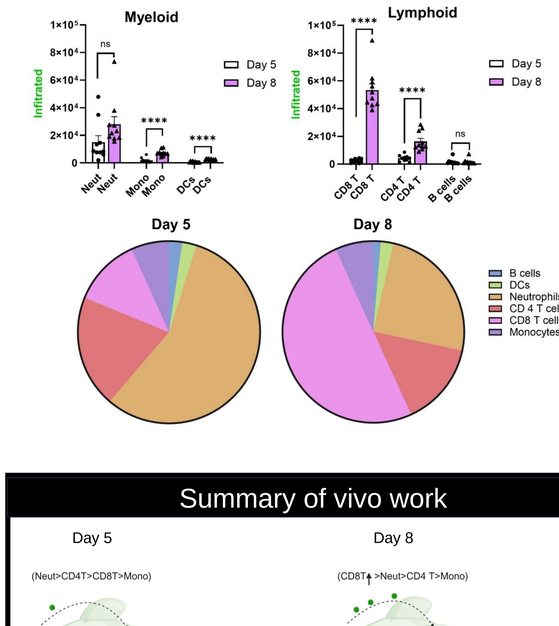
Dynamic shifts in leukocyte subtypes that retain within TME



Dynamic shifts in leukocyte subtypes that exit from TME



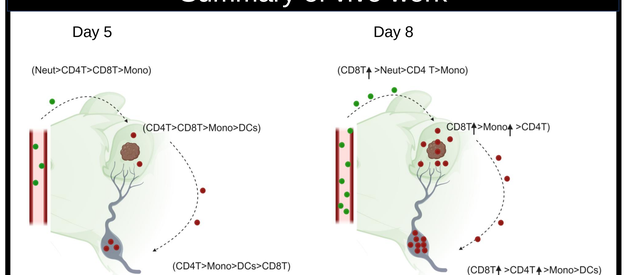
Dynamic shifts in leukocyte subtypes that infiltrate to TME



Future directions

- Investigate lymphatics *in vivo* in PDAC ear model
  - structure, number and drainage function
- Investigate leukocyte trafficking in the context VEGF pathway
  - VEGF pathway inhibitors
  - Prox1-CreERT2; VEGFR-2e3loxP transgenic mouse
- Combination therapy with PD-1/PDL-1
  Can we achieve tumor control with addition of immune check point blockade?
  What can we learn from trafficking pattern?

Summary of vivo work



		Day 5	Day 8
Ear	Infiltrated	Myeloid & Lymphoid (Neut>CD4T>CD8T>Mono)	Lymphoid & Myeloid (CD8T↑>Neut>CD4 T>Mono)
Ear	Retained	Lymphoid & Myeloid (CD4T>CD8T>Mono>DCs)	Lymphoid & Myeloid CD8T↑>Mono↑>CD4T)
dLN	Exit	Lymphoid & Myeloid (CD4T>Mono>DCs>CD8T)	Lymphoid (CD8T↑>CD4T↑>Mono>DCs)