Pathological perivascular phenotype contributes to hypoxia, impaired immune response and reduced therapeutic efficacy

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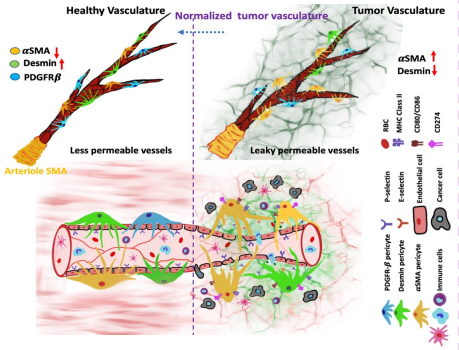
Abstract

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer death in men and women in the U.S., with a 5-year survival rate of 12%. One of the reasons for such a poor prognosis is its complex and dense tumor microenvironment (TME) and lack of effective treatment options. Within the PDAC TME, morphologically aberrant leaky vessels are responsible for hypoxia and impaired immune response, which likely reduces the efficacy of cancer therapies. Therefore, we hypothesize that correcting such undesirable phenotype via vascular normalization will alleviate the harsh TME and enhance the treatment efficacy. Our study revealed that tumor-associated pericytes across all PDAC tumor tissues exhibited ectopic αSMA expression, which was correlated with vascular leakiness and hypoxia. We also showed that such aberrant pericyte phenotype was due to pancreatic cancer cell-derived extracellular vesicles (PC-Exo). In addition, PC-Exo stimulated, tumor-conditioned αSMA+ pericytes present mechanical abnormalities and immune-suppressive features. *We performed single-cell RNA sequencing to further determine the pathological signature of tumor pericytes and identify potential target molecules toward vascular normalization*. Pericytes were isolated from WT pancreas and KPC tumors at 15-20 weeks to obtain a viable single-cell population for scRNA seq. In addition, *to define the influence of pericyte phenotypes on PDAC TME evolution, multispectral imaging was utilized*. Various combinations of pericytes and immune markers are examined to study the relationship between pericyte phenotype and immune composition in TME.

Working Hypothesis



Results

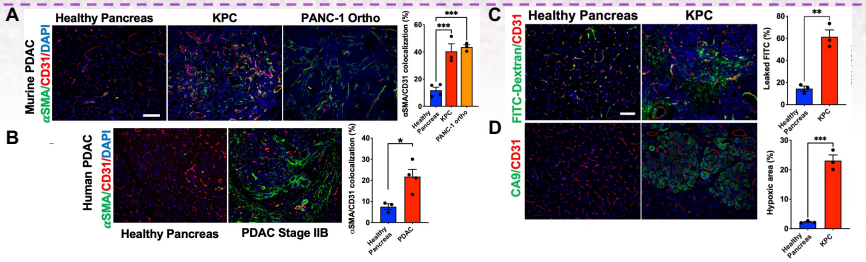
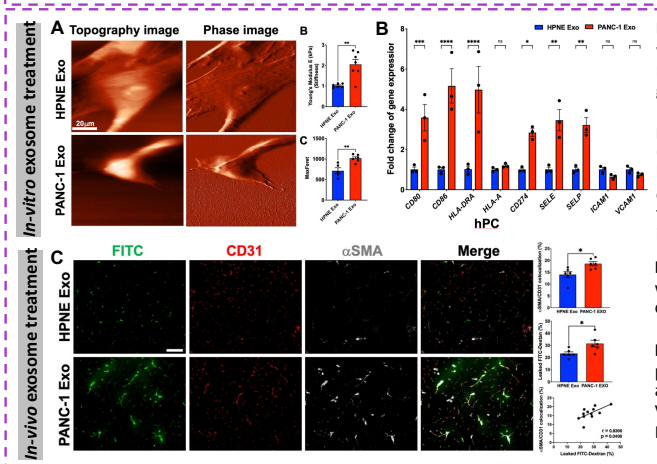


Figure 1. PDAC associated pericytes exhibit ectopic αSMA expression in both murine models of PDAC (A) and human PDAC TMA (B), which is significantly correlated with vascular leakiness (C) and hypoxia (D).



Experiment: Normal human pericytes were treated with PC-Exo and morphological changes and differential gene expression were analyzed.

Figure 2A-B. (A) Atomic Force Microscopy (AFM) shows significant changes in cell height, stiffness, and length of pericytes open treatment with PC-Exo. (B) PC-Exo treated pericytes also exhibited immunomodulatory properties.

Experiment: NSG mice were injected with HPNE Exo and PANC-1 Exo every other day for four weeks.

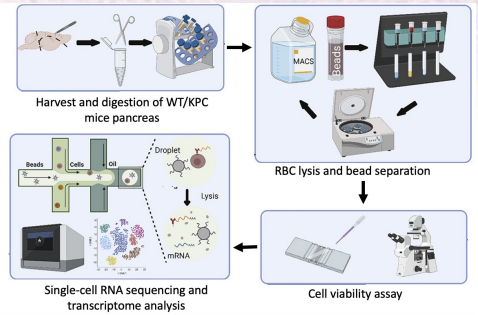
Figure 2C. Representative images of pancreatic tissues from exosome-treated animals stained for CD31 and αSMA. Vascular permeability is measured by FITC-Dextran leakage to tissues.

Conclusions

- Tumor-associated pericytes across all PDAC tumor tissues exhibit higher ectopic αSMA expression than the normal pericytes(Fig.1A-B).
- Perivascular phenotype shift correlated with vascular integrity/function and hypoxia (Fig.1C-D).
- PC-Exo treated pericyte exhibited cellular stiffness (Fig. 2A) and immunoregulatory phenotype (Fig. 2B)
- PC-Exo induce ectopic α-SMA expression in pericytes leading to abnormal pericyte phenotype in vivo. (Fig.2C)

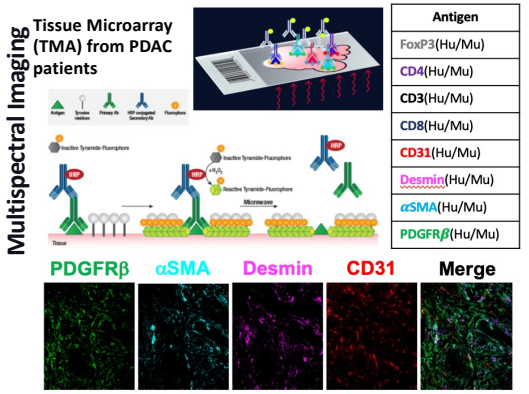
New approach #1

Single-cell RNA sequencing



New approach #2

Multispectral Imaging



Future Direction

- Single cell sequencing to understand perivascular heterogeneity.
- Novel mechanism to normalize tumor vasculature.
- To enhance chemo and immunotherapeutic efficacy.

References

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Acknowledgements

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