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Are basal microvilli on the microvasculature of pancreatic ductal adenocarcinoma a tumor specific target for therapies?

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) may be a uniformly lethal malignancy with near 6 months median survival. it's a stromarich, vascular-poor and hypo-perfused tumor, which was considered to prevents efficient drug or nutrient delivery in tumor microenvironment. Paradoxically, the tumor cells have robust glucose uptake and rare necrosis, suggesting that the microvasculature has might adopted an alternate way for nutrient uptake and cellular trafficking. Using adapted thick tumor section immunostaining and three-dimensional (3D) construction imaging in human fresh tissue samples, we identified an undiscovered feature of the mature microvasculature in advanced PDAC tumors; long, hair-like projections on the basal surface of micro vessels that we visit as 'basal microvilli'. Basal microvilli were also observed in intrahepatic cholangiocarcinoma (ICC) and metastatic pancreatic neuroendocrine tumor (panNET), but not in hepatocarcinoma, glioblastoma and renal clear cell carcinoma. Basal microvilli in PDAC are richer and denser than ICC and panNET. Functionally, these basal microvilli have an actin-rich cytoskeleton and endocytic and exocytic properties and contain glucose transporter-1 (GLUT-1)-positive vesicles. Clinically, as demonstrated by PET-CT, the tumor microvasculature with the longest and most abundant basal microvilli correlated with high glucose uptake of the PDAC tumor itself. additionally, these basal microvilli were found in regions of the tumor with low GLUT-1 expression, suggesting that their presence may be dependent upon the glucose concentration within the tumor milieu.

Similar microvasculature features which contain glucose were also observed during a K-Ras-driven model of murine PDAC. Altogether, these basal microvilli mark a completely unique pathological feature of PDAC microvasculature and ICC and panNET. Because basal microvilli are pathological features with endo and exocytic properties, they'll provide a non-conventional method for cellular trafficking in PDAC tumors. The autochthonous PCs of GEMMs that harbor KRAS and TP53 or CDKN2A or SMAD4 mutations contain abundant dense stroma and hypomicrovascularity. The pathophysiology and drug response of autochthonous PCs in GEMMs are concerning human PC.The autochthonous PCs in KPC and KPIC present basal microvilli. KIC mouse that harbor KRAS and Ink4 mutation form a highly lethal PC with near 2-month survival, and also the PCs in KIC present hypomicrovascularity and rich stroma. Thus, it's possible that the microvasculature of autochthonous PC in KIC also present basal microvilli. to determine if the microvasculature within the PCs of KIC presents basal microvilli, we've stained the PCs of KIC with a CD34 antibody. like the microvasculature of human pancreatic cancers, autochthonous KPC, and KPIC tumors, the microvasculature in KIC tumors also presents basal microvilli. per the characteristics of human basal microvilli microvasculature,we observed that the basal microvilli microvasculature in both KPC and KIC tumors features a lower level of VEGFR2 and pVEGFR2 (Y966) in comparison to the microvessels within the near-normal tissue. The cytoskeleton of the basal microvilli in human PC contains actin filaments.To observe the cytoskeleton of the basal microvilli in KIC PC, we stained the basal microvilli with a CD34 antibody and also the cytoskeleton with phalloidin. The results showed that the cytoskeleton of basal microvilli in KIC is actin based. These data support the notion that the basal microvilli microvasculature in KIC and KPC tumors structurally and functionally resembles the human basal microvilli microvasculature, indicating that KIC, KPC, and KPIC are suitable models for exploring the physiology of basal microvilli microvessels.

To characterize the basal microvilli microcirculation, we selected tumor-bearing KIC mice and perfused them with a mix of Lectin-Alexa 633 and CD31-FITC. Our scanning data of thick sections showed that the perfused labeling of Lectin-Alexa 633 in healthy pancreas tissues of KIC mice completely overlapped therewith of CD31-FITC and efficiently showed the microvasculature . However, the perfused labeling of Lectin-Alexa 633 within the precursor lesions partially overlapped therewith of CD31-FITC, the overlapping decreased from the precursor lesions to tumor regions, and a few microvessels labeled with Lectin-Alexa 633 were absent with CD31-FITC labeling and the other way around . To further test if some microvessels in KIC tumors are completely unlabeled by Lectin-Alexa 633, we stained the tissues perfused by Lectin-Alexa 633 with a CD34 antibody. We found that perfused labeling with Lectin-Alexa 633 failed to

show a big number of microvessels in PCs of KIC, especially the microvessels that present basal microvilli. to work out if limited or nonbinding of the lectin to the endothelial cells in PCs is common, we perfused tumor-bearing KPC mice with Lectin-Alexa 633. After immunostaining with a CD34 antibody, we also constructed entire microvessels with high-resolution imaging confocal microscopy. Contrary to microvessels within the healthy pancreas and kind of like KIC tumors, we observed that the lumen of basal microvilli microvessels contains a limited amount of lectin and is visible as scattered dots. These data suggest that the basal microvilli microvessels within the PC of GEMMs have blood flow. to look at the connection of blood flow with basal microvilli, we cultured human PC tissues with high glucose uptake medium in dishes. By comparing it with its freshly fixed counterpart, we found that the basal microvilli within the cultured PC tissues became shorter and thinner compared to the freshly fixed tissue after surgery.

This observation indicated that blood flow within the microvasculature could be necessary for the expansion of basal microvilli. to look at the characteristics of blood flow within the microvasculature of PCs, we analyzed videos of the human pancreas and PC microvasculature taken by probe-based confocal laser endoscopy (Cellvizio) within the clinic. We observed that the diameters of microvessels were thinner than those of a healthy pancreas . to see whether the luminal surface of the endothelial cells in PCs has characteristics that facilitate macropinocytosis, we evaluated TEM images of PC microvessels and observed multiple longer projections on the luminal surface of microvessels, resembling the macropinocytic filopodia . This observation indicated that the fast blood flow within the PC microvasculature may well be how to beat the high interstitial pressure within the stroma, and macropinocytosis can be some way to exchange nutrients and waste.

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