Journal of Pathology
J Pathol 2015; **236:** 133–135
Published online 30 March 2015 in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/path.4526

INVITED COMMENTARY

Feeding cancer's sweet tooth: specialized tumour vasculature shuttles glucose in pancreatic ductal adenocarcinoma#

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#Invited Commentary for Saiyin H *et al.* Identification of novel vascular projections with cellular trafficking abilities on the microvasculature of pancreatic ductal adenocarcinoma. *J Pathol* 2015; 236: 142–154.

Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal neoplasm characterized by a 'fortress' of thick collagen fibres, abundant myofibroblasts, and paradoxically reduced vascularization compared to normal pancreas. Despite these features, PDAC shows no reduction in the uptake of glucose that fuels tumour cell survival. In new work published in *The Journal of Pathology*, Saiyin and colleagues have identified a novel adaptation of PDAC tumour endothelium; namely, 'hairy-like' basal microvilli that increase the total vascular surface area and correlate with regions of highest glucose uptake. Since basal microvilli are not present on normal pancreatic blood vessels, their presence may add diagnostic value and blocking their function is a potential new treatment strategy for PDAC. This novel finding of basal microvilli on PDAC endothelium is a striking example of how phenotypic plasticity in tumour blood vessels contributes to tumour growth and progression, independent of conventional modes of angiogenesis.

Keywords: tumour angiogenesis; tumour endothelial cells; endothelial heterogeneity; endothelial plasticity; tumour microenvironment; pancreatic cancer

Received 11 February 2015; Revised 11 February 2015; Accepted 21 February 2015

No conflicts of interest were declared.

Structural and functional abnormalities of tumour blood vessels

Morphological differences in the vascular endothelium between tissues and organs have been long recognized. For example, fenestrated capillaries in the kidney and liver contrast with the continuous and tightly adjoined endothelium of the blood–brain barrier. Some of these phenotypic characteristics are encoded during development, but most are transmitted by paracrine and juxtacrine-dependent signalling specific to the organ microenvironment [1,2]. In the 'tumour organ', the microenvironment is aberrant and characterized by nutrient deprivation, hypoxia, acidosis, and chaotic blood flow [3]. This environment co-ordinates with well-characterized morphological and functional abnormalities in the tumour vasculature, including excessive sprouting, intercellular gaps, and a defective endothelial cell barrier [4]. Microarray studies of freshly isolated tumour-derived endothelial cells from different tumour types show changes in the expression of hundreds of genes and numerous cytogenetic and epigenetic anomalies [4,5]. It is presumed that many of these abnormalities in tumour blood vessels result from inappropriate signalling from tumour cells and other stromal cells in the tumour microenvironment.

Identification of 'hairy-like' projections on PDAC tumour vessels

PDAC is a notoriously lethal cancer that is refractory to therapy [6]. A cardinal feature of PDAC is the accumulation of a dense 'fortress' of desmoplastic stroma consisting of myofibroblasts, inflammatory cells, and extracellular matrix which contributes to their 'rock hard' texture and deficient vascularization [7]. Indeed, unlike other tumour types, angiogenesis inhibitors are virtually ineffective for the treatment of PDAC. Hypo-vascularization of PDAC may impair the delivery of chemotherapy such as gemcitabine, which has motivated treatment strategies that promote

vascularization and improve drug perfusion by attacking the stromal cells (eg myofibroblasts) [8]. In the study by Saiyin *et al*, the authors found fewer proliferating endothelial cells and sprouting tip cells in PDAC tumours than in normal pancreas or liver tumours [9]. If PDAC tumours are poorly vascularized, how then do they acquire sufficient nutrients to support their growth? Using thick (45 μm) sections and 3D construction imaging, the authors noticed numerous 'hairy-like' projections (0.8–1.2 μm in diameter and 3–41 μm in length) on the surface of PDAC micro-vessels. After careful characterization of these structures using immunohistochemistry for markers typically used to identify cilia, the authors termed these structures 'basal microvilli'. Remarkably, basal microvilli were only found in aggressive and metastatic PDAC tumours, but were not present in non-invasive precursor lesions or normal pancreas. Although sprouting tumour endothelial cells extend abluminal filopodia during tip cell specification, basal microvilli appear to be unique because they do not express VEGFR-2 or the tip cell marker UNC5B; nor are they actively proliferating, as indicated by the absence of Ki67 staining.

Basal microvilli are $CD34^+$ but do not express CD31/PECAM. They do not contain tubulin, but they do contain actin microfilaments that are linked to actin fibres within the cell body. Importantly, basal microvilli express clathrin and β-COP, two markers of transporter vesicles. Further analysis by TEM showed that basal microvilli also harbour pinocytic and exocytic vesicles as well as rough ER and mitochondria. These features led the authors to reason that these structures might be involved in the uptake of glucose, other nutrients, and/or aid in the clearance of waste products. Conventional ^{18}FDG-PET was used to semi-quantitatively measure glucose uptake in PDAC tumours *in vivo*. In seven patients, tumours with the highest glucose uptake had longer and denser basal microvilli, while tumours with lower glucose uptake had shorter and fewer basal microvilli. Notably, neoplastic cells closely positioned to basal microvilli had lower GLUT-1 expression, presumably because the glucose concentration was highest, whereas the highest GLUT-1 expression was found in tumour cells more distant from the structures. Thus, basal microvilli may impart novel functions to the tumour endothelium directly, and they might also impact the growth and behaviour of nearby tumour cells by distributing glucose. While correlative, these results indicate that basal microvilli may be a unique adaptation of the PDAC tumour vasculature that supports tumour growth independent of other well-characterized modes of tumour angiogenesis (Figure 1).

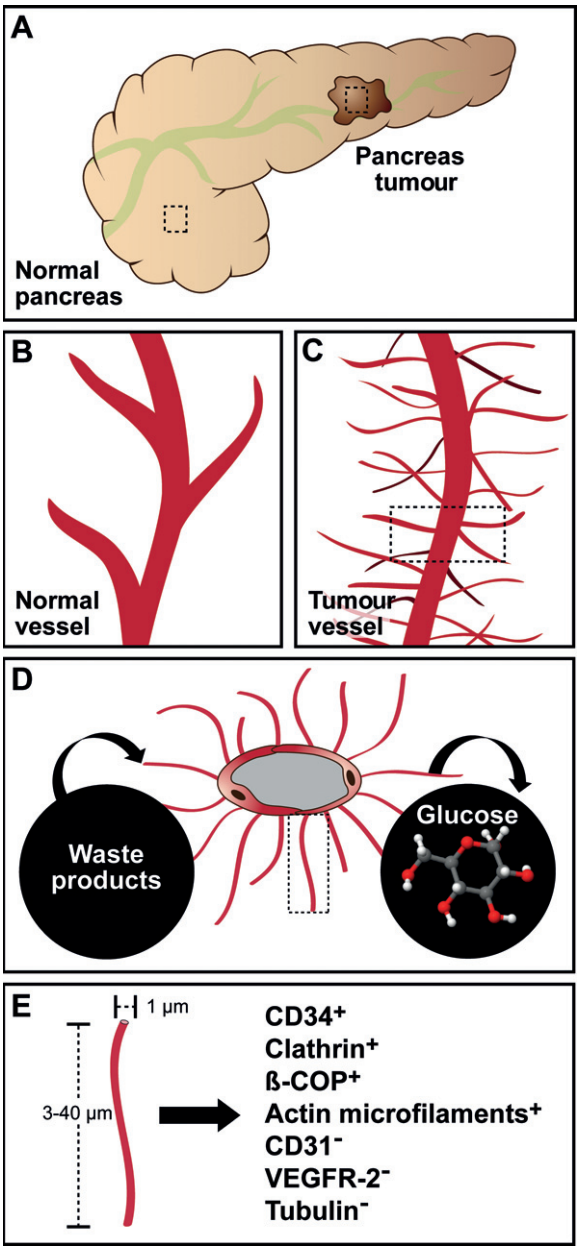

Figure 1. Identification of basal microvilli in PDAC tumour vasculature. (A) Schematic diagram showing regions of normal pancreas (boxed and zoomed in panel B) and pancreas tumour (boxed and zoomed in panel C). To identify basal microvilli, the authors used thick tissue sections and 3D construction imaging. Basal microvilli appear as 'arborescent-like' projections extending from the abluminal endothelial cell surface. (D) Based on ^{18}FDG-PET analysis, the authors conclude that basal microvilli act as a conduit for the transport of glucose to nearby tumour cells and may also facilitate the uptake and clearance of metabolic waste products. (E) Basal microvilli range from ~3 to 40 μm in length and ~1 μm in diameter, and express markers including CD34, clathrin, β-COP, and actin microfilaments but do not express CD31, VEGFR-2, or tubulin.

Perspective

A lingering question is how and why basal microvilli form only in PDAC capillaries but not in normal pancreas or blood vessels of other tumour types. The function of cilia in vascular endothelial cells may offer a clue. In other vascular beds, ciliated endothelium functions as a mechanosensor and is thought to mediate responses to shear stress. In good accord with this idea, the distribution of cilia in the cardiovascular system depends on the pattern of blood flow [10]. Interestingly, endothelial cell cilia have been linked

to shear stress-induced endothelial-to-mesenchymal transition, providing a functional relationship between cilia and flow-mediated changes in EC behaviour [11]. Thus, there may be chaotic blood flow patterns or reperfusion injury in dysfunctional PDAC vessels that mediate the formation of basal microvilli, with an obvious difference being that cilia are positioned at the luminal vascular surface, whereas basal microvilli are localized to the abluminal side.

Could basal microvilli therefore represent a novel adaptation of PDAC tumour vessels resulting from specific features of the PDAC tumour microenvironment? PDAC tumours are distinct in that vessels are sparse and the stroma is replete with collagen-producing myofibroblasts that distort and contract the extracellular matrix (ECM). These features alone might be sufficient to alter gene expression patterns in the underlying endothelial cells, which are intimately associated with and receive signals from the ECM. It would be interesting to characterize the gene expression signature of freshly isolated PDAC tumour endothelial cells, as has been done for other tumour types, to shed light onto why PDAC vessels display their unique phenotype. Because the authors have also identified basal microvilli in the KPC (LSL-$Kras^{G12D/+}$; LSL-$Trp^{53R172H/+}$; Pdx1-Cre) murine model which closely mirrors human PDAC pathology, this should allow for deeper mechanistic or genomics-type studies that will provide insight into how basal microvilli are acquired [12]. Moreover, a murine model of PDAC that recapitulates human disease creates an exciting prospect for developing new therapeutic strategies that specifically target the basal microvilli.

Finally, exactly how glucose might be transferred from basal microvilli into the PDAC tumour microenvironment is uncertain. Although basal microvilli are tethered to GLUT-1-positive endothelium and contain GLUT-1^+ vesicles, GLUT-1 typically transports glucose *inside* the cell from the external microenvironment, not the other way around. Glucose may exit basal microvilli via conventional mechanisms of nutrient transport, and the enhanced spatial uptake of glucose may be related to the increased surface area created by these structures. It is noted that the oncogenic $KRas^{G12D}$ mutation itself, which is present in more than 90% of PDAC patients, is known to reprogramme tumour metabolism and stimulate ribose biogenesis [13]. Thus, both the cancer cells and the stroma, in this case specialized tumour vasculature, may join forces to shuttle and utilize glucose as a fuel for PDAC tumour growth.

Acknowledgments

ACD is supported by a grant from the National Institute of Health/NCI (RO1-CA177875), and VLB by grants from the NIH (R01HL-43174; HL116719). We thank Kristin Johnson for the excellent artwork.

Author contribution statement

ACD and VLB wrote and edited the manuscript.

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