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# The Spectrum of Microvasculature Basal Microvilli in Human Solid Tumors: A Pilot Study

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**Abstract**

In our pervious study, we reported that basal microvilli with cellular trafficking ability exist in pancreatic ductal adenocarcinoma (PDAC) and murine PDAC models, which a remarkable example of how endothelial plasticity in tumor blood vessels feed tumor cells. As human solid tumors in different organs are driven by similar oncogenic mutations, basal microvilli may exist in other human solid tumors. Here we have done a small pilot study in human solid tumors to see whether other solid tumors take this approach and surprisingly found that basal microvilli-like structure observed in human cholangiocarcinoma, metastatic nonfunctional pancreatic neuroendocrine tumor (panNET) and breast cancer, but not in human hepatocellular carcinoma, glioblastoma, renal clear cell carcinoma and oral squamous cell carcinoma. Consistent with characteristics of basal microvilli in PDAC, immuno-staining results showed that the expression of VEGFR2 was decreased in the microvasculature with basal microvilli in panNET. This finding indicates that basal microvilli might be a common way to get nutrients from or clear waste to the microvasculature in aggressive and metastatic tumors.

**Keywords:** Basal microvilli, microvasculature, Pancreatic ductal adenocarcinoma, Metastatic tumors

## Introduction

Tumor is a novel organ what forms in adult body, contains aggressive and destructive tumor cells, stroma cells, circulation system and immune cells, and often originates from host cells by accumulating oncogenic mutations [1,2]. Oncogenic mutations reprogram tumor cellular metabolism to Warburg affect, and provide a survival advantage over host cells [3,4]. Tumor is a growing organ in adult, which not only occupies the space of host organ but destroys the structure of host organ and other organ where they reached or seeded. The new organ also reshaps the circulation of host organ, and constructs its own circulation system to meet its metabolic need. However, how the new organs reshape the host circulation remains unknown.

In our previous study, we have identified a tumor specific endothelial projection, referring as "basal microvilli", with cellular trafficking ability in PDAC tissues **[5]**. As the subtle anatomical changes in epithelial tissues are coincident with the metabolic status or demands of organs, such as microvilli in human intestine and kidney proximal tubule [6], we reasonably infer that basal microvilli should have an important function in PDAC metabolism. Consistent with the function of epithelial projections in tissue metabolism, PDAC with the highest glucose uptake value had longer and denser basal microvilli, while PDAC with lower glucose uptake value had shorter and fewer basal microvilli. PDAC are characterized with dense desmoplastic stroma, rare microvascularity and high mortality [7,8]. Basal microvilli were only observed in aggressive and metastatic PDAC tumors, but were not present in non-invasive precursor lesions or normal pancreas [5]. Some of human tumors, such as cholangiocarcinoma and some type of breast cancers, not only histopathologically resemble PDAC tissues [9,10], but harbor the same oncogenic mutations [11-13]. It implied that these tumors might grow the basal microvilli on microvasculature to support its metabolic demands.

In this pilot study, we have screened basal microvilli in several types of human solid tumors, and compared their characteristics with the basal microvilli in PDAC.

## Materials and Methods

### Patient samples

All PDAC samples, cholangiocarcinoma, local panNETs and metastatic panNETs were collected from surgical tumor resection at Department of General Surgery, Zhongshan Hospital, Fudan University; Breast cancer samples were collected from Changhai Hospital; Renal clear cell carcinoma samples were collected from Department of Urology, Shanghai General Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai by Dr. Moren; Two brain glioblastoma samples and oral squamous cell carinoma were kindly provided by Dr. Ying Wang and Dr. Qiao Zhen from Huashan hospital, Fudan University (China).

### RNA extraction and quantitative real-time PCR

Total tumor tissues RNA was extracted from tissues Trizol reagent, and were reverse transcribed by using Super Script II by reverse transcriptase kit (Invitrogen) by following the manufacturer's protocol. Quantitative real-time PCR was done by using SYBR Green Supermix kit (Takara, Dalian, China) with the ABI 7900HT detection system.

The primer of VEGFR2

Forward: GGTATGGTTCTTGCCTCAG

Reverse: CTTCAGATGCCACAGACTC

### Immunostaining and confocol microscopy scanning

Please refer to our previous work [5].

## Results

### The spectrum of basal microvilli in human solid tumors

To see whether basal microvilli also exist in the microvasculature of other solid tumors, we have used the same staining method [5]. We found that basal microvilli-like projections exist some of breast cancers (2/5), all metastatic panNET in liver (2/2) and cholangiocarcinoma microvasculature (3/3), but not in renal clear cell carcinoma (2/2), glioblastoma (2/2) and local panNET (7/7). In consistent with the growth patterns of basal microvilli, these projections protruded from the stem of microvasculature, not from the branching points or the tip of microvessels. Strikingly, the basal microvilli-like projections in metastatic nonfunctional panNETs in liver were observably denser when compared to basal microvilli in cholangiocarcinoma and breast cancers, more resembled to the basal microvilli in PDAC with high glucose uptake, were characterized with branched and extended to the inner side of tumor cells. 2/5 of breast cancers have basal microvilli-like structure on endothelium, and the basal microvilli are observably shorter and thinner when compared to the basal microvilli in cholangiocarcinoma and metastatic panNET (Figure 1 and Table 1).

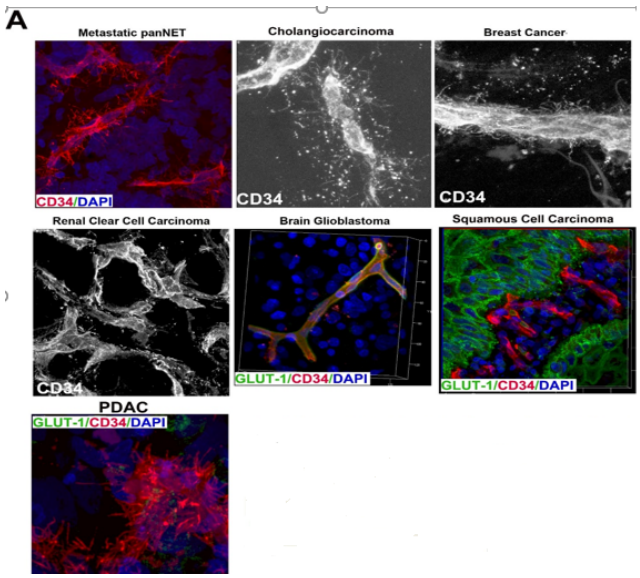


**Figure 1.** The spectrum of basal microvilli in human solid tumor. A, 3D construction of tumor microvasculature with CD34 staining showed that metastatic panNETs, cholangiocarcinoma and breast cancer have basal microvilli-like structure, whereas renal clear cell carcinoma, glioblastoma and squamous cell carcinoma didn't have this kind of structure (glioblastoma and squamous cell carcinoma, 3D channel; others, max intensity projections). Scale bar, 20 μm.

| Tumor Type | BM tumor/Total | BM Density | Range of BM lengths |
|---|---|---|---|
| Local panNET | 0/7 | N.A | N.A |
| Metastatic panNET to liver | 2/2 | High | 5-30μm |
| Cholangiocarcinomna | 2/2 | Moderate | 3-33μm |
| Breast cancer | 2/5 | Low | 3-18μm |
| Renal clear cell carcinoma | 0/2 | N.A | N.A |
| Glioblastoma | 0/2 | N.A | N.A |
| Squamous cell carcinoma | 0/2 | N.A | N.A |
| Hepatocellular carcinoma | 0/7 | N.A | N.A |

**Table 1:** The characteristics of basal microvilli in human different solid tumors.

### VEGFR2 are down-regulated in the microvasculature with basal microvilli of metastatic panNET

The endothelial tip cells with filopodia in angiogenic microvasculature expressed higher levels of vascular endothelial growth factor receptor 2 (VEGFR2) and phosphorylated VEGFR2 [14], whereas the microvasculature with basal microvilli had lower levels of VEGFR2 and phosphorylated VEGFR2 **[5]**. panNETs was known as angiogenic, the angiogenesis in local panNETs are dependent on VEGFR2 signaling pathway, and partially responded to Sunitinib, a VEGFR2 inhibitor [16,17]. To test the angiogenic levels in metastatic panNETs, we have detected VEGFR2 gene mRNA transcription levels in PDAC, local panNET, metastatic panNET by RT-PCR. We found that the VEGFR2 gene mRNA transcription levels in PDAC and metastatic panNET are lower than that of local panNET (Figure 2A). To test if the microvasculature with basal microvilli in metastatic panNET have also down-regulated VEGFR2 expression, we co-immunostained for VEGFR2 with CD34 in local panNET, metastatic panNET, glioblastoma and PDAC. Consistent with VEGFR2 expression in the microvasculature with basal microvilli in PDAC, and the co-immunostaining result showed that the VEGFR2 expression in the microvasculature with basal microvilli are observably lower than that of the microvasculature of local panNET and glioblastoma (Figure 2B). These data showed that metastatic panNETs are angiostatic and the function of basal microvilli-like structures in panNET may be similar with the basal microvilli in PDAC.

## Conclusion

In this pilot study, we found that cholangiocarcinoma, metastatic panNET and some of breast cancers contain basal microvilli-like structure in their tumor microvasculature. RT-PCR and VEGFR2 immunostaining results support that these endothelial projections in panNETs resembled PDAC basal microvilli. Cholangicarcinoma, metastatic panNET in liver are an incurable deadly tumor with grim prognosis [18-20]. Consistent with histopathological characteristics of PDAC tissues, cholangiocarcinoma and breast cancers contain rich desmoplastic stroma and hypomicrovascularity, and the richer stroma in these two tumors often linked to poor prognosis of patients [10,21]. These findings indicated that PDACs are not the only one to use basal microvilli to support their metabolism and sustain its growth, but

other deadly tumors also adopt this way to support their growth and aggressive behaviors [22-26].

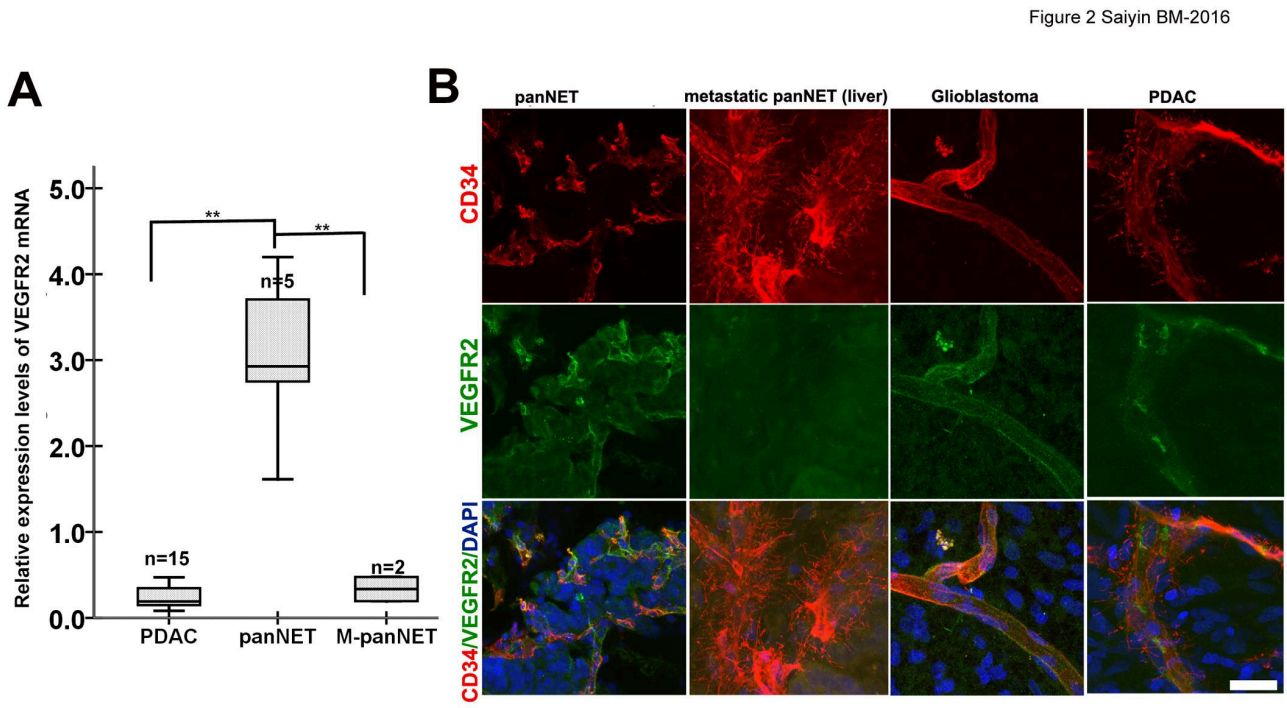


**Figure 2.** VEGFR2 expression in human solid tumors with "hairy" microvasculature and normal microvasculature. A: the VEGFR2 gene mRNA transcription levels in PDAC, local panNETs and metastatic panNETs. B: VEGFR2 and CD34 antibodies immunostaining in PDAC, glioblastoma, local panNETs and metastatic panNETs to liver. Scale bar, 20μm.

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