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Review article

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Understanding tumour endothelial cell heterogeneity and function from single-cell omics

Qun Zeng[1], Mira Mousa[2,5], Aisha Shigna Nadukkandy[1,3,5], Lies Franssens[1,5], Halima Alnaqbi[2,5], Fatima Yousif Alshamsi[4,5], Habiba Al Safar[2,4] ✉ & Peter Carmeliet[1,2,3] ✉

Abstract

Anti-angiogenic therapies (AATs) are used to treat different types of cancers. However, their success is limited owing to insufficient efficacy and resistance. Recently, single-cell omics studies of tumour endothelial cells (TECs) have provided new mechanistic insight. Here, we overview the heterogeneity of human TECs of all tumour types studied to date, at the single-cell level. Notably, most human tumour types contain varying numbers but only a small population of angiogenic TECs, the presumed targets of AATs, possibly contributing to the limited efficacy of and resistance to AATs. In general, TECs are heterogeneous within and across all tumour types, but comparing TEC phenotypes across tumours is currently challenging, owing to the lack of a uniform nomenclature for endothelial cells and consistent single-cell analysis protocols, urgently raising the need for a more consistent approach. Nonetheless, across most tumour types, universal TEC markers (*ACKR1*, *PLVAP* and *IGFBP3*) can be identified. Besides angiogenesis, biological processes such as immunomodulation and extracellular matrix organization are among the most commonly predicted enriched signatures of TECs across different tumour types. Although angiogenesis and extracellular matrix targets have been considered for AAT (without the hoped success), the immunomodulatory properties of TECs have not been fully considered as a novel anticancer therapeutic approach. Therefore, we also discuss progress, limitations, solutions and novel targets for AAT development.

Sections

[1]Laboratory of Angiogenesis and Vascular Metabolism, Department of Oncology, KU Leuven and Center for Cancer Biology, VIB, Leuven, Belgium. [2]Center for Biotechnology, Khalifa University of Science and Technology, Abu Dhabi, UAE. [3]Laboratory of Angiogenesis and Vascular Heterogeneity, Department of Biomedicine, Aarhus University, Aarhus, Denmark. [4]Department of Biomedical Engineering, Khalifa University of Science and Technology, Abu Dhabi, UAE. [5]These authors contributed equally: Mira Mousa, Aisha Shigna Nadukkandy, Lies Franssens, Halima Alnaqbi, Fatima Yousif Alshamsi. ✉e-mail: habiba.alsafar@ku.ac.ae; peter.carmeliet@kuleuven.be

Introduction

Endothelial cells (ECs) line a single layer (that is, the endothelium) at the inner surface of blood vascular and lymphatic vessels. Besides controlling the exchange of gases and metabolites between vessels and tissues, ECs regulate blood flow dynamics, coagulation, angiogenesis[1] and inflammation[2], in both healthy and pathological conditions. Depending on their anatomic location in the vascular tree, ECs in quiescent tissues can be categorized into arterial (aECs), venous (vECs), capillary (cECs) and lymphatic (LECs)[3]. ECs exhibit tissue-specific properties, for instance, fenestrated ECs in the liver and kidney glomeruli, lymphocyte extravasation-facilitating high endothelial venules (HEVs) in lymphoid tissues or blood–brain barrier (BBB) ECs with tight junctions in the brain[4]. Tumour ECs (TECs), regardless of the organ in which the cancer arises, serve as a key component of the tumour microenvironment (TME) promoting tumour progression and metastasis[5]. As angiogenesis is one of the hallmarks of cancer, anti-angiogenic therapies (AATs) are widely used for multiple types of cancers[6,7]. By suppressing angiogenesis and survival of ECs[8,9], AATs markedly reduce tumour vascular density in preclinical animal models[10]. AATs have exhibited clinical and survival benefits in patients with cancer[7,11], which results from complex physiological and pathological mechanisms, including reversing vascular endothelial growth factor (VEGF)-induced systemic syndrome in non-tumour organs[11,12]. However, the use of AATs is restricted by insufficient efficacy and resistance, varying across tumours and patients[6,7], suggesting intertumour and intratumour variability of EC phenotypes. It has also been long recognized that ECs are heterogeneous and have specialized phenotypes to carry out unique functions in different vascular beds and organs[3,4], probably contributing to the limitations of AATs[13,14].

During vessel sprouting in tumours and other diseases, ECs differentiate in response to various angiogenic cues into: (1) migratory tip cells (guiding and navigating the vessel sprout); (2) proliferating stalk cells (trailing behind the tip cell to elongate a sprout) and (3) quiescent phalanx cells (lining the newly established perfused vessel)[15]. The identity of tip cells and stalk cells is highly dynamic, with ECs expressing the lowest VEGF receptor 1 (VEGFR1; also known as FLT1) to VEGFR2 (also known as KDR) ratio leading the tip position[16], presumably to ensure that the most (metabolically) fit EC leads the sprouting vessel.

TECs also differ functionally from normal ECs (NECs). Early bulk RNA-sequencing (RNA-seq) studies revealed that TECs are more resistant to apoptosis and have distinct transcriptome signatures[17,18]. For instance, TECs from highly metastatic tumours in mice are more pro-angiogenic and invasive than TECs from less metastatic tumours[19], and after chemotherapy in patients, TECs were reported to increase the expression of a drug efflux transporter, ABC transporter B family member 1 (ABCB1)[20], contributing to tumour metastasis and resistance to treatment. However, traditional methods for analysing TECs have a low throughput (for example, immunofluorescence staining) or cannot distinguish the heterogeneity of TECs at the single-cell level (for example, bulk RNA-seq). Recently, EC heterogeneity at the single-cell whole transcriptomic level has begun to be delineated owing to the development of single-cell omics technologies.

By using single-cell RNA-seq (scRNA-seq) and bioinformatic analysis tools (Box 1), Kalucka et al.[21] sequenced more than 32,000 ECs freshly isolated from 11 organs of healthy C57BL6/J mice to generate a comprehensive atlas of mouse ECs. Up to 78 distinct subclusters were identified, the heterogeneity of which is hierarchically determined by the organ (most dominant factor) followed by the vascular bed. Interestingly, ECs from capillaries show more extensive transcriptomic diversity than ECs from arteries, veins and lymphatics[21]. Similar findings were also reported in another multi-organ scRNA-seq study comprising 12 organs in mice from the Tabula Muris consortium[22]. This organ heterogeneity was also prominent in humans as identified in the Tabula Sapiens consortium[23] but not in a pig EC atlas[24]. Further studies across species are needed to understand this discrepancy and to rule out any possible bioinformatic confounders. Because ECs constitute only a small fraction of most tissues, many scRNA-seq studies are underpowered and unable to capture detailed EC phenotypes, whereas studies that enrich for ECs are able to[21,25,26]. Integration of scRNA-seq data and meta-analyses of ECs from different studies could be an approach to address this problem. For example, Schupp et al.[27] extracted human EC data from six data sets and created an atlas of ECs of the human lung. Empowered by the abundance of ECs, previously indistinguishable subpopulations were identified. Nevertheless, only a few similar efforts have been generated to date. Besides, there is no standard nomenclature system as reference to annotate TECs properly, which has posed a problem. A more comprehensive EC atlas specific for the comparison of NECs and TECs in multiple organs could facilitate a better understanding of tumour insensitivity and resistance to AATs, as well as the development of novel EC-centric cancer therapies.

A seminal scRNA-seq study by Goveia et al.[25] revealed the surprising finding that only <10% of all TECs in human lung cancer exhibited angiogenic features of tip or proliferating ECs (the presumed main targets of clinically approved VEGF-blockade therapy[28,29], even though these therapies also target the survival of ECs), raising the fundamental question of whether this low number of VEGF targets may contribute to the insufficient efficacy and resistance to anti-VEGF therapies. Instead, this study identified previously underappreciated properties of TECs in immunomodulation, suggesting that future therapeutic approaches should be focused not only on pruning the tumour vasculature but also on 're-tuning' its phenotypic features, for instance, by rendering it more immunostimulatory.

In this Review, we focus on outlining EC heterogeneity at the single-cell level using scRNA-seq data in different human cancers as well as their translational relevance. Instead of providing an all-inclusive historical overview, we discuss only curated studies characterizing at least 500 high-quality sequenced TECs for sufficient power of analysis. Key points related to the heterogeneity of ECs in healthy tissues are summarized in Box 2, and a more detailed review of ECs in health and other diseases can be found elsewhere[30]. We focus on those TECs whose abundance is either increased or decreased in tumours and summarize top-ranking TEC markers, clinical trials targeting these markers, as well as possible predicted functions of TECs in different tumour types in Table 1 and Fig. 1. We also discuss limitations, new technologies and possible future directions to further delineate EC heterogeneity. Finally, we propose strategies to take advantage of EC single-cell omics data for therapy development.

Endothelial cell heterogeneity in cancer

Digestive system

Liver. In healthy livers, discontinuous liver sinusoidal ECs (LSECs) are specialized according to their zonal locations, and their heterogeneity also arises as a result of these zonal locations. EC heterogeneity in the liver has been documented in several studies, and their molecular signatures have been illustrated at single-cell resolution in various diseases of the liver[31–34]. An scRNA-seq study focusing on EC–immune cell crosstalk in human primary hepatocellular carcinoma (HCC) identified 11 different EC clusters, of which the phospholipid phosphatase 3$^+$ (*PLPP3*$^+$),

Box 1

Basic principles of single-cell RNA-sequencing technology

Single-cell RNA sequencing (scRNA-seq) is a technology that can dissect the gene expression within individual cells, reveal the cellular composition of a tissue, infer biological and molecular activities and predict interactions among different cell types within tissues and organs across different organisms[208]. scRNA-seq has become the method of choice for exploring the fundamental biological question of cellular heterogeneity as bulk RNA-seq can only provide the average gene expression across thousands of cells. The process of scRNA-seq includes single-cell isolation and capture, cell lysis, reverse transcription, cDNA amplification and library preparation, high-throughput sequencing and single-cell transcriptome analysis[209]. A typical analysis module includes quality control, data processing and clustering, cell annotation as well as exploratory analyses at the gene, cell and spatial levels[209,210]. Depending on the research questions, different downstream analysis tools are available. Detecting cell subtypes that are present in different data sets may be challenging owing to the inevitable batch effects, which are uncontrolled technical variations (for example, different sample sizes, laboratory conditions and analysis protocols) in data that occur as a consequence of handling cells in separate batches[211]. A single-cell data integration approach can attempt to correct for these technical differences and generate a cohesive version of the data for subsequent analysis (for example, cross-condition comparison)[212].

Key tools to analyse tumour endothelial cells

Cell–cell interactions: Cellular communication between endothelial cells (ECs) and their neighbouring cells (for example, pericytes, cancer-associated fibroblasts (CAFs) and immune cells) is vital for maintaining vascular homeostasis and remodelling. CellChat[213] and CellPhoneDB[214] are popular tools to gain insights into the interactions among cells in the tumour microenvironment (TME) that are mediated by receptors, ligands and cofactors. Conversely, NicheNet[53] focuses on the intracellular responses on the 'receiving end' as a result of predicted receptor–ligand interactions. These interactions can be more accurately studied when spatial information is obtained (discussed in the section 'Single-cell spatial transcriptomics'). Interaction analyses can also provide valuable insights into tumour EC (TEC)–immune cell interactions[91] and responses to potential combinations of anti-angiogenic therapies and immunotherapies[96].

Trajectory analysis: Cancer development manifests as a continuum of dynamic changes in the cellular state. The same applies to cells within the TME, such as TECs. Even if tissues are obtained at the same single time point, ECs are not found to be synchronous because of composite factors, such as the heterogeneity of marker gene expression[30], different surrounding cells in the TME[215] and angiocrine signalling gradients[216], to name a few. Computational algorithms of trajectory inference, pseudo-time or RNA velocity can provide clues on the dynamics even with snapshots of TECs. These algorithms are based on dimensionality reduction, gene space or RNA splicing events[180]. Popular packages include Monocle 3 (ref. 217), continuous-state hidden Markov models (CSHMMs)[218] and scVelo[48], among others. With stringent bioinformatic analysis and biological validation, the trajectory analysis can predict novel targets to prevent relapse or resistance[179].

SCENIC analysis: The single-cell regulatory network inference and clustering (SCENIC) analysis reconstructs gene regulatory networks and identifies cell states by analysing co-expression of transcription factors and/or cofactors and their downstream target genes[219]. For example, Lambrechts et al.[125] demonstrated using the SCENIC analysis that the upregulation of friend leukaemia integration 1 (*FLI1*) and TEA domain transcription factor 1 (*TEAD1*) might be responsible for TEC phenotypes.

insulin-like growth factor binding protein 3$^+$ (*IGFBP3*$^+$) and plasmalemma vesicle-associated protein$^+$ (*PLVAP*$^+$) ECs were enriched in TECs[35] (Table 1). In other studies, liver TECs were annotated in different ways[35–39], which is unfortunately a common problem for EC annotation and hinders cross-study interpretation. For example, *IGFBP3*$^+$ ECs were also identified by Xue et al.[38] and their signature was associated with immune exclusion (Fig. 1). However, it remains unclear whether *IGFBP3*$^+$ ECs in both studies represent the same EC cluster. The zonation of TECs in liver was also poorly characterized[35–39].

PLVAP marks an enriched TEC population in liver tumours[36,37,39]. This protein is pivotal for the formation of fenestral diaphragms in ECs[40,41], which regulate the permeability of vessels to large molecules in peripheral organs[41–43], and controls the access of lymphocytes to inflammation sites and lymph nodes[43,44]. PLVAP mRNA and protein are also enriched in TECs in other organs (discussed subsequently)[45], possibly suggesting a potential role for PLVAP in tumour angiogenesis[46,47]. *PLVAP*$^+$ ECs have also been identified in fetal liver tissues, suggesting a fetal-like reprogramming of ECs in HCC[35]. *PLVAP*$^+$ ECs are not a homogeneous cluster and were further subclustered into three groups, including an intermediate EC subpopulation[35], as predicted by the RNA velocity analysis[48]. Interestingly, two subpopulations of *PLVAP*$^+$ ECs express major histocompatibility complex class II (*MHC-II*), suggesting possible immunomodulatory roles[35]. Moreover, hepatocyte-derived VEGF signalling induced *PLVAP*$^+$ TECs, which expressed the canonical NOTCH ligand delta-like protein 4 (DLL4) and which colocalized with NOTCH2$^+$ tumour-associated macrophages (TAMs); *PLVAP*$^+$ TECs in HCC were shown to promote monocyte differentiation into TAMs via this Delta–Notch signalling[35]. These data support the concept that other cell types in the TME can influence the gene signature of TECs. In a small study, an antibody targeting both PLVAP and tissue factor (a prime initiator of coagulation) repressed the growth of subcutaneous Hep3B liver cancer cells in immunodeficient SCID mice, whereas an PLVAP monoclonal antibody alone did not[49]. Therefore, whether targeting PLVAP in liver cancer as a single modality (early phase I trial: NCT04601428 (ref. 50)), as opposed to in combination with other treatments, would be clinically beneficial requires more investigation.

ECs do not have a fixed predetermined genetic fate but can alter their phenotypes in response to different stimuli. LSECs lose their fenestrae and gain a capillary phenotype during fibrosis and HCC development[51]. A remarkable reduction of the LSEC population

(95% in normal liver versus 39% in liver tumours) and a substantial increase in continuous ECs were observed in a mouse HCC model, unveiling a progressive replacement of LSECs by continuous ECs with more mural cell coverage during hepatocarcinogenesis[52] (Fig. 1). An scRNA-seq study analysed ligand–receptor interactions between continuous TECs and pericytes in human malignant liver tumours using NicheNet[53], based on induction of downstream target genes, and highlighted a potential role for SLIT–Roundabout (ROBO) signalling[36], which is important for endothelial–pericyte attraction in the formation of blood vessels in tumours[54]. Additional pathways identified included platelet-derived growth factor subunit B (PDGFB)–PDGF receptor β (PDGFRβ) signalling, which facilitates pericyte recruitment[55], Delta–Notch signalling and VEGF signalling[36], both of which are essential for blood vessel formation[56].

With the power of scRNA-seq, ECs with intermediate phenotypes were also captured. For example, Zhao et al.[34] identified two clusters of chimeric cells that expressed both myeloid and endothelial cell markers after re-clustering Kupffer cells and ECs in mouse liver tumours. The chimeric ECs were further confirmed by the co-expression of platelet and endothelial cell adhesion molecule 1 (PECAM1; also known as CD31) and C-type lectin domain family 4 member F (CLEC4F) proteins by immunostaining. However, one should be cautious when differentiating intermediate EC phenotypes from doublet cells as ECs expressing markers of other cells are usually removed as doublets from the downstream analysis. Validation at the protein level using animal and/or patient tissues is necessary to confirm the existence of intermediate EC phenotypes.

Colorectum. Many scRNA-seq studies on colorectal cancer (CRC) have been published but only a few characterized ECs, partly owing to the limited number of detected ECs. In human CRC, EC heterogeneity includes tip(-like) ECs, HEVs, vECs, cECs, aECs, LECs, stalk-like ECs and

Box 2

Endothelial cell heterogeneity in healthy tissue

Multi-organ single-cell RNA-sequencing (scRNA-seq) studies have enabled unprecedented comparison of endothelial cells (ECs) across different organs and vascular beds. Blood vascular ECs show high interorgan heterogeneity and cluster into organ-specific groups, whereas lymphatic ECs from different organs tend to group together[21–23]. Markers of human and mouse ECs from different organs, vascular beds and species are summarized by Trimm and Red-Horse[220]; a comprehensive overview of EC subtypes in the mouse can be found elsewhere[21]. Notably, organs with specialized vasculatures tend to have more unique EC markers and phenotypes, that is, lung, liver, heart, uterus, pancreas, fat and muscle ECs in humans[23] and lung, liver, brain, testis, spleen and kidney ECs in mice[21,22]. By hierarchical clustering and gene set variation analysis, however, similarities between mouse ECs across organs have been identified. For instance, mouse liver and spleen ECs share similar expression of genes involved in scavenging and immune regulation, whereas mouse muscular and cardiac ECs share genes involved in redox homeostasis. Conversely, brain ECs display zone-dependent changes in their transcriptional profile[221], and this type of zonation is also observed in liver sinusoids[37]. Interestingly, the metabolic transcriptome signatures partially contribute to the interorgan heterogeneity of mouse blood ECs, as hierarchical clustering using only metabolic genes resulted in the same structure of organ grouping[21]. Multi-organ scRNA-seq studies could act as a source for existing knowledge on the interorgan overlapping gene expression and gene enrichment data[21–23].

The heterogeneity of capillaries contributes profoundly to interorgan EC heterogeneity. In an effort to identify organ-conserved mouse EC markers of different vascular beds, it was demonstrated that few capillary markers are conserved, and most capillary markers are organ-specific compared with those of arteries, veins and lymphatics[21]. During dehydration, mouse medullary capillary ECs (cECs) were predicted to be affected the most[141], but this would need to be validated by immunostaining. In chronic obstructive pulmonary disease, human cECs show the largest number of differentially expressed genes among all other ECs and contribute to the inflammatory process[222]. In human lung tumours, activated cECs and alveolar cECs are diminished and intermediate cECs are enriched compared with peritumour tissues[25]. Together, cECs might show such high interorgan heterogeneity to meet organ-specific needs.

scRNA-seq has facilitated the discovery of new EC subsets. Aerocyte cECs, which express carbonic anhydrase 4 (CA-IV) and require vascular endothelial growth factor-α (VEGFα) secreted from alveolar epithelial cells for their maintenance, are distinct from general cECs in mouse lungs[223,224]. These two cEC phenotypes are also identified in human lung scRNA-seq data[27] and are altered (with aerocyte cECs levels being decreased) in both human and mouse lung tumours[25,224]. Lipid-processing ECs, a type of cEC expressing fatty acid binding protein 4 (FABP4) found in healthy breast tissue, were recently identified to be diminished in breast cancer tissue and are predicted to be involved in lipid transport, metabolism and catabolism (for further details see section 'Breast')[26]. Aquaporin 7$^+$ cECs, which are predicted to be involved in the uptake and metabolism of lipids, were newly identified in a mouse multi-organ scRNA-seq study[21]. Unexpectedly, angiogenic, proliferating and interferon-activated cECs were also identified in the steady state of mice[21]. Furthermore, previously undistinguishable vascular ECs could be subclustered into pulmonary-venous and systemic-venous ECs on the basis of the expression of collagen α-1(XV) chain (COL15A1) and other markers in human lungs[27]. Notably, the existence of these newly identified or unexpected ECs has been validated by immunostaining.

Vascular bed heterogeneity varies with age and gender. The Tabula Muris consortium identified variation between both genders in EC gene expression in mouse organs such as the brain, heart and lung[22]. Studies have also shown that EC phenotype can be affected by ageing. For example, interactions between fibroblasts and ECs in the heart are influenced by age whereby aged fibroblasts exhibit higher expression of serpins, which consequentially has an anti-angiogenic effect on ECs[225].

Table 1 | Tumour-enriched endothelial cell subsets across organs

Organ	Tumour-enriched endothelial cell subset	Predicted function	Predicted inducer	Relevant clinical trials
Liver	*PLVAP*[a], further subclustered into *HLA-DR*$^{+}$ (*ACKR1*$^{+/-}$)[a] and *KDR*$^{+}$ (ref. 35)	Polarization of tumour-associated macrophages through Delta–Notch signalling (validated)	VEGF signalling (validated)	Anti-PLVAP: NCT04601428, phase I
	IGFBP3 (ref. 35)[a]	Immune cell exclusion[38]	NA	NA
	PLPP3 (ref. 35)	Anti-inflammatory and anti-migratory?[202] (curated by literature search)	NA	NA
Colorectal	*PLVAP*[a], *IGFBP7*, *HSPG2* (ref. 58)	Angiogenic and metabolically active	*HOXB6*, among others	NA
	Tip cells: *ESM1*, *NID2*, *RGCC*, *RAMP3*, *HSPG2* (refs. 57,58,61)	Angiogenesis	*MEF2D*, among others	NA
	ACKR1[a], *SELP*[58]	HEV-like	*HMGN3*, among others	NA
	BIRC5, CKS1B[57]	Proliferative	NA	NA
Pancreas	TECs are distinct from NECs: *IGFBP3*[a], *SPP1*, *CFH*, *IGLL5*, *TIMP1* (ref. 70)	Angiogenesis, cell migration, ECM organization, hypoxia	NA	Anti-IGF1R: receptor of IGFBP3, NCT00769483, phase I/II, prolonged overall survival[72] NCT01231347, phase III, no improvement to standard of care[76] NCT02399137, phase II, no improvement to standard of care[75]
Stomach	*IGFBP5*, *STC1*, *IGFBP3*[a], *CD93*, *ADAMTS1* (refs. 82,83)	Angiogenic, MYC pathway, EMT pathway	NA	NA
	ACKR1 (refs. 83,85)[a]	Immunomodulatory	NA	NA
	MHC-II low, COL4A1, COL4A2 (ref. 86)	Immunomodulatory	NA	NA
Oesophagus	TECs are distinct from NECs: *VEGFRs*, *ANGPT2*, *PDGFB*[89]	Angiogenic, immunomodulatory, promote myofibroblast transition	NA	Tyrosine kinase inhibitors + anti-PD1: NCT04879368 (ref. 94), phase III
Brain	Tip cell: *PLVAP*[a], *COL4A1*, *CD93*, HSPG2 (ref. 102)	Angiogenesis, ECM remodelling, cytoskeletal rearrangements, metabolically active	*SOX4*, *ETS1*	Anti-CD93: NCT05496595 (ref. 103), phase I for solid tumours
	PLVAP[a], *ACTB*, *GAPDH*, *VIM*[102]	Cytoskeletal and ribosomal protein expression, metabolically active	*SOX4*, *ETS1*	Anti-vimentin: NCT04396717 (ref. 104), phase I
	PLVAP[a], *ACKR1*[a], *IL1B*, *SELE*, *SELP*[102]	Immunomodulatory	*SOX4*, *ETS1*	NA
Breast	Tip-like and stalk-like cells: *APLNR*, *INSR*, *ESM1*, *KDR*, *VWA1*, *COL4A1*, *COL4A2* (ref. 26)	Angiogenesis, ECM remodelling, OXPHOS	NA	Insulin receptor modulator: metformin[111] KDR: reviewed elsewhere[110]
	Activated post-capillary venules: *POSTN*[26]	Angiogenesis, ECM remodelling, OXPHOS	NA	NA
Prostate	CXCL12 (ref. 117)	Pro-angiogenesis (validated)	NA	CXCR4: CXCL12 receptor, NCT05465590 (ref. 118), phase I, for solid tumours
Lung	Tip cell: *COL4A1*, *ESM1*, *ANGPT2*, *INSR*, *CXCR4*, and many more[25]	Angiogenesis, ECM remodelling, EC migration	NA	Insulin receptor modulator: metformin[203]
	Stalk-like immature cell: *PLVAP*[a], *ENG*, *HES1* (ref. 25)	Notch signalling, maturation of newly formed vessels and vessel barrier integrity	NA	Anti-endoglin: NCT01332721 (ref. 131) and NCT03780010 (ref. 132), phase I, +anti-VEGF bevacizumab, completed NCT03181308 (ref. 133), phase I, +anti-PD1, completed NCT05401110 (ref. 134), phase I, +EGFR inhibitor
	ACKR1[a], *POSTN*, *SELP*, *CCL14* (ref. 25)	HEV-like: immunomodulation	NA	NA
	IGFBP3[a], *ACKR1* (ref. 125)[a]	Increased MYC pathways and decreased antigen presentation and leukocyte homing	*FLI1*, *TEAD1*	Anti-IGF1R: IGFBP3 receptor, several clinical trials failed[137–139]
	SPRY1 (ref. 125)	Increased MYC pathways and decreased antigen presentation and leukocyte homing	FLI1, TEAD1	

Table 1 (continued) | Tumour-enriched endothelial cell subsets across organs

Organ	Tumour-enriched endothelial cell subset	Predicted function	Predicted inducer	Relevant clinical trials
Kidney	*PLVAP*[a], *VWF*, *HSPG2*, *EDNRB*[142]	EC growth and regeneration; decreased IFNγ response[142,204]	NA	Anti-endothelin receptor type B: NCT04205227 (ref. 144), phase I/II for solid tumours
	ACKR1[a], *SELP*[142]	Decreased sensitivity to AATs?	NA	NA
Skin (keloids)	*ACKR1*[a] or *CXCL12* or *CXCL3* (ref. 153)	Chemotaxis, antigen presentation and endothelium development	VEGFR, MAPK and WNT signalling	Anti-CXCR4: CXCL12 receptor, NCT02823405, phase I, completed[159]
Thyroid	Arterial: *FBLN5*, *GJA5*, *JAG1*, *PPP1R14A*[160]	Arterial remodelling and development	*SOX17*, *HES5* and so on	NA
	Stalk-like immature cell: *HES1*, *ID2*, *ENG*, *APLNR*, *HSPG2*, *PLVAP*[a], *IGFBP3* (refs. 160,162)[a]	Notch signalling and barrier integrity	*VAX2*, *JUNB* and so on	NAS
	Tip cell: *FLT1*, *KDR*, *NRP1*, *ENPP2*, *THY1*, *PLVAP* (refs. 160,162)[a]	Angiogenesis and cell migration	*ZEB1*, HOXB5, *STAT1* and so on	FLT1 and KDR: reviewed elsewhere[161]
	Venous: *ACKR1*[a], *VWF*, *SELE*, *POSTN*[160]	Leukocyte recruitment and adhesion	*NNHLH1*, *FOXA3*, *MYBL2* and so on	

AAT, anti-angiogenic therapy; EC, endothelial cell; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; HEV, high endothelial venule; HLA, human leukocyte antigen; MHC-II, major histocompatibility complex class II; IFNγ, interferon-γ; NA, not applicable; NEC, normal endothelial cell; OXPHOS, oxidative phosphorylation; PLVAP, plasmalemma vesicle-associated protein; TEC, tumour endothelial cell; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; VWF, Von Willebrand factor. [a]Common tumour endothelial cell markers.

proliferative ECs[57,58]. *PLVAP* again was enriched in tumour tip and cECs[58] (Table 1). However, owing to the lack of a standard EC nomenclature in CRC, some annotations may be vague and inconsistent, or perhaps context-dependent and/or species-dependent. For example, regulator of cell cycle (*RGCC*) and receptor activity modifying protein 3 (*RAMP3*) were proposed to be markers of tip cells in humans CRC[57], whereas other studies identified these genes as markers of cECs in mice[21,59]. Atypical chemokine receptor 1 (*ACKR1*) and P-selectin (*SELP*), which are markers of vECs[27,60], were used as markers of stalk-like cells[57].

Within the EC population, tip ECs and HEVs are enriched in CRC tissues[57,58], in contrast to the enriched carbonic anhydrase 4^{+} (*CA4*$^{+}$) cEC subset in healthy colorectal tissue[58]. These results are in agreement with the study of Pelka et al.[61], who showed that the fraction of vascular ECs and pericytes is higher in tumour tissue, whereas tip cells and proliferating ECs[57] were only detectable in the tumour. At the transcriptional level, different factors involved in angiogenesis were identified. Higher expression of the pro-angiogenic factor heparan sulfate proteoglycan 2 (*HSPG2*) was, for example, detected in tumour cECs in comparison to healthy cECs[58]. TAM and epithelial cell-derived *VEGFA* might contribute to increased angiogenesis in CRC[62]. Additionally, tip and stalk ECs in CRC tumours exhibited an over-representation of genes involved in 'regulators of angiogenesis'[57] and an under-representation of markers of 'antigen processing and presentation' when compared with healthy tissues[57,61] (Table 1 and Fig. 1).

EC metabolism is pivotal for angiogenesis and is driven in part by different signalling pathways[63]. Single-cell omics alongside metabolic analyses have demonstrated several metabolically distinct TEC subtypes in CRC. For instance, Qian et al.[58] revealed distinct metabolic gene signatures of glycolysis and oxidative phosphorylation (OXPHOS), which promote vessel sprouting[63], as upregulated in tumour tip ECs and cECs compared with healthy cECs[58].These findings suggest that TECs in CRC have more angiogenic phenotypes. To date, however, no clinical benefit has been observed for anti-VEGF treatment in the adjuvant setting in primary CRC in which samples for scRNA-seq were taken, whereas this treatment improves patient survival in metastatic CRC[64]. A detailed comparison of TECs between primary and metastatic CRC might help resolve this confusion and gain insights into overcoming the ineffectiveness of AATs in primary CRC.

Pancreas. Several scRNA-seq studies have investigated the heterogeneity of the TME in human pancreatic cancers[65–69], albeit with only a few studies focused on ECs[70,71]. The proportion of ECs in the TME varies considerably across different studies, ranging from 0% to 17%, possibly owing to different sample preparation protocols and the small cohort size (Box 3). Regardless, ECs are consistently characterized by high *PLVAP* expression[65–70]. Whether PLVAP is enriched in TECs to a higher level than in NECs was not reported. In one paper on human pancreatic ductal adenocarcinoma (PDAC), 17 EC clusters were identified, with TECs segregating from NECs[70], a finding that needs to be validated in larger cohorts and controlled for possible batch effects (Boxes 1 and 3). IGFBP3, which is enriched in TECs of other organs, was also increased in TECs compared with NECs in PDAC[70]. In a phase II clinical trial, an antibody targeting insulin-like growth factor 1 receptor (IGF1R), the receptor of IGFBP3, in combination with the chemotherapeutic agent gemcitabine prolonged the overall survival of patients with PDAC compared with chemotherapy alone[72] (Table 1), although these effects could be due to the blocking of IGF1R binding to multiple ligands[73] and/or IGFBP3 expression from multiple sources besides TECs[74] and/or be antibody-specific as two other IGF1R antibodies showed no improvement in patient survival[75,76]. TECs in PDAC also upregulate genes involved in ECM organization, angiogenesis and hypoxia responses[70].

Shiau et al.[71] reanalysed published single-nucleus RNA-sequencing data of human PDAC and detected a reactive endothelial-to-mesenchymal transition (EndMT) lineage that expressed leukocyte adhesion molecules (intercellular adhesion molecule 1 (*ICAM1*), vascular cell adhesion molecule 1 (*VCAM1*) and E-selectin (*SELE*)) and expanded after neoadjuvant chemoradiotherapy. Furthermore,

this EndMT lineage was associated with poor prognosis[71]. Whether EndMT-inhibiting regimens (such as the tyrosine kinase inhibitor nintedanib, the endothelin inhibitor macitentan, or rapamycin since EndMT involves multiple pathways[77]) could synergize with neoadjuvant chemoradiotherapy[78] is worth further exploration. Similarly, in a transgenic mouse model of PDAC, 'activated' ECs expressing leukocyte adhesion molecules were more abundant in late-stage than in early-stage PDAC. Importantly, these activated ECs express cytokines (including chemokines and growth factors such as colony-stimulating factor 3 (*Csf3*)) and could interact with dendritic cells, natural killer cells and neutrophils[79]. All these findings from scRNA-seq studies of PDAC support the postulate that TECs likely have a role in the recruitment of immune cells and could be immunomodulatory in nature (Fig. 1). Indeed, current strategies in clinical trials for pancreatic cancer are using AATs to normalize the tumour vasculature and to improve the delivery of chemotherapies or immunotherapies[80]. More in-depth characterization of TECs in pancreatic cancer might also reveal novel targets for vascular normalization.

Stomach. Gastric cancer development involves multiple processes ranging from inflammation to carcinogenesis[81], in which TECs expressing different markers in the TME undergo dynamic (evolutionary) changes. For instance, Yin et al.[82] generated a dynamic transcriptome map of human ECs during multistage disease from non-atrophic gastritis, chronic atrophic gastritis, intestinal metaplasia to gastric cancer using scRNA-seq data and indicated phenotypic convergence of ECs during gastric cancer progression. This map identified four EC clusters, with cluster 4 (*IGFBP5*$^+$) only appearing with gastric cancer and expressing enriched MYC targets and epithelial-to-mesenchymal transition signatures[82]. Another scRNA-seq study on human gastric cancer also identified a tumour-specific angiogenic *IGFBP5*$^+$ *IGFBP3*$^+$ cluster[83]. Using the cancer genome atlas (TCGA) stomach adenocarcinoma cohort, patients with a high expression of *IGFBP5*$^+$ *IGFBP3*$^+$ TEC signature genes, including *CD93* (which encodes the complement component C1q receptor) and a disintegrin and metalloproteinase with thrombospondin motifs 1 (*ADAMTS1*), showed worse overall survival than those with low gene expression signature[83]. Nevertheless, not all gastric cancer studies have reported the same enrichment of these types of TECs[84], which could be due to the different tumour subtypes analysed and sampling sites used or other technical differences. Tip-like cells[85], ACKR1$^+$ TECs[85], MHC-II^{low} ACKR1^{low} TECs[86] and EndMT TECs[87] have been reported in different studies (Table 1). The different

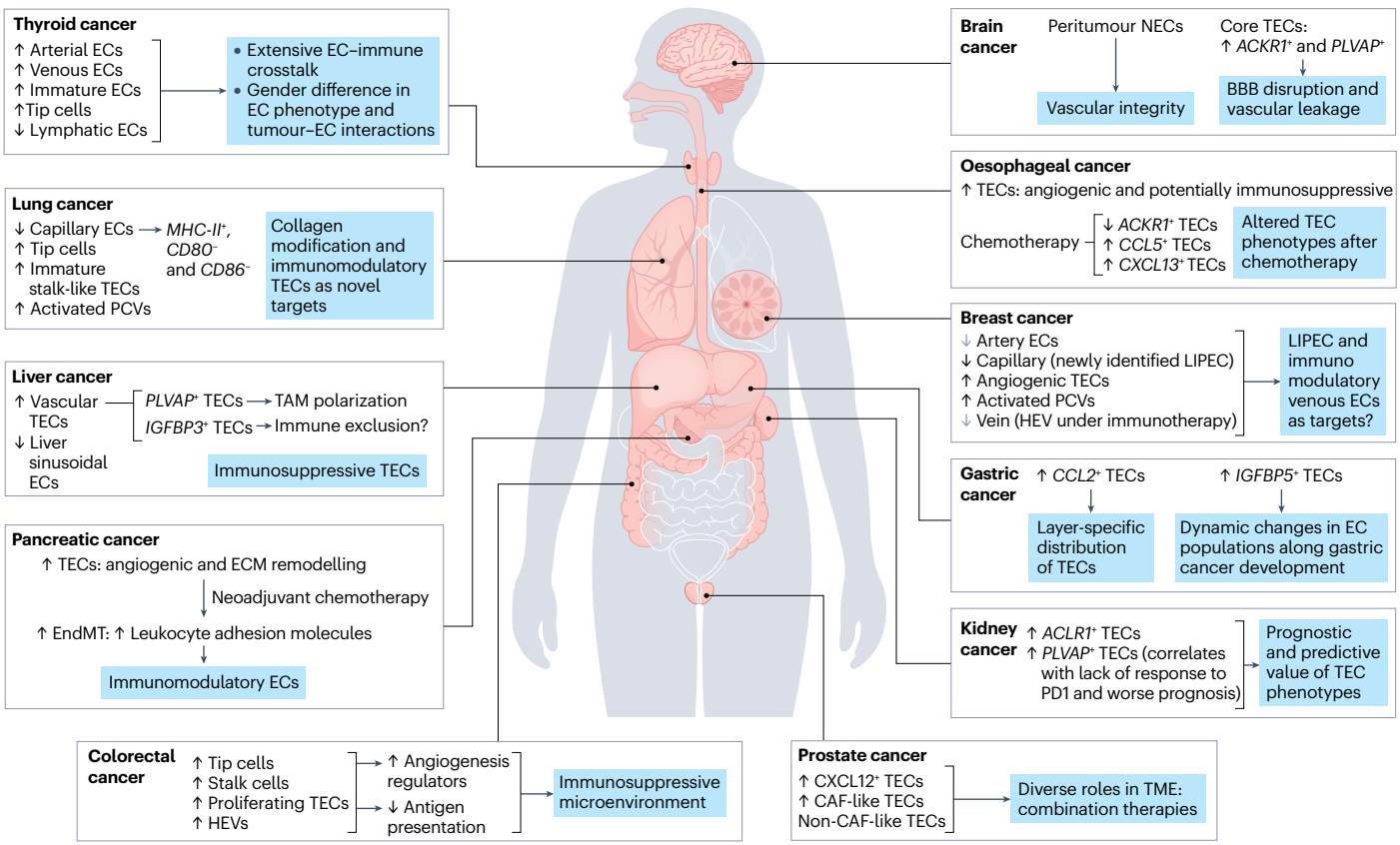

Fig. 1 | Body map of tumour endothelial cells characterized by single-cell RNA-sequencing in different cancer types. Overview of the key findings in different types of cancers, particularly highlighting the common increased tip cells and altered immunomodulatory function of tumour endothelial cells (TECs). This cross-organ comparison indicates the need for combination therapies targeting angiogenic, immunomodulatory and extracellular matrix (ECM) remodelling TECs. BBB, blood–brain barrier; CAF, cancer-associated fibroblast; EC, endothelial cell; EndMT, endothelial-to-mesenchymal transition; HEV, high endothelial venule; *IGFBP3*, insulin-like growth factor binding protein 3; LIPEC, lipid-processing endothelial cell; MHC-II, major histocompatibility complex class II; NEC, normal endothelial cell; PCV, post-capillary venule; PLVAP, plasmalemma vesicle-associated protein; TAM, tumour-associated macrophage; TME, tumour microenvironment. Grey arrow: trend without statistical significance.

Box 3

Limitations of single-cell RNA-sequencing studies

Bias during sample preparation
Different cells are lost at different rates during single-cell preparation. Immune cells are usually over-represented, whereas cancer cells and other stroma cells are under-represented[226]. Determining the best practice strategy for tissue dissociation through heuristic optimization for different tissue types is therefore important. Cell enrichment before library preparation can also allow researchers to specifically focus on rare cell types.

Technical noise
Limitations in the availability of samples and the vast amplification of RNA even when present in a small amount create higher technical noise when compared with bulk RNA sequencing[227].

Drop-out events
These can occur as a result of absence, lack of detection or amplification of the transcript during single-cell RNA-sequencing. It is largely dependent on the sequencing technology. Drop-out events can have a detrimental effect on the downstream analysis performed after sequencing[228].

Bias in bioinformatic analyses
Bioinformatic analyses involve both biomedical and computational expertise, both of which can be empirical. Different parameters used might affect the outcome and interpretation of results, for example, the number of clusters is highly variant among studies. Benchmark studies are therefore instructive for enabling researchers to adhere to appropriate analysis pipelines.

Batch effect
Variation in gene expression is influenced by imbalanced experimental designs. This can lead to incorrect data integration and interpretation and thus spurious results[229]. Batch-effect removal is pivotal to minimize false-positive findings[229].

Sample size
Scarcity in the availability of samples, especially clinical samples, often reduces a sample of interest to an inconsequential outlier[230].

Sampling region
Tissue samples used for single-cell RNA-sequencing library preparation are usually small, either obtained from a (needle) biopsy or part of a resection. Therefore, the level of heterogeneity resolved will be biased by the size and location of the sampling region[84] and may not represent the whole tissue or tumour, leading to false-positive hits in translational research. Hits should be validated in large cohorts before in vivo investigation.

Interpatient and intrapatient variation
Massalha et al.[36] showed that only the tumour cell clusters from hepatocellular carcinoma were changed with each new patient added to the analysis, demonstrating that the liver tumour microenvironment exhibits recurring gene expression signatures that are more uniform among patients. This uniformity in the tumour microenvironment compared with the interpatient heterogeneity of cancer epithelial cells was also broadly documented in other tumour types[58,125,231,232]. Interestingly, compared with tumour endothelial cells, adjacent 'normal' endothelial cells from three different tumour types (colorectal, ovarian and lung cancer) had higher patient and tissue specificity[58], which has also been documented in the mouse endothelial cell atlas[21]. Whether this lack of interpatient and intertissue variability stands true for tumour endothelial cells from all tumour types warrants further efforts by integrated analyses. Batch-effect correction should be carried out when strong patient variation is observed[229].

Spatial and temporal state of the cell
As the cells are lysed before profiling, any data obtained lack knowledge of the spatial environment of the cell and its dynamic behaviour within it[233]. However, technologies to incorporate this information have now been developed[183–186,189–192].

Abnormal normal tissues
It is noteworthy that some studies have considered tissues adjacent to cancerous lesions (the so-called adjacent normal) as normal tissue for comparing endothelial cell gene signatures with tumour tissues. Yet, this adjacent portion might contain small tumours that were not visible by gross inspection. Moreover, patients with cancer usually experience systemic changes whereby affected organs are not normal. For example, Zhao et al.[34] observed distinct liver sinusoidal endothelial cell clusters and gene expression differences between naive liver and the so-called adjacent normal liver tissues. Specifically, leucine-rich HEV glycoprotein 1 (*Lrg1*), which encodes a mitogen demonstrated to promote angiogenesis in the presence of transforming growth factor β1, was highly expressed in tumour ECs and upregulated in adjacent normal endothelial cells, whereas this mitogen was mostly absent from naive ECs. This finding suggests that the tumour might also influence the transcriptome of adjacent normal endothelial cells.

descriptions of TEC phenotypes may not be comprehensive and might lead to biased TEC target prediction (Box 3).

EC abundance and subtypes also varied depending on the tumour depth (superficial and deep layers) in patients with diffuse-type gastric cancer[84] (Fig. 1). ECs were notably more abundant in deep layers. Furthermore, the deep-layer-enriched TECs expressed higher levels of *IL6*, C-C motif chemokine ligand 2 (*CCL2*), *ICAM1* and *ACKR1* when compared with superficial TECs and NECs, thereby likely representing more inflammatory EC subsets. This enrichment of $CCL2^{+}$ ECs in the deep layers was confirmed by duplex RNA in situ hybridization[84]. Interestingly, *CCL2* expression correlated with gene signature scores of dendritic cells and immunosuppressive exhausted regulatory T cells, all

of which negatively correlated with the survival of patients with gastric cancer[84]. Although these possible interactions were not validated at the protein level, these findings suggest an involvement of *CCL2*$^+$ ECs in establishing an immunosuppressive TME in deep layers of gastric cancer[84]. Although current AATs did not show a beneficial effect in gastric cancer, continuous efforts have shown encouraging results of combining AATs with immunotherapies in this cancer type[88].

Oesophagus. In a large scRNA-seq study using oesophageal squamous cell carcinoma (ESCC) samples from 60 patients, six EC subtypes were identified, among which three different TEC subtypes were identified as being derived only from tumour tissues; possibly owing to a large imbalance between the number of samples taken from tumours and normal oesophageal tissues (only four patients)[89] (Box 3). These three TEC subtypes exhibited a lower expression of antigen presentation and cell adhesion genes (for example, *ICAM1* and *VCAM1*) and a higher expression of angiogenesis-related molecules (for example, *VEGFR* and *PDGFB*) compared with NECs[89] (Table 1). The low expression of cell adhesion genes might possibly be implicated in the suppression of immune cell infiltration into tumour tissues[90,91]. Additionally, two TEC subtypes had a notably increased expression of angiopoietin 2 (*ANGPT2*), which disrupts pericyte–EC interactions to enable angiogenesis and to promote vascular leakage[92]. Interestingly, the results also predicted that TECs might contribute to reshaping the TME by inducing the transition of pericytes to myofibroblasts. Specifically, higher expression of *PDGFB*, a gene whose protein product has a pivotal role in tumour angiogenesis by promoting pericyte-to-myofibroblast transition[93], was detected in TECs and correlated with the accumulation of myofibroblasts in the tumour[89]. Targeting both the angiogenic and immunomodulatory arms of TECs might offer a unique advantage, and a phase III clinical trial in gastro-oesophageal cancer combining regorafenib (a tyrosine kinase inhibitor (TKI), a type of AAT) and a PD1 antibody is currently ongoing (NCT04879368 (ref. 94); Table 1).

Furthermore, EC–cancer cell interactions might affect the sensitivity of cancer cells to neoadjuvant chemotherapy. A single-cell study compared the TME dynamics in patients with ESCC who received or did not receive preoperative combination paclitaxel and platinum chemotherapy, in which the treatment group displayed a lower proportion of TECs[95]. *ACKR1*$^+$ periostin (*POSTN*)$^+$ TECs were enriched in untreated patients, whereas the *CCL5*$^+$ C-X-C motif chemokine ligand 13$^+$ (*CXCL13*$^+$) ECs with potential immunomodulatory functions were enriched in the treated patients. Interestingly, *CCL5*$^+$ *CXCL13*$^+$ ECs altered their phenotype after chemotherapy, switching from an enrichment in genes involved in 'biological regulation' and 'cell growth' to an enrichment of genes involved in 'cellular and immune responses' in treated patients[95], suggesting a mounted immune response after chemotherapy (Fig. 1). The study did not explore the link between changes in TECs and response of patients to treatment, a question worth investigating as it will be helpful for future treatment strategies and for determining whether the *ACKR1*$^+$ TECs and *CCL5*$^+$ TECs might be immunostimulatory or immunosuppressive. Introducing AATs to these patients receiving chemotherapy might be an option to promote immune cell infiltration and antitumour immunity[96]. Nonetheless, over-inhibiting angiogenesis could reduce tumour uptake of administered chemotherapeutics, highlighting the need to investigate additional treatment strategies such as intermittent treatment schedules to maximize chemotherapeutic drug exposure[97].

Despite these two studies investigating TEC phenotypes in ESCC, the heterogeneity of TECs in oesophageal adenocarcinoma, the most common subtype of oesophageal cancer, is still to be explored. Mouse models do not or only poorly mimic human gastro-oesophageal physiology owing to the absence of oesophageal submucosal glands[98]. Moreover, isolating submucosal glands in fresh human tissue is technically challenging[99]. Therefore, more research effort is needed to reveal the heterogeneity of ECs in oesophageal adenocarcinoma.

Brain

The BBB, a unique vasculature formed by highly specialized ECs characterized by tight junctions with neighbouring ECs and expression of BBB-related transporters[100], is a considerable obstacle to effective drug delivery as it restricts the passage of most drug molecules into the brain parenchyma, creating a barrier to therapeutic intervention. Therefore, therapeutic targeting of primary and secondary (that is, those that have metastasized from other organs) brain cancers remains difficult[101]. Notably, a molecular atlas of human ECs from patients with glioblastoma identified five unique EC phenotypes with three TEC subpopulations largely localized in the tumour core and two NEC subpopulations largely from the peritumour brain[102] (Table 1). However, this distinct difference between TEC and NEC phenotypes remains to be validated in larger cohorts. ECs showed a heterogeneous expression of junctions and transporters, representing diverse states of EC activity and BBB dysfunction in tumour and peritumour tissues[102]. The results suggest that vascular leakage in glioblastoma is driven by two mechanisms: an increase in paracellular transport through the modification of tight junctions between ECs and/or an enhanced transcellular transport through changes in vesicular transcytosis[102].

Specifically, NEC subpopulations were characterized by high expression of genes involved in vascular integrity and BBB function[102]. Conversely, TEC subpopulations, particularly the *HSPG2*$^+$ tip cells, had high expression of angiogenic gene signatures such as basement membrane remodelling, cytoskeletal rearrangements, angiogenic sprouting and tip cell formation[102] (Table 1). TECs also displayed an enrichment of gene signatures associated with glycolysis, the citric acid cycle and OXPHOS, which reflects the high energy demand of angiogenesis in the TME[102]. Two phase I clinical trials targeting, respectively, CD93 (NCT05496595 (ref. 103) in patients with advanced or metastatic solid tumours, including glioblastoma) and vimentin (an intermediate filament protein) (NCT04396717 (ref. 104) in brain cancer), both of which are enriched in TECs, have been initiated (Table 1). By contrast, TECs partially lose the expression of BBB-related transporters, suggesting that the BBB is dysfunctional[102] (Fig. 1). Interestingly, PLVAP, a vascular marker of BBB disruption induced in pathological conditions in the brain[105] and also a marker of vascular permeability in peripheral organs[41–44], is highly expressed in TECs and could potentially be a marker for brain TECs[102].

Immunomodulatory ECs (IMECs) were also shown to be present and had distinct phenotypes in the tumour core and peritumour brain regions of patients with glioblastoma. *ACKR1*$^+$ TECs were highly enriched in the brain tumour core and expressed *IL1B*, *SELE* and *VCAM1* (Table 1), suggesting a leukocyte adhesion and inflammatory phenotype[102]. In peritumour brain regions, immunomodulatory NECs expressed high levels of MHC-II, *CCL4* and *CCL3*, suggesting an antigen presentation phenotype. However, these two IMEC subsets were predominantly derived from individual patients[102]. Whether these IMECs ubiquitously exist in patients with glioblastoma and whether their function could be therapeutically targeted remains to be uncovered in a larger scRNA-seq cohort.

The functional heterogeneity of TECs in brain cancer is far from being fully characterized as the brain is highly regionalized. Whether TECs, transdifferentiating from cancer cells through a process named vascular mimicry[106] (a process also identified in other tumour types[107]), can be identified at the single-cell level and whether the *trans*-differentiation process can be molecularly unravelled using in silico lineage tracing remain to be studied. Currently, AATs show limited efficiency in some patients with brain tumours as VEGF is not the only factor regulating angiogenesis in brain cancer[108]. More clinical trials are starting to investigate the combination of AATs (mainly anti-VEGFA) with immunotherapies[109]. Whether there exist biomarkers to predict patient responses or alternative angiogenic targets to VEGFA to avoid resistance warrants more research into TECs in brain cancer.

Reproductive system

Breast. A recent human scRNA-seq study has provided in-depth characterization of TECs in breast cancer and documented that angiogenic ECs and activated post-capillary venule ECs are enriched in tumour tissues compared with healthy breast tissues. Angiogenic ECs were found to express the VEGFR2 and the insulin receptor INSR, both of which are being targeted in clinical trials[110,111] (Table 1 and Fig. 1). Several ECM remodelling and OXPHOS-related genes were found to be upregulated in TECs compared with peritumour ECs in breast cancer[26,112]. Notably, ECM-associated genes are also overexpressed in ECs of other types of cancers, especially in CRC and lymphomas[112], raising the question of whether ECM-associated genes have a role as universal TEC markers (perhaps more than previously realized) and whether therapeutic strategies focusing on ECM targets should be considered in more detail. Interestingly, a new EC subset, lipid-processing ECs, which express peroxisome proliferator-activated receptor γ-regulated lipid-processing genes, was found to be under-represented in breast cancer[26]. A retrospective study on a very large cohort of patients with breast cancer, followed for more than a decade, revealed that treatment with an indirect peroxisome proliferator-activated receptor γ activator (metformin) offered a long-lasting survival benefit (in fact, as large as the standard of care hormonal therapy), which, interestingly, was associated with an increased fraction of lipid-processing ECs in the healthy breast tissue[26].

Analyses performed on previously published human scRNA-seq data also identified a higher fraction of tip-like TECs in tumours compared with adjacent tissues[113], indicating angiogenic activities in breast cancer. The fraction of tip ECs within the tumour was reported to correlate with the age of the patient, with younger patients showing a higher presence of tip TECs[113]; this observation was reported in other cancer types such as ovarian, liver cancer and melanoma as well[113].

A comparison between ECs of breast and lung cancer identified distinct phenotypic differences in TECs at the level of capillaries and veins, while exhibiting similar gene signatures of TECs involved in vessel sprouting[26]. Tissue-specific differences were also observed in the expression of immunomodulatory genes in human lung and breast cancers[26]. Although vein TECs in breast cancer showed higher expression of immunomodulatory genes, capillary TECs in lung cancer showed higher levels of genes involved in antigen processing and presentation[25,26,114]. This might be owing to the higher probability of capillaries encountering pathogens during exchange of gases, whereas shear stress and low flow rate characteristic of the veins in breast cancer might contribute to higher immune cell interactions[26]. Furthermore, compared with peritumour vECs, tumour vECs expressed lower levels of MHC-II and molecules involved in immune recruitment and inflammation[26]. Moreover, a recent study that combined omics studies with EC fate mapping and multiplex immune profiling identified the *trans*-differentiation of mouse venules into inflamed HEVs in the presence of anti-angiogenic immune modulating therapy in the PyMT and E0771 breast tumour mouse models[115]. Continuous paracrine signalling from $CD8^+$ T cells and natural killer cells was essential for the maintenance of these HEVs in the tumour. Encouragingly, the HEV gene signature in human breast tumours correlates with a response to immune checkpoint blockade (ICB) therapy[115] (Fig. 1). Anti-VEGFR2 therapy also synergizes with ICB therapy (anti-PD1) in breast cancer[116]. Therefore, mining scRNA-seq analyses for novel TEC targets holds promise in improving the efficacy of immunotherapies.

Prostate. In one scRNA-seq study of human prostate cancer, four EC clusters were identified: aECs, vECs, immature ECs and tip cells[117]. A few TEC markers were identified, for example, *CXCL12*, placenta-specific protein 9 (*PLAC9*) and prostaglandin I2 receptor (*PTGIR*). Interestingly, *CXCL12* is highly enriched in arterial TECs. Receptor–ligand interactome analyses predicted that arterial TECs interact with tip cells via a *CXCL12*–*CXCR4* axis to promote angiogenesis[117]. Heidegger et al.[117] showed that AMD3100, a CXCR4 inhibitor, suppresses human TEC proliferation and migration in vitro and results in decreased vessel number and density in a mouse prostate tumour model. Notably, the effects of AMD3100 therapy on CXCR4 regulation are observed only on TECs and not on NECs[117]. Currently, a phase I clinical trial is investigating a paclitaxel–CXCR4 peptide antagonist in advanced solid tumours (NCT05465590 (ref. 118); Table 1). Whether the CXCR4 inhibitor is a promising therapeutic target, especially combined with other treatment options in prostate cancer, remains to be determined.

Another human prostate cancer scRNA-seq study identified six TEC subsets[119], among which four TEC subsets, remarkably, expressed markers of cancer-associated fibroblasts (CAFs). The difference in TEC populations observed between these two prostate cancer studies might be attributed to the different tumour stages, in which the tumours containing CAF-like TECs are more progressive[117,119]. The CAF-like TECs were confirmed by flow cytometry staining and were shown to be enriched in castration-resistant prostate cancers compared with primary prostate cancers[119]. Chen et al.[119] predicted cell–cell interactions by CellPhoneDB and found that the CAF-like TECs have the highest number of interactions with epithelial cells compared with other cells. In vitro, a prostate cancer cell line cocultured with CAF-like TECs was more invasive than prostate cancer cells cocultured with parental fibroblasts, suggesting a role for CAF-like TECs in promoting tumour invasion and metastasis[119]. In addition, pathway enrichment and interactome analysis revealed that the CAF-like TECs were enriched in genes involved in ECM–receptor signalling and focal adhesions, whereas the non-CAF-like *ACKR1*$^+$ TECs could be immunomodulatory and were enriched in immune-related pathways such as chemotaxis[119] (Fig. 1). Despite the critical role of angiogenesis in prostate cancer[120], the VEGF antibody bevacizumab yields poor outcomes in castration-resistant prostate cancer[121], and so clinical interest has moved to explore the synergistic effect of AATs with anti-PDL1 therapies in prostate cancer (NCT05000294 (ref. 122) and NCT05489211 (ref. 123)), a combination which is supported by the identification of both tip cells (mentioned earlier) and immunomodulatory non-CAF-like *ACKR1*$^+$ TECs from scRNA-seq studies.

Lung

Despite being derived from a highly vascularized organ, lung ECs are usually understudied in scRNA-seq studies that look at the entire tumour[124–129]. To characterize TECs in lung cancer in depth, Goveia et al.[25]

enriched for ECs using the magnetic-activated cell sorting system by CD45$^+$ cell depletion and subsequent PECAM1$^+$ cell enrichment from both human and mouse lung tumours for scRNA-seq, resulting in the identification of 17 known and 16 previously unrecognized EC phenotypes. Further investigation of this TEC heterogeneity revealed a correlation between angiogenic signatures and the survival of patients with non-small-cell lung carcinoma (NSCLC)[25]. Specifically, patients with NSCLC, who expressed high levels of gene set signatures of angiogenic tip, immature, activated post-capillary or lymphatic TECs, had shorter overall survival, presumably because these signatures reflect active angiogenesis and lymphatic spread.

Peritumour cECs displayed a transcriptomic profile that included the expression of MHC-II, suggesting a role in immune surveillance[25]. This finding, along with the absence of *CD80* and *CD86*, both of which encode T lymphocyte activation ligands, suggested a role for these cECs as semi-professional antigen-presenting cells as previously identified by Raemer et al.[130]. Interestingly, the tip TECs, enriched in ECM remodelling and EC migration pathways, and the *PLVAP*$^+$ endoglin (*ENG*)$^+$ immature TEC phenotype, resembling stalk-like TECs, were only detectable in the tumour tissues and made up 8.1% and 10.4% of total TECs, respectively[25]. Three phase I clinical trials using a combination therapy to target endoglin and VEGF or PD1 in lung cancer have recently been completed[131–133], whereas another one is ongoing[134] (Table 1). It remains to be explored whether *ENG*$^+$ TECs make up a sufficiently large enough fraction of TECs to render such a therapeutic approach sufficiently efficacious. An activated post-capillary vein TEC phenotype was also present at higher numbers in lung tumour tissues when compared with peritumour non-malignant lung tissues. This subset expresses *ACKR1* and upregulates immunomodulatory factors and ribosomal proteins[25], which are features of HEVs in inflamed tissues[135] (Table 1 and Fig. 1).

Furthermore, the AAT using a VEGF antibody in a Lewis lung carcinoma mouse model showed that migratory tip and 'breach' TECs (a subset of TECs expressing genes related to basement-membrane remodelling and breaching) were more sensitive to VEGF blockade than post-capillary vein and capillary TECs, tuning these TEC subsets to promote a more quiescent and mature tumour vasculature with homeostatic functions[25]. This finding is consistent with another study by Wu et al.[114], predicting strong VEGF-mediated signals of interactions between cancer cells and tip cells, vECs and aECs in patients with NSCLC. Intratumour cellular and molecular interaction analysis also suggested a role for tumour-generated chemokines in angiogenesis, that is, a gene signature of the CXCL12–CXCR4 signalling axis between tumour cells and sprouting ECs was activated in patients with NSCLC.

Both bulk RNA-seq analysis and RT–PCR yielded a higher level of genes associated with collagen modification in lung TECs from patients with NSCLC compared with NECs[25]. Using genome-scale metabolic models, collagen biosynthesis-related genes were predicted to have an essential role in choroidal (part of the vascular layer of the eye) and lung tumour angiogenesis[59]. This finding is further strengthened by a meta-analysis of data sets from patients with five different tumour types including NSCLC, revealing that transcripts encoding collagen-modifying enzymes were enriched and ranked among the top 1–5% of most consistently upregulated genes in TECs[25]. This finding was then verified by the proteomics analysis using samples from four different human tumours including NSCLC, showing that collagen-modifying enzymes lysyl oxidase-like 2 (LOXL2), procollagen-lysine,2-oxoglutarate 5-dioxygenase 1 (PLOD1) and PLOD2 were highly upregulated in TECs, together suggesting that collagen modification could be considered a candidate angiogenic pathway in lung cancer[25] (Fig. 1).

Characterization of TECs in other scRNA-seq studies of human lung tumours is relatively scarce[124,125,136]. Nonetheless, a consistent downregulation of genes involved in immune activation was reported in *ACKR1*$^+$ *IGFBP3*$^+$ TECs and *SPRY1*$^+$ TECs[124,125], reinforcing the hypothesis that TECs may have a role in promoting immune tolerance in lung tumours. Several clinical trials targeting IGF1R without targeting the immunomodulation arm have failed in metastatic and advanced NSCLC[137–139] (Table 1), raising the question of whether a combination with immunotherapy might yield a better outcome. Decreased expression of FOS, JUN and E74-like factor 3 (*ELF3*) along with increased expression of friend leukaemia integration 1 (*FLI1*) and TEA domain transcription factor 1 (*TEAD1*) in TECs was predicted to be responsible for this immunomodulatory phenotype of TECs[124] (Table 1). Additionally, TECs in lung adenocarcinoma (LUAD) express high levels of angiogenic genes accommodating neovascularization and vascular changes[124]. On the contrary, TECs of ground glass nodules, an early type of LUAD with good prognosis, exhibited decreased angiogenic signatures compared with solid LUAD, which might be owing to the low-grade malignancy state[126]. Overall, scRNA-seq studies on lung cancer suggest that ECs may have a prominent role in tumour growth by promoting angiogenesis and suppressing antitumour immunity.

Excretory system

Kidney. In the kidney, renal endothelial cells of the glomerular, cortical and medullary compartments are exposed to distinct microenvironmental factors for supporting different kidney functions, such as vasodilation, renin production, osmolarity gradient formation and immune surveillance[140]. A single-cell atlas developed by Dumas et al.[141] identified intercompartment and intracompartment renal EC heterogeneity in the mouse kidney. Specifically, 24 different renal EC phenotypes in healthy mice (of which 8 were novel) were identified, highlighting extensive heterogeneity of these cells between and within the cortex, glomeruli and medulla.

Expression profiling of human clear cell renal cell carcinoma (ccRCC) and benign kidney tissues using scRNA-seq revealed that the tumour vasculature predominantly comprised two distinct subpopulations of ECs marked by *PLVAP*[142] and *ACKR1* (refs. 142,143), respectively, both of which have also been reported in other tumour types (Table 1). *PLVAP*$^+$ TECs in ccRCC tumour tissues, which consist of multiple small clusters in dimension reduced graphs and therefore might be more heterogeneous than reported, expressed higher levels of endothelin receptor type B (*EDNRB*; a phase I trial targeting endothelin receptor type B in combination with a PDL1 antibody to solid tumours has been initiated (NCT04205227 (ref. 144); Table 1)), Von Willebrand factor and *HSPG2*, but lower levels of genes associated with the interferon γ response than *PLVAP*$^+$ NECs in benign tissues[142]. In addition, compared with *PLVAP*$^+$ TECs, *ACKR1*$^+$ TECs were less abundant, mainly derived from tumour tissues, and showed decreased expression of VEGF receptors, including *KDR* and *FLT1*, suggesting that this EC subtype might be able to evade AATs[142].

Strikingly, a high fraction of *PLVAP*$^+$ ECs in ccRCC negatively correlates with patient survival and clinical benefit from nivolumab (a PD1 antibody)[142]. By comparing the bulk RNA-seq data of tumours from patients who responded to nivolumab (7 patients) with that from tumours of patients who did not respond (19 patients), a set of predictive genes were identified[142]. When validated in the scRNA-seq data, genes associated with non-response were predominantly expressed by pericytes, *PLVAP*$^+$ ECs and vascular smooth muscle cells[142]. Instead, *ACKR1*$^+$ TECs expressed genes associated with both response and non-response[142]. By orthogonal

immunohistochemical staining of PECAM1 (a marker of EC intercellular junctions), nivolumab responders were shown to have fewer TECs than non-responders[142]. Moreover, the survival benefit is more pronounced in patients who were predicted to have high levels of $CD8^+$ T cells and low levels of *PLVAP*$^+$ ECs compared with those patients who were predicted to have high numbers of *PLVAP*$^+$ ECs[142] (Fig. 1).

Combination therapies (of PDL1 antibodies and TKIs) have achieved better but limited responses in advanced RCC compared with TKIs alone[145,146]. The results of Zhang et al.[142] add exciting insight that signatures of *PLVAP*$^+$ ECs could have predictive values for immunotherapy responses. Possibly, current combination therapies might also benefit from stratifying patients on the basis of the *PLVAP*$^+$ EC signature. Although another scRNA-seq data set of human ccRCC failed to identify *PLVAP*$^+$ ECs[147] (raising concerns of reproducibility (Box 3)), the strategies of integrating scRNA-seq analyses with bulk RNA-seq analyses and clinical data as adopted by Zhang et al.[142] might be useful to answer some outstanding questions in the future, for instance, (i) which subcluster (subclusters) in *PLVAP*$^+$ ECs and which gene (genes) have prognostic or predictive values in ccRCC? (ii) Via which mechanism do *PLVAP*$^+$ ECs function? (iii) Can we target the protein products of the gene (genes) to improve current combination therapies?

Integumentary system

Several scRNA-seq studies have focused on different aspects of skin cancer[148–152], but only a few studies have analysed the EC compartment[151,153,154]. Notable expansion of three vascular EC subpopulations expressing *ACKR1*, *CXCL12* and *CXCL3* was found in human keloids (a dermal fibrotic disorder, exhibiting biological features similar to malignant tumours) compared with 'normal' skin tissue[153]. Notably, the expanded *ACKR1*$^+$ ECs represented antigen-presenting ECs expressing MHC-IIs (Table 1), and in all vascular ECs, the VEGFR signalling pathway was activated[153]. In addition, tumour-related signalling pathways such as oncogenic MAPK, WNT and PTEN signalling pathways were also activated in vascular ECs in keloids, suggesting that overlap exists in the dysregulated pathways between keloid and malignant tumours[153].

In human cutaneous squamous cell carcinoma, a tumour-specific keratinocyte (TSK) population unique to cancer and localized to a fibrovascular niche was predicted to modulate the endothelium through interactions between placental growth factor and FLT1, placental growth factor and neuropilin 2 (NRP2) and ephrin B1 and ephrin type-B receptor 4 (ref. 151). At the same time, ECs were found to express ligands for TSKs, such as transforming growth factor β (*TGFB*) and integrin β1 (*ITGB1*)[151]. The high expression of TSK markers (plasminogen activator, urokinase (*PLAU*) and *ITGB1*) was correlated with lower progression-free survival after treatment with PD1 inhibitors, suggesting a possible immunosuppressive activity of TSKs or an intrinsic resistance to immune attack[155]. Further studies have demonstrated the influence of the immunomodulatory function of ECs in skin cancer. The CXCL12–CXCR4 pathway in mice contributes to angiogenesis in skin cancer[156] and inhibits immune cell infiltration and activation in melanoma[157]. A phase I clinical trial combining anti-CXCR4 and anti-PDL1 (NCT02823405 (ref. 158); Table 1) was well tolerated in patients with advanced melanoma and led to an increased interferon γ gene signature in the tumour[159], supporting further investigation of this combination.

Endocrine system

An scRNA-seq study identified a potential vascular–immune crosstalk in human papillary thyroid carcinoma (PTC)[160]. Almost all the *PLVAP*$^+$ tip cells, aECs, *ACKR1*$^+$ vECs and *PLVAP*$^+$ *IGFBP3*$^+$ immature ECs were found to be located in primary or metastatic tumour samples, whereas only LECs were enriched in normal thyroid tissues[160] (Table 1 and Fig. 1). Furthermore, TECs were predicted to interact with immune cells in various ways. For instance, vECs, immature ECs and aECs were predicted to interact with immune cells through expression of *ICAM1*, which was reduced in tip and LECs. Instead, tip ECs interacted with immune cells mainly through the key angiogenic VEGF–VEGFR signalling pathway, which has been targeted in multiple clinical trials for thyroid cancer[161]. scRNA-seq performed on human PTC tissue also identified ECs by high expression of *ENG* and *PLVAP*[162]. Furthermore, cytokine–receptor interactions were predicted in *ACKR1*$^+$ ECs within the PTC tumour[162], suggesting a role in the enhancement of lymphocyte transmigration as described previously in human liver cirrhosis[163]. Moreover, the immune infiltration in PTC tumours is closely related to the survival of patients with PTC[164].

Consistent with the gender difference in thyroid cancer epidemiology[165], female patients with PTC were shown to have a higher proportion of ECs with different and higher strength of interactions between malignant cells and ECs compared with male patients[166]. For example, the human leukocyte antigen–receptor interaction of fibroblasts and ECs with malignant epithelial cells was more prominent in females, whereas the TGFβ–receptor interactions were more common in male patients with PTC. This study suggests that gender might be a potential factor contributing to EC heterogeneity.

Cross-organ comparison

ECs from healthy tissues exhibit considerable differences across organs and vascular beds (Box 2). In each cancer type described earlier, it is also clear that TECs are highly heterogeneous. However, some TECs share the same marker genes or seem to have similar predicted functions across organs. Notably, *ACKR1*, *PLVAP* and *IGFBP3* are the three most frequently observed markers of TECs (Fig. 2a), although the reason for this remarkable observation remains unknown to date. We hypothesize that the pro-angiogenic and chronic inflammatory TME[167] might be implicated. PLVAP can be induced in TECs by VEGF signalling[35,45], whereas IGFBP3 regulates angiogenesis[168–170] and can be induced by tumour necrosis factor (TNF)[171] and TGFβ[74], two common inflammation regulatory cytokines in tumours[167]. *ACKR1*$^+$ ECs may contribute to the inflammatory microenvironment in tumours[172,173] and thus become selected for by the cancer. However, only PLVAP is being directly targeted in HCC (NCT04601428 (ref. 50)), albeit in clinical trials at a very early stage. It may be that the other markers cannot be targeted owing to the broad expression of ACKR1 (ref. 174) in non-TEC cells and the secreted nature of IGFBP3. However, these three markers are not mutually exclusive and the marked TECs can overlap in functions (Fig. 2a), for example, *PLVAP*$^+$ cells can be *ACKR1*$^+$ (ref. 35), and both *PLVAP*$^+$ TECs and *IGFBP3*$^+$ TECs were associated with immunosuppression in liver cancer[35,38].

Angiogenic tip cells, immunomodulatory TECs and ECM remodelling TECs are the most common functional subsets across different tumours (Fig. 2a). These three functional TEC subsets align with the cancer hallmarks, inducing or accessing vasculature, tumour-promoting inflammation or avoiding immune attack and activating invasion and metastasis[5], suggesting a harmonized cooperation between different TECs in promoting tumour progression. Angiogenic tip cells are the most commonly observed phenotype in different tumours and modulate angiogenesis in different ways (Fig. 2b). However, the resolution of tip cell classification varies among different studies, that is, some tip cell subsets could be further subclustered. This variation

results in varying percentages of tip cells (5–60%) identified in different studies[25,26,82,83,102,160]. This discrepancy could reflect true biological differences or, instead, be due to technical differences and the lack of standardization to identify these (and other) EC subtypes. Normally, tip cells only exist in active sprouting angiogenesis. Angiogenic tip cell-targeting AATs have shown some therapeutic benefit in various cancer types[7]. Knowing the percentage of true tip cells is important, because it might determine the sensitivity of tumours to and efficacy of AATs. The other two functions of TECs have been less well explored. To date, ECM-targeting agents have shown limited efficacy in treating tumours in clinical trials[175,176]. But the combination of ECM-targeting agents and immunotherapies, especially for the immune-excluded tumours, has not been broadly explored.

Single-cell studies revealed that 'IMECs'[91] might have relevant functions in controlling pro-tumour or antitumour immunity[35,115,142]. These cells express low levels of MHC-II but high levels of chemoattractant molecules for immune cells (Fig. 2b). It remains to be explored whether targeting IMECs may achieve a tumoricidal effect by reversing the immunosuppressive TME. Although IMECs have not been specifically targeted, low-dose AATs as vascular normalizing agents to enhance the efficacy of immunotherapies have been approved in several tumour types and are now undergoing more clinical trials in other tumour types[96]. However, current AATs only target the VEGF–VEGFR signalling pathway and do not exploit the full spectrum of novel angiogenic or immunomodulatory genes revealed by single-cell omics studies. By data mining differentially expressed genes or cell–cell interaction molecules in TECs from scRNA-seq studies (discussed subsequently), we foresee more potent TEC-targeting therapeutics to be used either as single modalities or in combination with immunotherapies.

Despite the observation of common TEC markers and predicted functions in different tumour types, a 'universal pan-tumour' TEC-targeting therapy might be challenging. In healthy humans and mice, ECs show organ heterogeneity[21,23]. Even in the absence of a TEC atlas of the whole human body (discussed subsequently), we can already observe some degree of organ-dependent, disease-dependent and treatment-dependent differences in TECs. For example, the IMEC phenotype is more prominent in capillaries in the lung, whereas it is more prominent in veins in the breast[25,26]. TECs showed dynamic changes in the relative abundance of their TEC phenotypes during gastric cancer development[82] and even changed phenotype after chemoradiotherapy[71] (Fig. 1). In different tumours or the same tumour at different stages or under treatment, the coordination and contribution of angiogenic tip, immunomodulatory and ECM remodelling TECs might be different (Fig. 2b), raising the question of whether this will ultimately determine therapeutic targeting efficacy.

Future opportunities and challenges

Strategies for tapping TEC single-cell omics data

TEC atlas. Most scRNA-seq studies use open science practice that enable public access and reuse for mining, even though General Data Protection Regulation (GDPR) legislation may prevent such accessibility. However, the abundance of data related to the vascular compartment in individual studies is often low, precluding a detailed and all-encompassing interrogation of its heterogeneity. Many studies have not investigated ECs in sufficient detail, limiting the studies we could review herein and leaving ECs unmined. A recent study integrated all cell types from 10 solid cancer types and identified a common EndMT process and TEC interactions with CAFs and macrophages[177].

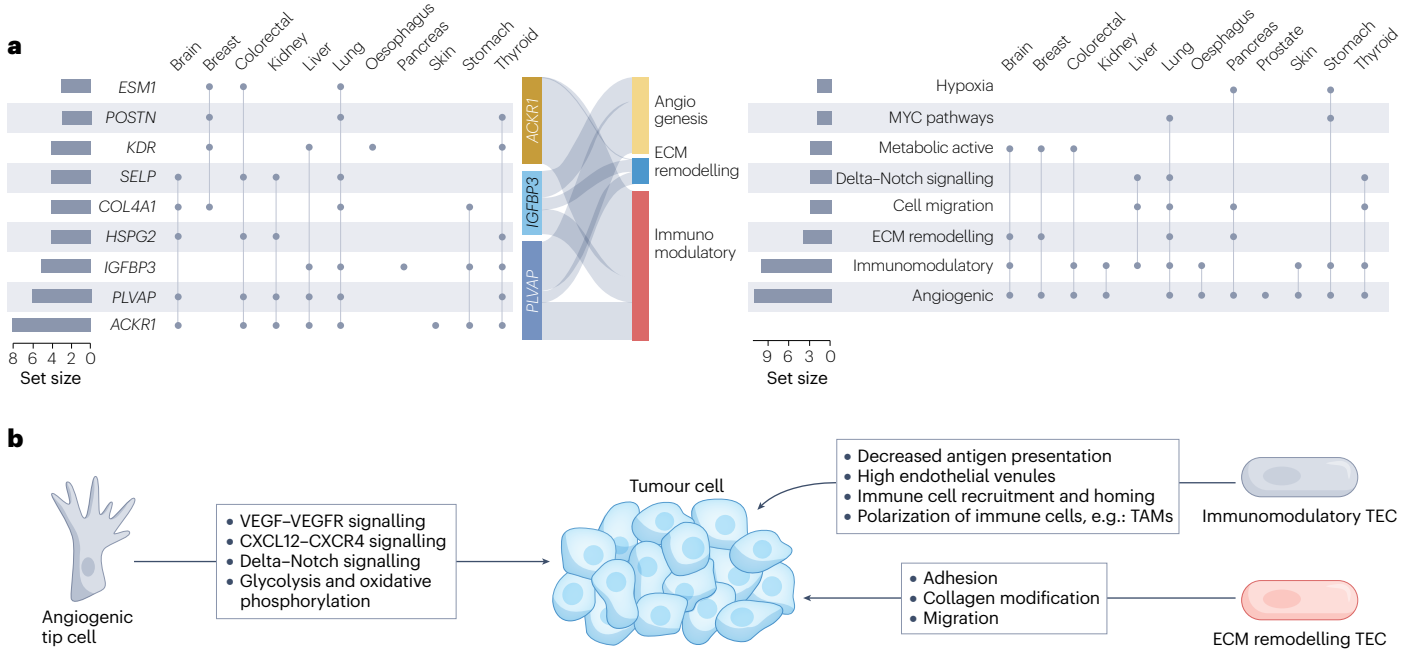

Fig. 2 | Common markers and functions of tumour-enriched endothelial cells. **a**, UpSet plot of top markers and functions of tumour-enriched endothelial cell subsets across different studies. The top 3 markers are mapped to the top 3 reported functions, as shown in the Sankey plot[25,26,35,57,58,61,70,82,83,85,86,102,117,125,142,153,160,162,204]. **b**, Summary of the ways in which tumour endothethial cells (TECs) can accomplish the top 3 functions to promote tumour progression. ECM, extracellular matrix; TAM, tumour-associated macrophage; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor. The data presented in part **a** were extracted from the studies presented in Table 1 and are available in the Supplementary Information. The UpSet plot was generated with UpSetR software[205] and modified by Inkscape. The Sankey plot was plotted using *R* and the code is available in the Supplementary Information.

Glossary

Angiocrine signalling
The paracrine or juxtacrine signalling between endothelial cells and the neighbouring cells to regulate tissue growth and repair.

Angiogenesis
The formation of new blood vessels from existing ones.

Batch effects
Systematic variations in experimental measurements that are not caused by the biological factors, but rather by technical factors such as differences in experimental conditions, instruments, reagents or equipment.

Chromium single-cell-fixed RNA profiling
A high-throughput single-cell gene expression profiling technique that uses oligonucleotide-conjugated antibodies to capture and barcode individual cells in fixed tissue samples.

Cytometry by time of flight
(CyTOF). A single-cell analysis technique that combines flow cytometry with mass spectrometry and differentiates the metal isotype-labelled antibodies by the time of flight.

Doublet cells
Two or more aggregated cells that are encapsulated into one reaction volume and tagged by the same barcode during a single-cell RNA sequencing experiment.

Duplex RNA in situ hybridization
A technique used to detect and visualize two RNA molecules simultaneously within a single sample.

Fenestral diaphragms
A thin protein barrier anchored in the fenestrae that is found in endothelial cells containing multiple small circular openings.

Gene set variation analysis
A computational method to calculate the gene enrichment score of a pathway in samples.

Genome-scale metabolic models
A mathematical modelling approach that predicts the metabolic network reconstructions, metabolic pathways and metabolite production rates of an organism.

Hierarchical clustering
A computational method to group similar cells and form a hierarchy of clusters.

In silico lineage tracing
A computational method to determine cell lineage and fate of individual cells on the basis of their gene expression profiles and/or epigenetic markers.

Kupffer cells
Specialized liver macrophages involved in maintaining liver homeostasis.

Mural cell
Specialized cells found in the walls of blood vessels, including vascular smooth muscle cells and pericytes.

RNA velocity
A computational method used to predict the direction and speed of cell differentiation by analysing the spliced and unspliced RNA molecules.

Shear stress
The parallel force applied on the endothelial surface of the blood vessel by flowing blood.

Vesicular transcytosis
The transportation of macromolecules from one side of an epithelial or endothelial cell to the other side through vesicles.

However, even in this study, TECs remained under-characterized (only four clusters identified). At the same time, existing studies have annotated ECs differently, making comparisons across studies or even across organs challenging. For example, in lung and lung cancer alone, Lambrechts et al.[125] numbered 6 EC clusters; Goveia et al.[25] annotated 13 human EC clusters, whereas another lung EC atlas study annotated 6 EC clusters according to the vascular bed[27], raising an urgent need to implement guidelines or standard nomenclature for ECs.

Increasing the magnitude of EC-derived single-cell data sets, by performing a joint analysis across all publicly available tumour studies, could offer a solution to this problem. In 2020, a mouse EC atlas of 11 healthy mouse tissues was published, which improved the understanding of EC heterogeneity across and within tissues[21]. This atlas by way of a reference contributed to the proper description of ECs in other studies. The development of a new EC atlas comprising human and/or mouse cancer tissues should facilitate the discovery and the identification of new putative ECs and novel gene expression in known EC phenotypes and the ability to compare (organotypic and/or vascular bed) EC types and abundance within tissues during pathological angiogenesis in cancer. The hope would be that such a TEC atlas might be useful in predicting the sensitivity of tumours to and efficacy of AATs by comparing the percentage of tip cells and the expression level of angiogenic receptors. This TEC atlas should also allow the assessment of age or gender as potential factors involved in the transcriptomic diversity among ECs from the same (cancer) organ.

To further benefit the scientific community, visualization tools for scRNA-seq data (and other omics data) are needed. Reanalysing scRNA-seq data requires bioinformatic expertise, which has a steep learning curve for biomedical scientists. Individual accompanying webtools (for example, those of the Carmeliet laboratory) or timely updates in the popular databases (such as CELLxGENE, Single Cell Portal, and Single Cell Expression Atlas) for published scRNA-seq data could enable non-bioinformatician scientists to validate the genes of interest within a few clicks.

Targeting endothelial cell immunomodulation. Accumulating evidence suggests that ECs are involved in immune responses[91,96], that is, IMECs[91]. Furthermore, AATs can enhance the infiltration of tumoricidal immune cells and synergize with immune-boosting therapies such as ICB[96]. More than 80 clinical trials have been initiated combining AATs with immunotherapies, with 5 combinations having been approved by the FDA[96]. Recent advances in scRNA-seq have further reinforced the notion that subsets of ECs are immunomodulatory (Table 1). Goveia et al.[25] discovered that subsets of ECs putatively regulate immune surveillance in lung cancer, having a transcriptome signature of (i) HEVs involved in immune cell recruitment, or (ii) antigen-presenting cells. In addition, others also found that TECs in lung cancer[125] and CRCs[57] downregulate antigen presentation gene signatures compared with NECs. Whether and how we can target these IMEC clusters for tumour immunotherapy needs further investigation.

Prioritization of targetable candidates. scRNA-seq data offer an unprecedented opportunity to discover candidate genes for therapeutic development in cancer. Performing meta-analyses across different platforms (for instance, scRNA-seq, cytometry by time of flight (CyTOF) and bulk transcriptomics and epigenetic analysis) and comparing data across species (for example, mouse, rat and human) can narrow down candidate cell types and genes with essential biological roles in

a pathological condition, rendering the prioritization of targetable candidates. This approach determines genes and proteins that are differentially regulated in the pathological condition, independent of the method and species used. For instance, an integrated meta-analysis of candidate gene expression across species, diseases and models identified *PLOD1* and *PLOD2* as novel angiogenic candidates[25]. Silencing each gene impaired in vitro and in vivo vessel sprouting, hence validating the therapeutic potential of the protein products of these genes. Other strategies for the prioritization of novel candidate genes have been reviewed elsewhere[30].

More recently, the Guidelines on Target Assessment for Innovative Therapeutics (GOT-IT) working group proposed five assessment blocks for prioritizing targets in a more translational and visionary approach. These assessment blocks are comprehensive and consider the target–disease linkage, safety aspects, microbial targets, strategic issues and technical feasibility[178]. Collectively, all these approaches demonstrate the possibility to prioritize potential cell types and targets on the basis of complex scRNA-seq data, to unravel important knowledge of EC subtypes and disease marker genes that are most relevant for further research in a cancer-specific context.

New technologies for future TEC studies

Temporal scRNA-seq. scRNA-seq captures the transcriptomic state of cells at specific time points. However, as biological systems are not static, the way TECs change phenotypically during tumour growth or treatment is barely understood. To evaluate the dynamic changes of TECs throughout such processes for translational target discovery (for example, for identifying genes whose protein products are involved in tumour initiation or resistance to therapies[179]), two approaches could be taken: computational (trajectory analysis) and experimental. However, the capacity of computational algorithms is limited and may not always correctly reconstruct the temporal ordering[180]. Therefore, experimental time-series data are required to complement the computational tools.

A conventional method to construct the dynamic models of TECs is to collect tumour samples at discrete intervals. For example, breast cancer tissues were collected before and during anti-PD1 treatment, and the intratumour immunophenotypes were scrutinized to discover useful markers to predict treatment responses[181], although phenotypic changes in TECs were not analysed. Nonetheless, choosing the time point of sampling and the number of time points is a great challenge for conventional temporal scRNA-seq studies. Currently, it is mainly based on the expertise of researchers and the availability of precious tumour tissues (usually only 2–3 time points). Batch effects can be another challenge but could be alleviated by computational integration[182] or technical multiplexing (such as with the use of chromium single-cell-fixed RNA profiling). By comparing TEC phenotypes from responders to AATs with that of non-responders, which is currently lacking, we would be able to discover approaches to predict responses of patients or overcome the resistance to AATs.

More recently, metabolic labelling of newly synthesized RNA with 4-thiouridine or 6-thioguanine enables one to construct the trajectory with higher resolution. Nevertheless, such strategies (for example, single-cell, thiol-(SH)-linked alkylation of RNA for metabolic labelling sequencing (scSLAM-seq)[183], new transcriptome alkylation-dependent single-cell RNA-sequencing (NASC-seq)[184] and single-cell metabolically labelled new RNA tagging sequencing (scNT-seq)[185]) can only track RNAs for a few hours. Live-seq represents another strategy that can sequentially sample the cytoplasmic biopsy of the same cell without inducing major cellular perturbations and therefore directly map the trajectory of a cell[186]. However, both strategies only apply to isolated cells or tissues, which is a barrier to studying TECs because they quickly lose their phenotypes ex vivo[25]. A further development that enables trajectory recording in vivo is demanded.

Single-cell spatial transcriptomics. Cellular communication between ECs and their neighbouring cells is vital for maintaining vascular homeostasis and remodelling. However, as samples are lysed after tissue isolation for scRNA-seq, the spatial information is lost. Therefore, single-cell spatial transcriptomics (scST) profiling of tumours is required to retain the spatial structure of TECs. The rationale for and comparisons between different scST technologies[187] and computational methods are reviewed elsewhere in detail[188]. Subsequently, we briefly highlight some of the key challenges faced by scST.

First, the resolution versus quantity (gene) trade-off needs to be resolved. 'Targeted' scST, such as multiplexed error-robust fluorescence in situ hybridization (MERFISH)[189] and sequential fluorescence in situ hybridization (seqFISH)[190], has subcellular resolution but can only measure a subset of the whole transcriptome, ranging from 10 to 10,000 genes. 'Untargeted' scST can sequence the whole transcriptome. However, current widely adopted untargeted scST technologies lack single-cell resolution. For example, Visium ST has a resolution of 55 µm, whereas GeoMX DSP has a resolution of several hundreds of micrometres, and the size of a cell is, on average, about 10 µm. By contrast, high-definition spatial transcriptomics reported a resolution of 2 µm[191], and spatial enhanced resolution omics-sequencing (Stereo-seq) reported a resolution of 0.5 µm[192]; however both methods are neither commercialized nor widely validated. Second, although many platforms are (gradually becoming) compatible with formalin-fixed or paraffin-embedded (FFPE) tissues, the detection capacity and efficiency of scST in FFPE tissues are lower than those in frozen tissues[187]. Third, despite the many available deconvolution algorithms[193], there is no benchmark study to date to compare their performances. Fourth, analysing and interpreting scST data requires special expertise. Interactive visualization of published data would greatly promote the spread and reutilization of scST data.

In general, scST is being increasingly adopted by scientists and will improve over time. However, to date, no publications using untargeted scST have focused on TECs. The high cost of untargeted scST and scarcity of ECs within a small piece of 2D tissue may be underlying reasons. More optimization using scST is therefore required to understand TECs within the original tumour milieu.

Single-cell multi-omics. A cell bears multidimensional information: genomic (DNA copy number), epigenomic (chromatin accessibility and DNA methylation), transcriptomic, proteomic, metabolomic, perturbational, spatial and temporal. Multi-omics technologies have emerged that are able to capture multiple dimensions from the same cell, which better reflects the complex networks of interactions that are responsible for cellular functions. For example, by conjugating DNA oligonucleotide barcodes to antibodies, cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq)[194] and RNA expression and protein sequencing (REAP-seq)[195] or intracellular staining and sequencing (INs-seq)[196] can measure surface or intracellular proteins and the transcriptome of a single cell simultaneously. Deterministic barcoding in tissue for spatial omics sequencing (DbiT-seq) co-maps mRNAs and proteins and gives spatial information to both 'omics' layers[197]. Additionally, other combinations of multi-omics have also

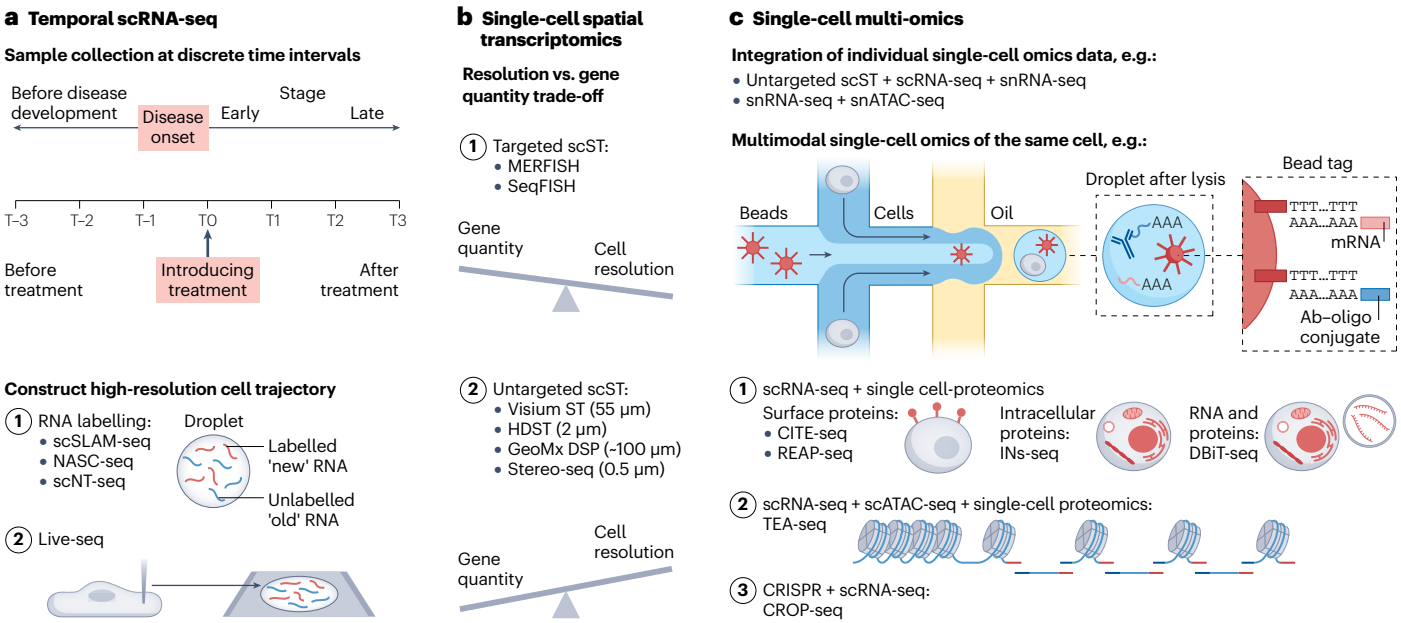

Fig. 3 | New technologies to delineate endothelial cell heterogeneity. This schematic outlines ways to delineate the endothelial cell heterogeneity in tumour microenvironments using existing and in-coming computational and experimental tools, or combinations of both. **a**, Experimental discrete time-series single-cell RNA sequencing (scRNA-seq) data can aid computational algorithms in constructing the temporal ordering of tumour endothelial cells (TECs) along the process of disease progression or treatment. Metabolic labelling of newly synthesized RNA also increases the resolution of trajectories. In addition, Live-seq poses a great tool to directly map the trajectory of a cell by sampling the cell continuously. **b**, Single-cell spatial transcriptomics (scST) could uncover the vascular communications between TECs and neighbouring cells during homeostasis and remodelling. ScST tools must often contend with the trade-off between resolution and gene quantity. Targeted scST offers subcellular resolution but can only measure 10–10,000 genes. Untargeted scST can sequence the whole transcriptome but lacks single-cell resolution. **c**, Combining omics measurements of a cell (genetic, epigenomic, transcriptomic, proteomic, metabolomic, perturbational, spatial and temporal information) allows the discovery of the full spectrum of heterogeneity of TECs and the complexity of intercellular and intracellular networks. This combination can be experimental (simultaneous measurement) or post-experimental (computational integration). By conjugating DNA oligonucleotide barcodes to antibodies (Ab), cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) and RNA expression and protein sequencing (REAP-seq) can simultaneously quantify 125 surface proteins and the transcriptome of a cell. Additionally, intracellular staining and sequencing (INs-seq) and deterministic barcoding in tissue for spatial omics sequencing (DBiT-seq) can detect intracellular proteins, transcriptional factors and signalling pathway activity. Other single-cell multi-omics approaches can expand the possible combinations further, such as TEA-seq, a trimodal assay that simultaneously measures transcriptomics (scRNA-seq), epitopes and chromatin accessibility (scATAC-seq) from thousands of single cells[206] and CRISPR droplet sequencing (CROP-seq)[207]. ATAC-seq, assay for transposase-accessible chromatin with sequencing; GeoMx DSP, GeoMx® Digital Spatial Profiler; HDST, high-definition spatial transcriptomics; INs-seq, intracellular staining and sequencing; MERFISH, multiplexed error-robust fluorescence in situ hybridization; NASC-seq, new transcriptome alkylation-dependent single-cell RNA sequencing; scNT-seq, single-cell metabolically labeled new RNA tagging sequencing; sc-SLAM-seq, single-cell, thiol-(SH)-linked alkylation of RNA for metabolic labelling sequencing; seqFISH, sequential fluorescence in situ hybridization; snRNA-seq, single-nucleus RNA sequencing; stereo-seq, spatial enhanced resolution omics-sequencing; TEA-seq, transcription, epitopes, and accessibility sequencing; Visium ST, Visium spatial transcriptomics.

been developed recently (Fig. 3) and have been well described in other studies[198–201]. Besides measuring multi-omics of the same cell, integrating different single-cell omics data of the same tissue provides an alternative way for more possible 'omics' combinations. However, the way in which different types of single-cell omics data should be integrated is a hot topic in the field[182].

For studying TECs, unfortunately, no work integrating multimodal single-cell omics data has yet been published. Such works in the future might include looking at transcription factors or signalling pathways that control the heterogeneity of TECs (for example, using scRNA-seq or INs-seq together with single-cell assay for transposase-accessible chromatin with sequencing (scATAC-seq)) and possible interactions with other stromal cells before and after AAT treatment (for example, using AAT treatment with DbiT-seq) to understand AAT resistance and for further development of novel TEC-targeting therapies. Figure 3 provides an illustration of ways in which EC heterogeneity in TMEs can be delineated using existing or in-coming computational and experimental tools or combinations of both.

Concluding remarks

Single-cell omics technologies are powerful for answering the question of heterogeneity. Almost all types of cancers have been profiled by scRNA-seq. However, because of the scarcity of ECs in tumour tissues and the lower recovery of non-immune stromal cells compared with tumour-infiltrating immune cells, many scRNA-seq studies have failed to characterize the small fraction of ECs in sufficient detail. Current available data suggest that ECs in cancer are heterogeneous, and one or more TEC clusters are transcriptomically separated from NECs. These TEC clusters express a remarkably altered gene signature with pro-tumour properties, particularly related to angiogenesis, ECM remodelling and

immunomodulation, such as through the downregulation of MHC-II. Tip cells are the most consistently observed TECs, reflecting the elevated need for blood supply in tumours. Other subclusters of TECs are, however, less consistently characterized among different types of tumour (Table 1), partly owing to the different annotations, warranting the need for an integrated TEC atlas and a standard EC nomenclature.

An increasingly large set of single-cell omics data can be mined for novel target discovery. Current clinically approved AATs primarily target the VEGF–VEGFR signalling pathway with insufficient therapeutic efficacy. Publicly available single-cell sequencing data have the potential to enable the identification of novel TEC targets or interactions that are only present in tumours but not in normal tissues. However, despite the power of scRNA-seq, limitations remain (Box 3). RNA levels do not necessarily reflect or correlate with functions as cells regulate functions on multiple levels, calling for the combination of multi-omics and functional validations. Additional single-cell omics data with temporal and spatial dimensions from patients before and after treatments will be instrumental. Retrospective analyses linking the single-cell omics phenotype with responses of patients will also facilitate the discovery of predictive markers for treatment response[142] and new regimens or combinations to overcome resistance.

Finally, there are outstanding questions surrounding TEC functions. For example, do TECs show less cross-organ heterogeneity than NECs? Do cancer cell-derived TECs, exhibiting vascular mimicry, exist and are they functionally different from other TECs? Do metastatic cancer cells imprint TECs differently from non-metastatic cancer cells and if so, how is this achieved? What is the importance of non-tip cell TECs in the TME and can they be therapeutically targeted? What specific roles do TECs have in modulating antitumour immunity? Are they less immunogenic or less tolerogenic[91]? Can we modulate TECs (for example, metabolically) to make them more immunogenic? Answering these questions by tapping publicly available and performing well-designed single-cell omics studies combined with functional validation will be pivotal for developing the next generation of TEC-targeting therapies.

Data availability

Markers and functions of TECs were extracted from the original referenced work. The summarized tables for plotting Fig. 2a and the source code used in this paper and written by Q.Z. are available as Supplementary Information.

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References

1. Potente, M., Gerhardt, H. & Carmeliet, P. Basic and therapeutic aspects of angiogenesis. *Cell* **146**, 873–887 (2011).
2. Pober, J. S. & Sessa, W. C. Evolving functions of endothelial cells in inflammation. *Nat. Rev. Immunol.* **7**, 803–815 (2007).
3. Potente, M. & Mäkinen, T. Vascular heterogeneity and specialization in development and disease. *Nat. Rev. Mol. Cell Biol.* **18**, 477–494 (2017).
4. Augustin, H. G. & Koh, G. Y. Organotypic vasculature: from descriptive heterogeneity to functional pathophysiology. *Science* https://doi.org/10.1126/science.aal2379 (2017).
5. Hanahan, D. Hallmarks of cancer: new dimensions. *Cancer Discov.* **12**, 31–46 (2022).
6. Jayson, G. C., Kerbel, R., Ellis, L. M. & Harris, A. L. Antiangiogenic therapy in oncology: current status and future directions. *Lancet* **388**, 518–529 (2016).
7. Garcia, J. et al. Bevacizumab (Avastin®) in cancer treatment: a review of 15 years of clinical experience and future outlook. *Cancer Treat. Rev.* **86**, 102017 (2020).
8. Yang, Y. et al. Anti-VEGF– and anti-VEGF receptor-induced vascular alteration in mouse healthy tissues. *Proc. Natl Acad. Sci. USA* **110**, 12018–12023 (2013).
9. Cao, Y. VEGF-targeted cancer therapeutics — paradoxical effects in endocrine organs. *Nat. Rev. Endocrinol.* **10**, 530–539 (2014).
10. Goel, S., Wong, A. H.-K. & Jain, R. K. Vascular normalization as a therapeutic strategy for malignant and nonmalignant disease. *Cold Spring Harb. Perspect. Med.* https://doi.org/10.1101/cshperspect.a006486 (2012).
11. Cao, Y. Off-tumor target — beneficial site for antiangiogenic cancer therapy. *Nat. Rev. Clin. Oncol.* **7**, 604–608 (2010).
12. Xue, Y. et al. Anti-VEGF agents confer survival advantages to tumor-bearing mice by improving cancer-associated systemic syndrome. *Proc. Natl Acad. Sci. USA* **105**, 18513–18518 (2008).
13. Bergers, G. & Hanahan, D. Modes of resistance to anti-angiogenic therapy. *Nat. Rev. Cancer* **8**, 592–603 (2008).
14. Gacche, R. N. & Assaraf, Y. G. Redundant angiogenic signaling and tumor drug resistance. *Drug. Resist. Updat.* **36**, 47–76 (2018).
15. Carmeliet, P., De Smet, F., Loges, S. & Mazzone, M. Branching morphogenesis and antiangiogenesis candidates: tip cells lead the way. *Nat. Rev. Clin. Oncol.* **6**, 315–326 (2009).
16. Jakobsson, L. et al. Endothelial cells dynamically compete for the tip cell position during angiogenic sprouting. *Nat. Cell Biol.* **12**, 943–953 (2010).
17. Croix, B. S. et al. Genes expressed in human tumor endothelium. *Science* **289**, 1197–1202 (2000).
18. Bussolati, B., Deambrosis, I., Russo, S., Deregibus, M. C. & Camussi, G. Altered angiogenesis and survival in human tumor-derived endothelial cells. *FASEB J.* **17**, 1159–1161 (2003).
19. Ohga, N. et al. Heterogeneity of tumor endothelial cells: comparison between tumor endothelial cells isolated from high- and low-metastatic tumors. *Am. J. Pathol.* **180**, 1294–1307 (2012).
20. Kikuchi, H. et al. Chemotherapy-induced IL8 upregulates MDR1/ABCB1 in tumor blood vessels and results in unfavorable outcome. *Cancer Res.* **80**, 2996–3008 (2020).
21. Kalucka, J. et al. Single-cell transcriptome atlas of murine endothelial cells. *Cell* **180**, 764–779.e20 (2020).
This work pioneers an atlas of EC heterogeneity in the whole body of healthy mice.
22. Paik, D. T. et al. Single-cell RNA sequencing unveils unique transcriptomic signatures of organ-specific endothelial cells. *Circulation* **142**, 1848–1862 (2020).
23. Jones, R. C. et al. The tabula sapiens: a multiple-organ, single-cell transcriptomic atlas of humans. *Science* **376**, eabl4896 (2022).
24. Wang, F. et al. Endothelial cell heterogeneity and microglia regulons revealed by a pig cell landscape at single-cell level. *Nat. Commun.* **13**, 3620 (2022).
25. Goveia, J. et al. An integrated gene expression landscape profiling approach to identify lung tumor endothelial cell heterogeneity and angiogenic candidates. *Cancer Cell* **37**, 21–36.e13 (2020).
This work for the first time characterizes ECs in depth specifically in lung cancer and validates bioinformatic findings by integrated approaches.
26. Geldhof, V. et al. Single cell atlas identifies lipid-processing and immunomodulatory endothelial cells in healthy and malignant breast. *Nat. Commun.* **13**, 5511 (2022).
This work for the first time systematically analyses EC heterogeneity at single-cell resolution in breast cancer.
27. Schupp, J. C. et al. Integrated single-cell atlas of endothelial cells of the human lung. *Circulation* **144**, 286–302 (2021).
28. Gerhardt, H. et al. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. *J. Cell Biol.* **161**, 1163–1177 (2003).
29. Apte, R. S., Chen, D. S. & Ferrara, N. VEGF in signaling and disease: beyond discovery and development. *Cell* **176**, 1248–1264 (2019).
30. Becker, L. M. et al. Deciphering endothelial heterogeneity in health and disease at single cell resolution: progress and perspectives. *Cardiovasc. Res.* https://doi.org/10.1093/cvr/cvac018 (2022).
31. Cavalli, M. et al. A multi-omics approach to liver diseases: integration of single nuclei transcriptomics with proteomics and HiCap bulk data in human liver. *OMICS* **24**, 180–194 (2020).
32. Wang, H. et al. Integrative single-cell transcriptome analysis reveals a subpopulation of fibroblasts associated with favorable prognosis of liver cancer patients. *Transl. Oncol.* **14**, 100981 (2021).
33. Zhang, Y. et al. Single-cell transcriptome analysis reveals tumor immune microenvironment heterogenicity and granulocytes enrichment in colorectal cancer liver metastases. *Cancer Lett.* **470**, 84–94 (2020).
34. Zhao, Q. et al. Heterogeneity and chimerism of endothelial cells revealed by single-cell transcriptome in orthotopic liver tumors. *Angiogenesis* **23**, 581–597 (2020).
35. Sharma, A. et al. Onco-fetal reprogramming of endothelial cells drives immunosuppressive macrophages in hepatocellular carcinoma. *Cell* **183**, 377–394.e21 (2020).
This work identifies a conserved EC population in liver cancer and the fetal liver and demonstrates its important role in contributing to the immunosuppressive TME.
36. Massalha, H. et al. A single cell atlas of the human liver tumor microenvironment. *Mol. Syst. Biol.* **16**, e9682 (2020).
37. Aizarani, N. et al. A human liver cell atlas reveals heterogeneity and epithelial progenitors. *Nature* **572**, 199–204 (2019).
38. Xue, R. et al. Liver tumour immune microenvironment subtypes and neutrophil heterogeneity. *Nature* **612**, 141–147 (2022).
39. Xing, X. & Song, J. Identification of the different gene expression characteristics from liver cirrhosis to hepatocellular carcinoma using single-cell sequencing analyses. *J. Immunol. Res.* **2021**, 6619302 (2021).
40. Ioannidou, S. et al. An in vitro assay reveals a role for the diaphragm protein PV-1 in endothelial fenestra morphogenesis. *Proc. Natl Acad. Sci. USA* **103**, 16770–16775 (2006).

41. Stan, R. V. et al. The diaphragms of fenestrated endothelia: gatekeepers of vascular permeability and blood composition. *Dev. Cell* **23**, 1203–1218 (2012).
42. Jones, J. H., Friedrich, E., Hong, Z., Minshall, R. D. & Malik, A. B. PV1 in caveolae controls lung endothelial permeability. *Am. J. Respir. Cell Mol. Biol.* **63**, 531–539 (2020).
43. Rantakari, P. et al. The endothelial protein PLVAP in lymphatics controls the entry of lymphocytes and antigens into lymph nodes. *Nat. Immunol.* **16**, 386–396 (2015).
44. Keuschnigg, J. et al. The prototype endothelial marker PAL-E is a leukocyte trafficking molecule. *Blood* **114**, 478–484 (2009).
45. Strickland, L. A. et al. Plasmalemmal vesicle-associated protein (PLVAP) is expressed by tumour endothelium and is upregulated by vascular endothelial growth factor-A (VEGF). *J. Pathol.* **206**, 466–475 (2005).
46. Li, Z. et al. Single-cell transcriptome analyses reveal novel targets modulating cardiac neovascularization by resident endothelial cells following myocardial infarction. *Eur. Heart J.* **40**, 2507–2520 (2019).
47. Wang, Y. et al. Plasmalemma vesicle-associated protein promotes angiogenesis in cholangiocarcinoma via the DKK1/CKAP4/PI3K signaling pathway. *Oncogene* **40**, 4324–4337 (2021).
48. Bergen, V., Lange, M., Peidli, S., Wolf, F. A. & Theis, F. J. Generalizing RNA velocity to transient cell states through dynamical modeling. *Nat. Biotechnol.* **38**, 1408–1414 (2020).
49. Wang, Y.-H. et al. Plasmalemmal vesicle associated protein (PLVAP) as a therapeutic target for treatment of hepatocellular carcinoma. *BMC Cancer* **14**, 815 (2014).
50. US National Library of Medicine. *ClinicalTrials.gov* https://ClinicalTrials.gov/ct2/show/NCT04601428 (2020).
51. Poisson, J. et al. Liver sinusoidal endothelial cells: physiology and role in liver diseases. *J. Hepatol.* **66**, 212–227 (2017).
52. Thomann, S. et al. YAP orchestrates heterotypic endothelial cell communication via HGF/c-MET signaling in liver tumorigenesis. *Cancer Res.* **80**, 5502–5514 (2020).
53. Browaeys, R., Saelens, W. & Saeys, Y. NicheNet: modeling intercellular communication by linking ligands to target genes. *Nat. Methods* **17**, 159–162 (2020).
54. Wang, B. et al. Induction of tumor angiogenesis by Slit-Robo signaling and inhibition of cancer growth by blocking Robo activity. *Cancer Cell* **4**, 19–29 (2003).
55. Raza, A., Franklin, M. J. & Dudek, A. Z. Pericytes and vessel maturation during tumor angiogenesis and metastasis. *Am. J. Hematol.* **85**, 593–598 (2010).
56. Pitulescu, M. E. et al. Dll4 and Notch signalling couples sprouting angiogenesis and artery formation. *Nat. Cell Biol.* **19**, 915–927 (2017).
57. Lee, H. O. et al. Lineage-dependent gene expression programs influence the immune landscape of colorectal cancer. *Nat. Genet.* **52**, 594–603 (2020).
58. Qian, J. et al. A pan-cancer blueprint of the heterogeneous tumor microenvironment revealed by single-cell profiling. *Cell Res.* **30**, 745–762 (2020).
This work is an 'antetype' of a human tumour EC atlas and compares ECs from three different types of cancer.
59. Rohlenova, K. et al. Single-cell RNA sequencing maps endothelial metabolic plasticity in pathological angiogenesis. *Cell Metab.* **31**, 862–877.e14 (2020).
60. Thiriot, A. et al. Differential DARC/ACKR1 expression distinguishes venular from non-venular endothelial cells in murine tissues. *BMC Biol.* **15**, 45 (2017).
61. Pelka, K. et al. Spatially organized multicellular immune hubs in human colorectal cancer. *Cell* **184**, 4734–4752.e20 (2021).
62. Che, L.-H. et al. A single-cell atlas of liver metastases of colorectal cancer reveals reprogramming of the tumor microenvironment in response to preoperative chemotherapy. *Cell Discov.* **7**, 80 (2021).
63. Li, X., Kumar, A. & Carmeliet, P. Metabolic pathways fueling the endothelial cell drive. *Annu. Rev. Physiol.* **81**, 483–503 (2019).
64. Kuipers, E. J. et al. Colorectal cancer. *Nat. Rev. Dis. Prim.* **1**, 15065 (2015).
65. Lin, W. et al. Single-cell transcriptome analysis of tumor and stromal compartments of pancreatic ductal adenocarcinoma primary tumors and metastatic lesions. *Genome Med.* **12**, 80 (2020).
66. Wang, X. et al. Single-cell RNA-seq reveals the genesis and heterogeneity of tumor microenvironment in pancreatic undifferentiated carcinoma with osteoclast-like giant-cells. *Mol. Cancer* **21**, 133 (2022).
67. Peng, J. et al. Single-cell RNA-seq highlights intra-tumoral heterogeneity and malignant progression in pancreatic ductal adenocarcinoma. *Cell Res.* **29**, 725–738 (2019).
68. Chen, K. et al. Single-cell RNA-seq reveals dynamic change in tumor microenvironment during pancreatic ductal adenocarcinoma malignant progression. *eBioMedicine* https://doi.org/10.1016/j.ebiom.2021.103315 (2021).
69. Zhou, Y. et al. Single-cell RNA sequencing reveals spatiotemporal heterogeneity and malignant progression in pancreatic neuroendocrine tumor. *Int. J. Biol. Sci.* **17**, 3760–3775 (2021).
70. Chen, K. et al. Hypoxic pancreatic cancer derived exosomal miR-30b-5p promotes tumor angiogenesis by inhibiting GJA1 expression. *Int. J. Biol. Sci.* **18**, 1220–1237 (2022).
71. Shiau, C. et al. Treatment-associated remodeling of the pancreatic cancer endothelium at single-cell resolution. *Front. Oncol.* https://doi.org/10.3389/fonc.2022.929950 (2022).
72. Abdel-Wahab, R. et al. Randomized, phase I/II study of gemcitabine plus IGF-1R antagonist (MK-0646) versus gemcitabine plus erlotinib with and without MK-0646 for advanced pancreatic adenocarcinoma. *J. Hematol. Oncol.* **11**, 71 (2018).
73. Mutgan, A. C. et al. Insulin/IGF-driven cancer cell-stroma crosstalk as a novel therapeutic target in pancreatic cancer. *Mol. Cancer* **17**, 66 (2018).
74. Baxter, R. C. IGF binding proteins in cancer: mechanistic and clinical insights. *Nat. Rev. Cancer* **14**, 329–341 (2014).
75. Kundranda, M. et al. Randomized, double-blind, placebo-controlled phase II study of istiratumab (MM-141) plus nab-paclitaxel and gemcitabine versus nab-paclitaxel and gemcitabine in front-line metastatic pancreatic cancer (CARRIE). *Ann. Oncol.* **31**, 79–87 (2020).
76. Fuchs, C. S. et al. A phase 3 randomized, double-blind, placebo-controlled trial of ganitumab or placebo in combination with gemcitabine as first-line therapy for metastatic adenocarcinoma of the pancreas: the GAMMA trial†. *Ann. Oncol.* **26**, 921–927 (2015).
77. Choi, K. J., Nam, J.-K., Kim, J.-H., Choi, S.-H. & Lee, Y.-J. Endothelial-to-mesenchymal transition in anticancer therapy and normal tissue damage. *Exp. Mol. Med.* **52**, 781–792 (2020).
78. Choi, S.-H. et al. Tumour-vasculature development via endothelial-to-mesenchymal transition after radiotherapy controls CD44v6+cancer cell and macrophage polarization. *Nat. Commun.* **9**, 5108 (2018).
79. Schlesinger, Y. et al. Single-cell transcriptomes of pancreatic preinvasive lesions and cancer reveal acinar metaplastic cells' heterogeneity. *Nat. Commun.* **11**, 4516 (2020).
80. Hosein, A. N., Brekken, R. A. & Maitra, A. Pancreatic cancer stroma: an update on therapeutic targeting strategies. *Nat. Rev. Gastroenterol. Hepatol.* **17**, 487–505 (2020).
81. Fox, J. G. & Wang, T. C. Inflammation, atrophy, and gastric cancer. *J. Clin. Invest.* **117**, 60–69 (2007).
82. Yin, H. et al. A dynamic transcriptome map of different tissue microenvironment cells identified during gastric cancer development using single-cell RNA sequencing. *Front. Immunol.* https://doi.org/10.3389/fimmu.2021.728169 (2021).
This work is a good example of how one could take advantage of publicly available data and gain insights into the dynamic changes of ECs during cancer development.
83. Jiang, H. et al. Revealing the transcriptional heterogeneity of organ-specific metastasis in human gastric cancer using single-cell RNA Sequencing. *Clin. Transl. Med.* **12**, e730 (2022).
84. Jeong, H. Y. et al. Spatially distinct reprogramming of the tumor microenvironment based on tumor invasion in diffuse-type gastric cancers. *Clin. Cancer Res.* **27**, 6529–6542 (2021).
85. Li, Y. et al. Single-cell landscape reveals active cell subtypes and their interaction in the tumor microenvironment of gastric cancer. *Theranostics* **12**, 3818–3833 (2022).
86. Sun, K. et al. scRNA-seq of gastric tumor shows complex intercellular interaction with an alternative T cell exhaustion trajectory. *Nat. Commun.* **13**, 4943 (2022).
87. Li, X. et al. Single-cell RNA sequencing reveals a pro-invasive cancer-associated fibroblast subgroup associated with poor clinical outcomes in patients with gastric cancer. *Theranostics* **12**, 620–638 (2022).
88. Yu, X. et al. Tumor vessel normalization and immunotherapy in gastric cancer. *Ther. Adv. Med. Oncol.* **14**, 17588359221110176 (2022).
89. Zhang, X. et al. Dissecting esophageal squamous-cell carcinoma ecosystem by single-cell transcriptomic analysis. *Nat. Commun.* **12**, 5291 (2021).
90. Nagl, L., Horvath, L., Pircher, A. & Wolf, D. Tumor endothelial cells (TECs) as potential immune directors of the tumor microenvironment — new findings and future perspectives. *Front. Cell Dev. Biol.* **8**, 766–766 (2020).
91. Amersfoort, J., Eelen, G. & Carmeliet, P. Immunomodulation by endothelial cells — partnering up with the immune system? *Nat. Rev. Immunol.* https://doi.org/10.1038/s41577-022-00694-4 (2022).
This review article comprehensively revisits the immunomodulatory function of ECs in different organs and diseases.
92. Saharinen, P., Eklund, L. & Alitalo, K. Therapeutic targeting of the angiopoietin–TIE pathway. *Nat. Rev. Drug Discov.* **16**, 635–661 (2017).
93. Chen, Y. T. et al. Platelet-derived growth factor receptor signaling activates pericyte–myofibroblast transition in obstructive and post-ischemic kidney fibrosis. *Kidney Int.* **80**, 1170–1181 (2011).
94. US National Library of Medicine. *ClinicalTrials.gov*, http://www.clinicaltrials.gov/ct2/show/NCT04879368 (2021).
95. Chen, Z. et al. Dissecting the single-cell transcriptome network in patients with esophageal squamous cell carcinoma receiving operative paclitaxel plus platinum chemotherapy. *Oncogenesis* **10**, 71–71 (2021).
96. Huinen, Z. R., Huijbers, E. J. M., van Beijnum, J. R., Nowak-Sliwinska, P. & Griffioen, A. W. Anti-angiogenic agents — overcoming tumour endothelial cell anergy and improving immunotherapy outcomes. *Nat. Rev. Clin. Oncol.* https://doi.org/10.1038/s41571-021-00496-y (2021).
This is a comprehensive review article of the clinical applications of AAT and immunotherapy combinations.
97. Ma, J. & Waxman, D. J. Combination of antiangiogenesis with chemotherapy for more effective cancer treatment. *Mol. Cancer Ther.* **7**, 3670–3684 (2008).
98. Kapoor, H., Lohani, K. R., Lee, T. H., Agrawal, D. K. & Mittal, S. K. Animal models of Barrett's esophagus and esophageal adenocarcinoma — past, present, and future. *Clin. Transl. Sci.* **8**, 841–847 (2015).
99. Nowicki-Osuch, K. et al. Molecular phenotyping reveals the identity of Barrett's esophagus and its malignant transition. *Science* **373**, 760–767 (2021).
100. Arvanitis, C. D., Ferraro, G. B. & Jain, R. K. The blood–brain barrier and blood–tumour barrier in brain tumours and metastases. *Nat. Rev. Cancer* **20**, 26–41 (2020).

101. Peleli, M., Moustakas, A. & Papapetropoulos, A. Endothelial–tumor cell interaction in brain and CNS malignancies. *Int. J. Mol. Sci.* https://doi.org/10.3390/ijms21197371 (2020).
102. Xie, Y. et al. Key molecular alterations in endothelial cells in human glioblastoma uncovered through single-cell RNA sequencing. *JCI Insight* https://doi.org/10.1172/jci.insight.150861 (2021).
This work comprehensively characterizes EC heterogeneity in glioblastoma and demonstrates distinct TEC phenotypes in the tumour core.
103. US National Library of Medicine. *ClinicalTrials.gov* http://www.clinicaltrials.gov/ct2/show/NCT05496595 (2022).
104. US National Library of Medicine. *ClinicalTrials.gov* http://www.clinicaltrials.gov/ct2/show/NCT04396717 (2022).
105. Bosma, E. K., van Noorden, C. J. F., Schlingemann, R. O. & Klaassen, I. The role of plasmalemma vesicle-associated protein in pathological breakdown of blood–brain and blood–retinal barriers: potential novel therapeutic target for cerebral edema and diabetic macular edema. *Fluids Barriers CNS* **15**, 24 (2018).
106. Soda, Y. et al. Transdifferentiation of glioblastoma cells into vascular endothelial cells. *Proc. Natl Acad. Sci. USA* **108**, 4274–4280 (2011).
107. Wei, X. et al. Mechanisms of vasculogenic mimicry in hypoxic tumor microenvironments. *Mol. Cancer* **20**, 7 (2021).
108. Wälchli, T. et al. Shaping the brain vasculature in development and disease in the single-cell era. *Nat. Rev. Neurosci.* **24**, 271–298 (2023).
109. Jain, S., Chalif, E. J. & Aghi, M. K. Interactions between anti-angiogenic therapy and immunotherapy in Glioblastoma. *Front. Oncol.* https://doi.org/10.3389/fonc.2021.812916 (2022).
110. Zhang, M. et al. Anti-vascular endothelial growth factor therapy in breast cancer: molecular pathway, potential targets, and current treatment strategies. *Cancer Lett.* **520**, 422–433 (2021).
111. Cejuela, M., Martin-Castillo, B., Menendez, J. A. & Pernas, S. Metformin and breast cancer: where are we now? *Int. J. Mol. Sci.* **23**, 2705 (2022).
112. Sun, Z. et al. Single-cell RNA sequencing reveals gene expression signatures of breast cancer-associated endothelial cells. *Oncotarget* **9**, 10945–10961 (2018).
113. Wang, W., Wang, L., She, J. & Zhu, J. Examining heterogeneity of stromal cells in tumor microenvironment based on pan-cancer single-cell RNA sequencing data. *Cancer Biol. Med.* **19**, 30–42 (2021).
114. Wu, S. Z. et al. A single-cell and spatially resolved atlas of human breast cancers. *Nat. Genet.* **53**, 1334–1347 (2021).
115. Hua, Y. et al. Cancer immunotherapies transition endothelial cells into HEVs that generate TCF1+T lymphocyte niches through a feed-forward loop. *Cancer Cell* https://doi.org/10.1016/j.ccell.2022.11.002 (2022).
116. Li, Q. et al. Low-dose anti-angiogenic therapy sensitizes breast cancer to PD1 blockade. *Clin. Cancer Res.* **26**, 1712–1724 (2020).
117. Heidegger, I. et al. Comprehensive characterization of the prostate tumor microenvironment identifies CXCR4/CXCL12 crosstalk as a novel antiangiogenic therapeutic target in prostate cancer. *Mol. Cancer* **21**, 132 (2022).
118. US National Library of Medicine. *ClinicalTrials.gov* http://www.clinicaltrials.gov/ct2/show/NCT05465590 (2022).
119. Chen, S. et al. Single-cell analysis reveals transcriptomic remodellings in distinct cell types that contribute to human prostate cancer progression. *Nat. Cell Biol.* **23**, 87–98 (2021).
120. Ioannidou, E. et al. Angiogenesis and anti-angiogenic treatment in prostate cancer: mechanisms of action and molecular targets. *Int. J. Mol. Sci.* **22**, 9926 (2021).
121. Kelly, W. K. et al. Randomized, double-blind, placebo-controlled phase III trial comparing docetaxel and prednisone with or without bevacizumab in men with metastatic castration-resistant prostate cancer: CALGB 90401. *J. Clin. Oncol.* **30**, 1534–1540 (2012).
122. US National Library of Medicine. *ClinicalTrials.gov* http://www.clinicaltrials.gov/ct2/show/NCT05000294 (2021).
123. US National Library of Medicine. *ClinicalTrials.gov* http://www.clinicaltrials.gov/ct2/show/NCT05489211 (2022).
124. Kim, N. et al. Single-cell RNA sequencing demonstrates the molecular and cellular reprogramming of metastatic lung adenocarcinoma. *Nat. Commun.* **11**, 2285 (2020).
125. Lambrechts, D. et al. Phenotype molding of stromal cells in the lung tumor microenvironment. *Nat. Med.* **24**, 1277–1289 (2018).
126. Lu, T. et al. Single-cell transcriptome atlas of lung adenocarcinoma featured with ground glass nodules. *Cell Discov.* **6**, 69 (2020).
127. Ma, K. Y. et al. Single-cell RNA sequencing of lung adenocarcinoma reveals heterogeneity of immune response-related genes. *JCI Insight* https://doi.org/10.1172/jci.insight.121387 (2019).
128. Zhang, F. et al. Dynamics of peripheral T cell clones during PD-1 blockade in non-small cell lung cancer. *Cancer Immunol. Immunother.* **69**, 2599–2611 (2020).
129. Zhong, R. et al. Immune cell infiltration features and related marker genes in lung cancer based on single-cell RNA-seq. *Clin. Transl. Oncol.* **23**, 405–417 (2021).
130. Raemer, P. C. et al. Endothelial progenitor cells possess monocyte-like antigen-presenting and T-cell-co-stimulatory capacity. *Transplantation* **87**, 340–349 (2009).
131. US National Library of Medicine. *ClinicalTrials.gov* http://www.clinicaltrials.gov/ct2/show/NCT01332721 (2011).
132. US National Library of Medicine. *ClinicalTrials.gov* https://ClinicalTrials.gov/ct2/show/NCT03780010 (2018).
133. US National Library of Medicine. *ClinicalTrials.gov* https://ClinicalTrials.gov/ct2/show/NCT03181308 (2017).
134. US National Library of Medicine. *ClinicalTrials.gov* https://ClinicalTrials.gov/ct2/show/NCT05401110 (2022).
135. Girard, J.-P. & Springer, T. A. High endothelial venules (HEVs): specialized endothelium for lymphocyte migration. *Immunol. Today* **16**, 449–457 (1995).
136. Bischoff, P. et al. Single-cell RNA sequencing reveals distinct tumor microenvironmental patterns in lung adenocarcinoma. *Oncogene* **40**, 6748–6758 (2021).
137. Moran, T. et al. Activity of dalotuzumab, a selective anti-IGF1R antibody, in combination with erlotinib in unselected patients with non-small-cell lung cancer: a phase I/II randomized trial. *Exp. Hematol. Oncol.* **3**, 1 (2014).
138. Huang, C. H. et al. Impact study: MK-0646 (dalotuzumab), insulin growth factor 1 receptor antibody combined with pemetrexed and cisplatin in stage IV metastatic non-squamous lung cancer. *Front. Oncol.* https://doi.org/10.3389/fonc.2015.00301 (2016).
139. Langer, C. J. et al. Randomized, phase III trial of first-line figitumumab in combination with paclitaxel and carboplatin versus paclitaxel and carboplatin alone in patients with advanced non-small-cell lung cancer. *J. Clin. Oncol.* **32**, 2059–2066 (2014).
140. Molema, G., Zijlstra, J. G., van Meurs, M. & Kamps, J. A. A. M. Renal microvascular endothelial cell responses in sepsis-induced acute kidney injury. *Nat. Rev. Nephrol.* **18**, 95–112 (2022).
141. Dumas, S. J. et al. Single-cell RNA sequencing reveals renal endothelium heterogeneity and metabolic adaptation to water deprivation. *J. Am. Soc. Nephrol.* **31**, 118–138 (2020).
142. Zhang, Y. et al. Single-cell analyses of renal cell cancers reveal insights into tumor microenvironment, cell of origin, and therapy response. *Proc. Natl Acad. Sci. USA* **118**, e2103240118 (2021).
This work adopts ambitious strategies combining scRNA-seq data of RCC and patient survival and response to an immunotherapy to discover the prognostic and predictive values of PLVAP$^+$ EC signatures.
143. Young, M. D. et al. Single-cell transcriptomes from human kidneys reveal the cellular identity of renal tumors. *Science* **361**, 594–599 (2018).
144. US National Library of Medicine. *ClinicalTrials.gov*, https://ClinicalTrials.gov/ct2/show/NCT04205227 (2019).
145. Rini, B. I. et al. Pembrolizumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. *N. Engl. J. Med.* **380**, 1116–1127 (2019).
146. Motzer, R. J. et al. Avelumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. *N. Engl. J. Med.* **380**, 1103–1115 (2019).
147. Shi, Y. et al. Decoding the multicellular ecosystem of vena caval tumor thrombus in clear cell renal cell carcinoma by single-cell RNA sequencing. *Genome Biol.* **23**, 87 (2022).
148. Tirosh, I. et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science* **352**, 189–196 (2016).
149. Jerby-Arnon, L. et al. A cancer cell program promotes T cell exclusion and resistance to checkpoint blockade. *Cell* **175**, 984–997.e24 (2018).
150. Li, J. et al. Single-cell characterization of the cellular landscape of acral melanoma identifies novel targets for immunotherapy. *Clin. Cancer Res.* **28**, 2131–2146 (2022).
151. Ji, A. L. et al. Multimodal analysis of composition and spatial architecture in human squamous cell carcinoma. *Cell* **182**, 497–514.e22 (2020).
152. Durante, M. A. et al. Single-cell analysis reveals new evolutionary complexity in uveal melanoma. *Nat. Commun.* **11**, 496 (2020).
153. Liu, X. et al. Single-cell RNA-sequencing reveals lineage-specific regulatory changes of fibroblasts and vascular endothelial cells in keloids. *J. Invest. Dermatol.* **142**, 124–135.e111 (2022).
154. Brosseau, J. P. et al. Human cutaneous neurofibroma matrisome revealed by single-cell RNA sequencing. *Acta Neuropathol. Commun.* **9**, 11 (2021).
155. Miao, Y. et al. Adaptive immune resistance emerges from tumor-initiating stem cells. *Cell* **177**, 1172–1186.e1114 (2019).
156. Chen, G. S. et al. CXC chemokine receptor CXCR4 expression enhances tumorigenesis and angiogenesis of basal cell carcinoma. *Br. J. Dermatol.* **154**, 910–918 (2006).
157. Saxena, R., Wang, Y. & Mier, J. W. CXCR4 inhibition modulates the tumor microenvironment and retards the growth of B16-OVA melanoma and Renca tumors. *Melanoma Res.* **30**, 14–25 (2020).
158. US National Library of Medicine. *ClinicalTrials.gov* https://ClinicalTrials.gov/ct2/show/NCT02823405 (2016).
159. Andtbacka, R. H. I. et al. Mavorixafor, an orally bioavailable CXCR4 antagonist, increases immune cell infiltration and inflammatory status of tumor microenvironment in patients with melanoma. *Cancer Res. Commun.* **2**, 904–913 (2022).
160. Pu, W. et al. Single-cell transcriptomic analysis of the tumor ecosystems underlying initiation and progression of papillary thyroid carcinoma. *Nat. Commun.* **12**, 6058 (2021).
161. Melaccio, A. et al. Prognostic and therapeutic role of angiogenic microenvironment in thyroid cancer. *Cancers* **13**, 2775 (2021).
162. Pan, J. et al. Papillary thyroid carcinoma landscape and its immunological link with hashimoto thyroiditis at single-cell resolution. *Front. Cell Dev. Biol.* **9**, 758339 (2021).
163. Ramachandran, P. et al. Resolving the fibrotic niche of human liver cirrhosis at single-cell level. *Nature* **575**, 512–518 (2019).
164. Yang, Z. et al. A new risk factor indicator for papillary thyroid cancer based on immune infiltration. *Cell Death Dis.* **12**, 51 (2021).
165. Lee, Y. H. et al. Is male gender a prognostic factor for papillary thyroid microcarcinoma. *Ann. Surg. Oncol.* **24**, 1958–1964 (2017).

166. Peng, M. et al. Single-cell transcriptomic landscape reveals the differences in cell differentiation and immune microenvironment of papillary thyroid carcinoma between genders. *Cell Biosci.* **11**, 39 (2021).
167. Greten, F. R. & Grivennikov, S. I. Inflammation and cancer: triggers, mechanisms, and consequences. *Immunity* **51**, 27–41 (2019).
168. GRANATA, R. et al. Insulin-like growth factor binding protein-3 induces angiogenesis through IGF-I- and SphK1-dependent mechanisms. *J. Thromb. Haemost.* **5**, 835–845 (2007).
169. Kim, J.-H. et al. Antiangiogenic antitumor activities of IGFBP-3 are mediated by IGF-independent suppression of Erk1/2 activation and Egr-1-mediated transcriptional events. *Blood* **118**, 2622–2631 (2011).
170. Dallinga, M. G. et al. IGF-binding proteins 3 and 4 are regulators of sprouting angiogenesis. *Mol. Biol. Rep.* **47**, 2561–2572 (2020).
171. Anwar, A., Zahid, A. A., Scheidegger, K. J., Brink, M. & Delafontaine, P. Tumor necrosis factor-α regulates insulin-like growth factor-1 and insulin-like growth factor binding protein-3 expression in vascular smooth muscle. *Circulation* **105**, 1220–1225 (2002).
172. Horton, L. W., Yu, Y., Zaja-Milatovic, S., Strieter, R. M. & Richmond, A. Opposing roles of murine duffy antigen receptor for chemokine and murine CXC chemokine receptor-2 receptors in murine melanoma tumor growth. *Cancer Res.* **67**, 9791–9799 (2007).
173. Jenkins, B. D. et al. Atypical chemokine receptor 1 (DARC/ACKR1) in breast tumors is associated with survival, circulating chemokines, tumor-infiltrating immune cells, and African ancestry. *Cancer Epidemiol. Biomark. Prev.* **28**, 690–700 (2019).
174. Massara, M., Bonavita, O., Mantovani, A., Locati, M. & Bonecchi, R. Atypical chemokine receptors in cancer: friends or foes? *J. Leukoc. Biol.* **99**, 927–933 (2016).
175. Cox, T. R. The matrix in cancer. *Nat. Rev. Cancer* **21**, 217–238 (2021).
176. Jiang, Y. et al. Targeting extracellular matrix stiffness and mechanotransducers to improve cancer therapy. *J. Hematol. Oncol.* **15**, 34 (2022).
177. Luo, H. et al. Pan-cancer single-cell analysis reveals the heterogeneity and plasticity of cancer-associated fibroblasts in the tumor microenvironment. *Nat. Commun.* **13**, 6619 (2022).
178. Emmerich, C. H. et al. Improving target assessment in biomedical research: the GOT-IT recommendations. *Nat. Rev. Drug Discov.* **20**, 64–81 (2021).
This work systematically assesses the processes of translational research and provides helpful insights.
179. Su, Y. et al. Multi-omic single-cell snapshots reveal multiple independent trajectories to drug tolerance in a melanoma cell line. *Nat. Commun.* **11**, 2345 (2020).
180. Ding, J., Sharon, N. & Bar-Joseph, Z. Temporal modelling using single-cell transcriptomics. *Nat. Rev. Genet.* **23**, 355–368 (2022).
181. Bassez, A. et al. A single-cell map of intratumoral changes during anti-PD1 treatment of patients with breast cancer. *Nat. Med.* **27**, 820–832 (2021).
182. Miao, Z., Humphreys, B. D., McMahon, A. P. & Kim, J. Multi-omics integration in the age of million single-cell data. *Nat. Rev. Nephrol.* **17**, 710–724 (2021).
183. Erhard, F. et al. scSLAM-seq reveals core features of transcription dynamics in single cells. *Nature* **571**, 419–423 (2019).
184. Hendriks, G. J. et al. NASC-seq monitors RNA synthesis in single cells. *Nat. Commun.* **10**, 3138 (2019).
185. Qiu, Q. et al. Massively parallel and time-resolved RNA sequencing in single cells with scNT-seq. *Nat. Methods* **17**, 991–1001 (2020).
186. Chen, W. et al. Live-seq enables temporal transcriptomic recording of single cells. *Nature* **608**, 733–740 (2022).
187. Moses, L. & Pachter, L. Museum of spatial transcriptomics. *Nat. Methods* **19**, 534–546 (2022).
188. Liu, B., Li, Y. & Zhang, L. Analysis and visualization of spatial transcriptomic data. *Front. Genet.* https://doi.org/10.3389/fgene.2021.785290 (2022).
189. Xia, C., Fan, J., Emanuel, G., Hao, J. & Zhuang, X. Spatial transcriptome profiling by MERFISH reveals subcellular RNA compartmentalization and cell cycle-dependent gene expression. *Proc. Natl Acad. Sci. USA* **116**, 19490–19499 (2019).
190. Eng, C. L. et al. Transcriptome-scale super-resolved imaging in tissues by RNA seqFISH. *Nature* **568**, 235–239 (2019).
191. Vickovic, S. et al. High-definition spatial transcriptomics for in situ tissue profiling. *Nat. Methods* **16**, 987–990 (2019).
192. Chen, A. et al. Spatiotemporal transcriptomic atlas of mouse organogenesis using DNA nanoball-patterned arrays. *Cell* **185**, 1777–1792.e21 (2022).
193. Atta, L. & Fan, J. Computational challenges and opportunities in spatially resolved transcriptomic data analysis. *Nat. Commun.* **12**, 5283 (2021).
194. Stoeckius, M. et al. Simultaneous epitope and transcriptome measurement in single cells. *Nat. Methods* **14**, 865–868 (2017).
195. Peterson, V. M. et al. Multiplexed quantification of proteins and transcripts in single cells. *Nat. Biotechnol.* **35**, 936–939 (2017).
196. Katzenelenbogen, Y. et al. Coupled scRNA-Seq and intracellular protein activity reveal an immunosuppressive role of TREM2 in cancer. *Cell* **182**, 872–885.e19 (2020).
197. Liu, Y. et al. High-spatial-resolution multi-omics sequencing via deterministic barcoding in tissue. *Cell* **183**, 1665–1681.e18 (2020).
198. Ma, A., McDermaid, A., Xu, J., Chang, Y. & Ma, Q. Integrative methods and practical challenges for single-cell multi-omics. *Trends Biotechnol.* **38**, 1007–1022 (2020).
199. Ginhoux, F., Yalin, A., Dutertre, C. A. & Amit, I. Single-cell immunology: past, present, and future. *Immunity* **55**, 393–404 (2022).
200. Gault, J. et al. Combining native and 'omics' mass spectrometry to identify endogenous ligands bound to membrane proteins. *Nat. Methods* **17**, 505–508 (2020).
201. Specht, H. et al. Single-cell proteomic and transcriptomic analysis of macrophage heterogeneity using SCoPE2. *Genome Biol.* **22**, 50 (2021).
202. Touat-Hamici, Z. et al. Role of lipid phosphate phosphatase 3 in human aortic endothelial cell function. *Cardiovasc. Res.* **112**, 702–713 (2016).
203. Luo, X., Chen, X., Wang, L., Yang, B. & Cai, S. Metformin adjunct with antineoplastic agents for the treatment of lung cancer: a meta-analysis of randomized controlled trials and observational cohort studies. *Front. Pharmacol.* https://doi.org/10.3389/fphar.2021.639016 (2021).
204. Su, C. et al. Single-cell RNA sequencing in multiple pathologic types of renal cell carcinoma revealed novel potential tumor-specific markers. *Front. Oncol.* **11**, 719564 (2021).
205. Lex, A., Gehlenborg, N., Strobelt, H., Vuillemot, R. & Pfister, H. UpSet: visualization of intersecting sets. *IEEE Trans. Vis. Comput. Graph.* **20**, 1983–1992 (2014).
206. Swanson, E. et al. Simultaneous trimodal single-cell measurement of transcripts, epitopes, and chromatin accessibility using TEA-seq. *eLife* **10**, e63632 (2021).
207. Datlinger, P. et al. Pooled CRISPR screening with single-cell transcriptome readout. *Nat. Methods* **14**, 297–301 (2017).
208. Chen, G., Ning, B. & Shi, T. Single-cell RNA-seq technologies and related computational data analysis. *Front. Genet.* https://doi.org/10.3389/fgene.2019.00317 (2019).
209. Haque, A., Engel, J., Teichmann, S. A. & Lönnberg, T. A practical guide to single-cell RNA-sequencing for biomedical research and clinical applications. *Genome Med.* **9**, 75 (2017).
210. Hwang, B., Lee, J. H. & Bang, D. Single-cell RNA sequencing technologies and bioinformatics pipelines. *Exp. Mol. Med.* **50**, 1–14 (2018).
211. Mereu, E. et al. Benchmarking single-cell RNA-sequencing protocols for cell atlas projects. *Nat. Biotechnol.* **38**, 747–755 (2020).
212. Luecken, M. D. et al. Benchmarking atlas-level data integration in single-cell genomics. *Nat. Methods* **19**, 41–50 (2022).
213. Jin, S. et al. Inference and analysis of cell–cell communication using CellChat. *Nat. Commun.* **12**, 1088 (2021).
214. Efremova, M., Vento-Tormo, M., Teichmann, S. A. & Vento-Tormo, R. CellPhoneDB: inferring cell–cell communication from combined expression of multi-subunit ligand–receptor complexes. *Nat. Protoc.* **15**, 1484–1506 (2020).
215. De Palma, M., Biziato, D. & Petrova, T. V. Microenvironmental regulation of tumour angiogenesis. *Nat. Rev. Cancer* **17**, 457–474 (2017).
216. Ubezio, B. et al. Synchronization of endothelial Dll4-Notch dynamics switch blood vessels from branching to expansion. *eLife* **5**, e12167 (2016).
217. Cao, J. et al. The single-cell transcriptional landscape of mammalian organogenesis. *Nature* **566**, 496–502 (2019).
218. Lin, C. & Bar-Joseph, Z. Continuous-state HMMs for modeling time-series single-cell RNA-Seq data. *Bioinformatics* **35**, 4707–4715 (2019).
219. Aibar, S. et al. SCENIC: single-cell regulatory network inference and clustering. *Nat. Methods* **14**, 1083–1086 (2017).
220. Trimm, E. & Red-Horse, K. Vascular endothelial cell development and diversity. *Nat. Rev. Cardiol.* https://doi.org/10.1038/s41569-022-00770-1 (2022).
221. Vanlandewijck, M. & Betsholtz, C. in *Lymphangiogenesis: Methods and Protocols* (eds Guillermo, O. & Mark, L. K.) 309–324 (Springer, 2018).
222. Sauler, M. et al. Characterization of the COPD alveolar niche using single-cell RNA sequencing. *Nat. Commun.* **13**, 494 (2022).
223. Vila Ellis, L. et al. Epithelial Vegfa specifies a distinct endothelial population in the mouse lung. *Dev. Cell* **52**, 617–630.e6 (2020).
224. Gillich, A. et al. Capillary cell-type specialization in the alveolus. *Nature* **586**, 785–789 (2020).
225. Vidal, R. et al. Transcriptional heterogeneity of fibroblasts is a hallmark of the aging heart. *JCI Insight* https://doi.org/10.1172/jci.insight.131092 (2019).
226. Davies, P., Jones, M., Liu, J. & Hebenstreit, D. Anti-bias training for (sc)RNA-seq: experimental and computational approaches to improve precision. *Brief. Bioinform.* https://doi.org/10.1093/bib/bbab148 (2021).
227. Kim, J. K., Kolodziejczyk, A. A., Ilicic, T., Teichmann, S. A. & Marioni, J. C. Characterizing noise structure in single-cell RNA-seq distinguishes genuine from technical stochastic allelic expression. *Nat. Commun.* **6**, 8687 (2015).
228. Kharchenko, P. V., Silberstein, L. & Scadden, D. T. Bayesian approach to single-cell differential expression analysis. *Nat. Methods* **11**, 740–742 (2014).
229. Tran, H. T. N. et al. A benchmark of batch-effect correction methods for single-cell RNA sequencing data. *Genome Biol.* **21**, 12 (2020).
230. Kiselev, V. Y., Andrews, T. S. & Hemberg, M. Challenges in unsupervised clustering of single-cell RNA-seq data. *Nat. Rev. Genet.* **20**, 273–282 (2019).
231. Zhang, M. et al. Single-cell transcriptomic architecture and intercellular crosstalk of human intrahepatic cholangiocarcinoma. *J. Hepatol.* **73**, 1118–1130 (2020).
232. Maynard, A. et al. Therapy-induced evolution of human lung cancer revealed by single-cell RNA sequencing. *Cell* **182**, 1232–1251.e22 (2020).
233. Ilicic, T. et al. Classification of low quality cells from single-cell RNA-seq data. *Genome Biol.* **17**, 29 (2016).

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Author contributions

Q.Z., L.F., M.M., H.A., A.S.N. and F.Y.A. researched data for the article. Q.Z., L.F., M.M., H.A., A.S.N., F.Y.A., H.A.S. and P.C. contributed substantially to discussion of the content and wrote the article. All authors reviewed and/or edited the manuscript before submission.

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The authors declare no competing interests.

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