Trends in Endocrinology & Metabolism

CellPress
REVIEWS

Review

Metabolic Signatures of Distinct Endothelial Phenotypes

Sébastien J. Dumas,[1,2] Melissa García-Caballero,[1,2] and Peter Carmeliet[1,2,*,@]

Angiogenesis is crucial for the development of the blood vasculature during embryogenesis, but also contributes to cancer and other diseases. While therapeutic targeting of endothelial cells (ECs) through growth factor inhibition is limited by insufficient efficacy and resistance, a new paradigm for modulating angiogenesis by targeting EC metabolism has emerged. Findings from the past decade highlight how ECs adapt their metabolism to proliferate or migrate during vessel sprouting, or to maintain the vascular barrier and protect themselves against oxidative stress in the high-oxygen environment they are exposed to in healthy conditions. We overview key endothelial metabolic pathways underlying the different EC phenotypes, as well as potential opportunities for targeting EC metabolism in therapeutic settings.

Highlights

We postulated in 2009 that the formation of new blood vessels by ECs requires adaptations of EC metabolic pathways, a concept that now has been experimentally validated.

ECs exhibit remarkable plasticity both at the phenotypic and metabolic (transcriptome) levels.

Preclinical studies have revealed that targeting specific EC metabolic pathways can offer antiangiogenic benefits. The challenge will be to translate these findings to the clinic.

Introduction

Blood vessels are vital because they transport oxygen and nutrients for the development and maintenance of perivascular tissues [1]. They also remove metabolic waste, maintain fluid homeostasis (together with lymphatic vessels that drain extravasated fluid), and, when dysfunctional, can promote thrombosis and inflammation [1,2]. During development and later, for instance in adult malignancies, new blood vessels are formed via sprouting angiogenesis in response to proangiogenic stimuli. A current model of vessel sprouting postulates that a leading **tip cell** (see Glossary) migrates into avascular regions, and proliferating **stalk cells** elongate the new sprout [1,3]. When two nearby newly formed sprouts meet each other, they fuse, after which ECs differentiate into **quiescent phalanx cells** [1,3]. ECs exhibit a phenotypic plasticity, and tip, stalk, and phalanx cells do not have predefined fixed states, and instead represent dynamic interchangeable phenotypes [1,4]. An advantage of tip/stalk cell competition, where nonfit tip cells are overtaken by fitter stalk cells to become the new tip cell, is that the EC with the greatest tip cell competitivity always leads the sprout [1,4]. In addition, recent findings from the analysis of ECs at the single-cell level during angiogenesis *in vivo* demonstrated an unexpectedly high degree of phenotypic complexity [5,6]. These studies revealed additional activated postcapillary venule, immature, transitioning, **breach cell**, and neo-phalanx EC phenotypes that are associated with vessel formation in pathological situations [5,6]. However, how these different phenotypes contribute to sprouting angiogenesis needs to be further elucidated [5,6].

Growth factors, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), their cognate receptors, as well as many other signals, drive the angiogenic process [7]. Although targeting these factors has shown antiangiogenic effects in cancer and ocular diseases, such strategies often showed limited efficacy and resistance, partly because of compensatory takeover by other angiogenic signals [8]. In 2009, we postulated that, for angiogenic molecules to signal vessel formation, ECs must adapt their **metabolism** [9]. Recent advances have indeed uncovered that EC metabolism is a key determinant of the differentiation and functioning of tip, stalk, and phalanx EC phenotypes [10–22]. Cell metabolism is an evolutionarily conserved process that mediates the conversion (catabolism) of nutrients into energy and small metabolites,

[1]Laboratory of Angiogenesis and Vascular Metabolism, Department of Oncology, Katholieke Universiteit (KU) Leuven, Leuven, B 3000, Belgium
[2]Laboratory of Angiogenesis and Vascular Metabolism, Vesalius Research Center, Vlaams Instituut voor Biotechnologie (VIB), Leuven, B 3000, Belgium

*Correspondence: peter.carmeliet@kuleuven.vib.be (P. Carmeliet).
@Twitter: @CarmelietLab

which are used as building blocks to generate **biomass** (proteins, lipids, nucleotides) via anabolism. Quiescent ECs (QECs) or angiogenic ECs (tip and stalk cells) reprogram their metabolism to optimally meet their specific physiological needs. This metabolic heterogeneity represents a formidable resource of druggable metabolic targets (>4000 metabolic reactions), and offers unprecedented opportunities for developing novel anti- and proangiogenic treatments. Although we describe here the metabolic adaptations of quiescent, tip, and stalk ECs (Table 1, Key Table), there is evidence that other metabolic pathways are activated in both, tip and stalk cells, and it remains to be determined whether tip and/or stalk cells rely on other metabolic pathways.

Maintenance and Differentiation of Quiescent 'Phalanx' ECs: The Unexpected Role of Fatty Acid Metabolism

ECs remain quiescent for years in healthy adults [1]. They maintain tissue perfusion, counteract thrombosis and vascular inflammation, and modulate vascular tone and barrier function [22]. Although dysfunctional QECs contribute to several diseases affecting millions of people (including atherothrombosis, stroke, myocardial infarction, diabetes, and others), little is known about their metabolism. Nonetheless, by contrast to what one would perhaps expect, they are not hypometabolic and instead sustain basal levels of **glycolysis** and upregulate **fatty acid β-oxidation (FAO)** [22]. The main metabolic substrates used by QECs for biomass synthesis, energy production, and redox homeostasis are detailed below (Figure 1A and Table 1).

Glycolysis

Despite their direct exposure to high levels of oxygen in the blood that would allow them to metabolize glucose via the oxidative pathway, QECs mainly rely on anaerobic glycolytic metabolism for energy production, a metabolic pathway that is known to yield lower ATP production per mole of glucose than **oxidative phosphorylation (OXPHOS)** [21]. However, this might be a strategy to protect themselves against oxidative stress arising from oxidative metabolism, and also to transfer the maximal amounts of oxygen to perivascular cells. Although QECs display a high glycolytic rate, it is lower than in proliferating endothelial cells (PECs) [22].

Fatty Acid Oxidation

Intriguingly, compared with PECs, which utilize FAO for nucleotide synthesis [18], QECs (that do not require substantial nucleotide synthesis) upregulate FAO more than threefold, secondary to Notch-dependent upregulation of carnitine palmitoyltransferase 1a (CPT1a) expression (Figure 1B) [22]. Also, the pro-quiescence signal Notch promotes the hydrolysis of triglycerides as well as the uptake and transcellular transport of fatty acids to the parenchymal tissue by QECs, highlighting Notch as a key modulator of fatty acid metabolism in QECs [23]. Quiescence-induced FAO upregulation was also reported in non-EC types such as adult stem cells and cancer cells [24,25]. In QECs, FAO does not support biomass or energy production but sustains the **tricarboxylic acid (TCA) cycle** for redox homeostasis through isocitrate dehydrogenase- and malic enzyme-dependent NADPH regeneration (Figure 1B), a mechanism that is also documented in cancer cells [22,26,27]. Reactive oxygen species (ROS)-scavenging systems, such as glutathione peroxidase, require reduced glutathione (GSH), which is generated by glutathione reductase in a NADPH-dependent reaction (Figure 1B) [28]. QECs also use other metabolic strategies to produce NADPH, by upregulating genes involved in redox homeostasis (i.e., NADP synthesis, nicotinamide nucleotide transhydrogenase, oxidative pentose phosphate pathway enzymes, etc.) (Figure 1B) [22]. Interestingly, QECs reprogram their metabolism to increase regeneration of NADPH, at the same time as upregulating the expression of vasculoprotective genes, such as prostaglandin G/H synthase 1 (*PTGS1*), eNOS (*NOS3*), and glutaredoxin (*GRX*), among others, which require NADPH as cofactor, thereby protecting themselves against the high oxygen and oxidative stress-prone environment [22]. In agreement

Glossary

Anaplerosis: replenishment of carbons into the TCA cycle for biomass synthesis.
Biomass: all cellular constituents including lipids, proteins, carbohydrates, and nucleic acids.
Breach cells: endothelial cells (ECs) that create a breach in the basal lamina; these are presumably involved in the initiation of vessel sprouting by tip cells.
Fatty acid β-oxidation (FAO): a series of metabolic reactions that break down the long carbon chains of fatty acids into two-carbon units (acetate), which, combined with coenzyme A, generate acetyl-CoA molecules that can enter the TCA cycle.
Glycocalyx: a layer of glycolipids and glycoproteins (including the polysaccharides heparan sulfate, chondroitin sulfate, and its principal component, hyaluronan) at the luminal side of ECs that confers most of the functional aspects of the endothelium.
Glycolysis: cytosolic biochemical reactions that break down glucose and release energy as ATP and pyruvate.
Metabolism: >4000 biochemical reactions that convert nutrients into biomass, energy, redox homeostasis, and others, that are necessary for the maintenance of cellular life.
Mitochondrial respiration/oxidative phosphorylation (OXPHOS): a set of metabolic reactions occurring in mitochondria that require oxygen to produce energy as ATP molecules.
6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3): the enzyme that metabolizes the glycolytic intermediate fructose-6-phosphate (F6P) into fructose-2,6-bisphosphate (F2,6BP), a positive allosteric modulator of the glycolytic enzyme phosphofructokinase 1 (PFK1).
Quiescent phalanx cells: quiescent ECs (QECs) that establish a barrier to ensure blood perfusion; their metabolism is mainly directed towards the maintenance of redox homeostasis.
Stalk cells: proliferative ECs that contribute to vessel sprout elongation; they are characterized by metabolism mainly directed towards the production of biomass and energy to support proliferation.
Tip cells: ECs that lead the sprout during sprouting angiogenesis; tip cells are characterized by metabolism mainly directed towards energy production to support migration.

with the fact that elevated ROS levels can disrupt the integrity of the vascular barrier by disrupting intercellular junctions [22,29], Cpt1a inhibition or loss of Cpt1a in ECs *in vivo* increases oxidative stress, barrier leakage, vascular inflammation, and leukocyte infiltration, signs of EC dysfunction, explaining why endothelial Cpt1a loss aggravates inflammatory bowel disease [22,30].

Of note, FAO is also necessary to maintain blood vascular EC differentiation. Hence, blunting FAO by deletion of endothelial CPT1a promotes embryonic endothelial-to-mesenchymal transition (EndoMT) [31]. During EndoMT, ECs differentiate to mesenchymal, stem cell-like, or fibroblast cells. Although EndoMT contributes to heart valve formation, it also contributes to cancer, tissue fibrosis, pulmonary arterial hypertension (PAH), and atherosclerosis [32,33]. In cancer, EndoMT is a source (up to 40%) of cancer-associated fibroblasts (CAFs) which promote tumor growth and

Tricarboxylic acid (TCA) cycle: a series of biochemical reactions in which oxidation of acetyl-CoA molecules results in the production of CO_2, GTP, and reducing agents.

Tumor vessel normalization: the reversal of immature, tortuous, and leaky tumoral vessels to more mature, stable, and functional blood vessels.

Key Table

Table 1. Key Metabolic Pathways and Metabolites That Maintain the Different Endothelial Cell Phenotypes

EC phenotype	Metabolic pathways	Key metabolites	Roles in maintaining the EC phenotype	Refs
Quiescent 'phalanx' ECs	Glycolysis	ATP	Basal energy level to sustain EC function	[10,22]
	Oxidative pentose phosphate pathway	NADPH	Redox homeostasis, barrier integrity	[22]
	FAO	NADPH	Redox homeostasis, barrier integrity	[22,28–31,33,38]
		Acetyl-CoA	Maintenance of the endothelial phenotype	
	Organ-specific metabolic pathways	Various	Depends on the function and metabolic demands of the tissues	[39–42]
Migratory 'tip' ECs	Glycolysis (filopodia/lamellipodia)	ATP	Actin remodeling for cell motility	[6,11,16,46,48,49,52,53]
	Glutamine breakdown	Various	Necessary for EC migration	[12]
	Proline biosynthesis	Proline	Collagen synthesis and modification	[5,6]
	Fatty acid biosynthesis	Malonyl-CoA	Membrane fluidity	[14]
	Cholesterol transport	Cholesterol	Lipid raft formation	[15]
Proliferative 'stalk' ECs	Glycolysis (perinuclear)	ATP	Energy for cell proliferation	[6,11,16,53]
		TCA cycle intermediates, dNTPs, rNTPs, amino acids	DNA replication, RNA and protein synthesis	
	Pentose phosphate pathway	dNTPs, rNTPs	DNA replication and RNA synthesis	[22,54]
	Glutamine breakdown	TCA cycle intermediates, dNTPs, rNTPs	DNA replication, RNA and protein synthesis	[12,13,65–67]
		Amino acids	Protein biosynthesis, ER stress inhibition	
	Serine biosynthesis	Serine, dNTPs, rNTPs	DNA replication and RNA synthesis	[6,68]
		Glycine, heme	Redox homeostasis, maintenance of mitochondrial function	
	FAO	TCA cycle maintenance (in conjunction with an anaplerotic substrate), dNTPs, rNTPs, amino acids	DNA replication, RNA and protein synthesis	[18,70]
	Fatty acid synthesis	FASN substrate (malonyl-CoA)	Prevention of malonyl-CoA accumulation and protein malonylation (FASN activity)	[19]
	Mitochondrial respiration	NAD^+, aspartate	Amino acid biosynthesis	[6,20]

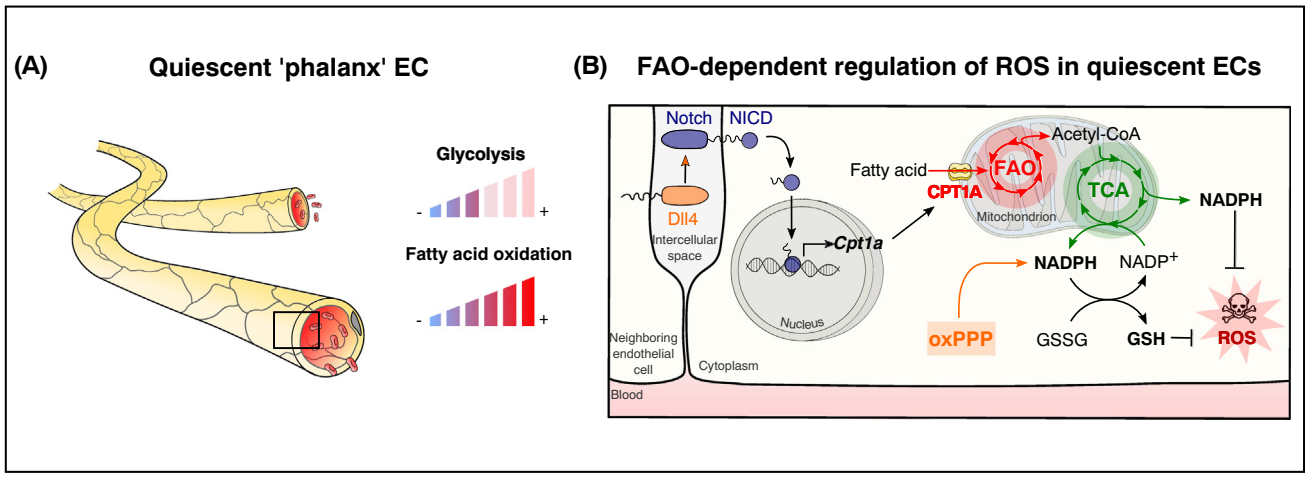


Figure 1. Metabolism of Quiescent 'Phalanx' Endothelial Cells (ECs). (A) Quiescent ECs (QECs) display a lower rate of glycolysis but increased fatty acid β-oxidation (FAO) relative to proliferating ECs (PECs). (B) QECs are characterized by a high rate of FAO, secondary to Notch activation by Dll4 and NICD-dependent *CPT1A* overexpression. Acetyl-CoA produced by FAO enters the tricarboxylic acid (TCA) cycle, resulting in NADPH production that exerts antioxidative effects for the maintenance of barrier integrity. The oxidative pentose phosphate pathway (oxPPP) generates more NADPH that can also be used to regenerate antioxidant reduced glutathione (GSH) from its oxidized form (GSSG). Abbreviations: NICD, Notch intracellular domain; ROS, reactive oxygen species.

spread [34]. Moreover, as a source of myofibroblasts, EndoMT promotes tissue fibrosis by producing extracellular matrix (ECM) molecules and by increasing tissue stiffness [35]. In PAH, EndoMT is partly responsible for the accumulation of neointimal smooth muscle-like cells, which occludes pulmonary arteries, and inhibiting EndoMT improves PAH [36,37]. EndoMT is also common in atherosclerosis where it is associated with neointima formation and plaque instability [37]. Transforming growth factor (TGF)-β is a key regulator of EndoMT by lowering FAO and acetyl-CoA levels, thereby impairing SMAD7 acetylation and signaling [31,33]. Furthermore, during the developmental transdifferentiation of venous ECs (VECs) into lymphatic ECs (LECs), the master transcription factor PROX1 upregulates CPT1a expression and hence FAO levels, which results in elevated acetyl-CoA levels [38]. Acetyl-CoA is then used as a substrate by histone acetyltransferase p300, which forms a complex with PROX1 and acetylates histones at lymphangiogenic genes (i.e., *VEGFR3, PROX1*), thereby favoring chromatin decondensation and promoting lymphatic gene expression [38].

Interestingly, recent studies on the (metabolic) transcriptome of QECs at single-cell resolution unraveled the tissue-specific profile of ECs [39] (Figure 2A). For instance, quiescent brain ECs upregulate glucose, amino acid, and fatty acid transporters [39] (Figure 2B). Genes involved in cholesterol metabolism are particularly enriched in splenic ECs, and lung ECs show upregulated expression of genes implicated in cAMP levels [39] (Figure 2B). Furthermore, cardiac and muscle ECs display an enriched gene signature related to lipid uptake and metabolism [39] (Figure 2B). In line with these findings, bulk RNA sequencing of cardiac ECs revealed TCF2- and MEOX2-dependent enriched expression of the *CD36* and *FABP4* genes that encode fatty acid transporters necessary for fatty acid uptake [40]. Metabolic heterogeneity of ECs isolated from heart, lung, liver, and kidney was further interrogated *in vitro*, revealing higher glycolysis and OXPHOS in cardiac ECs [41]. In addition to the observed inter-tissue EC metabolic heterogeneity, arterial, capillary, and venous ECs, as well as other EC phenotypes within a single tissue also demonstrate different expression levels of metabolic genes [39] (Figure 2B). For instance, ECs from the choroid plexus in the brain display lower expression of transporters relative to other

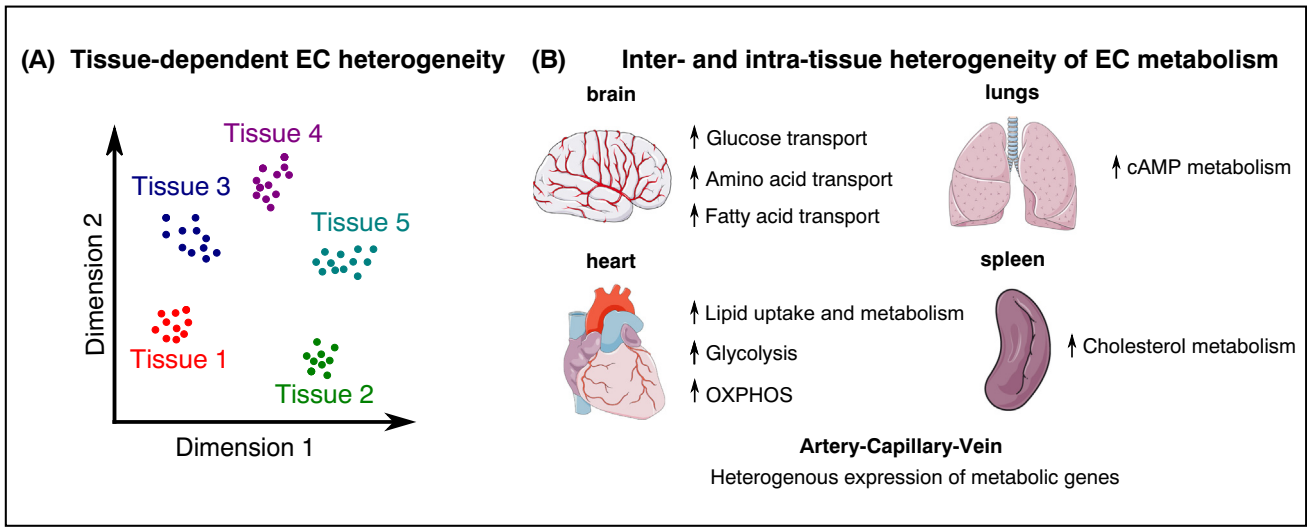


Figure 2. Heterogeneity of Quiescent 'Phalanx' Endothelial Cells (ECs). (A) Quiescent ECs (QECs) from different tissues show distinct transcriptome profiles. The plot shows the dimensionality reduction of the transcriptome profiles of QECs isolated from five hypothetical tissues/organs. Dots represent either pools of QECs or single ECs, according to the transcriptomic method used (for bulk or single-cell RNA-seq respectively). Dimensions 1 and 2 contain the most differentially expressed genes between the different samples/cells. They vary according to the dimensionality reduction method used. (B) Metabolic heterogeneity of QECs between and within tissues. Quiescent brain ECs upregulate glucose, amino acid, and fatty acid transporters; lung ECs upregulate genes involved in cAMP levels; splenic ECs increase the expression of cholesterol metabolism genes; cardiac and muscle ECs show an enriched gene expression signature for lipid uptake and metabolism. ECs from arteries, capillaries, and veins within the same organ also show differential metabolic gene expression. Abbreviation: OXPHOS, oxidative phosphorylation.

brain EC phenotypes [39]. In another example, ECs from the renal medulla upregulate OXPHOS to survive to the rise in extracellular osmolarity induced by water deprivation [42]. These results suggest that QEC metabolism is likely driven by the function and metabolic demands of each tissue. Moreover, the metabolic plasticity of QECs is crucial as an adaptive response to changes in the microenvironment.

Migratory Endothelial 'Tip' Cells: The Glucose-Powered Locomotive

In sprouting angiogenesis, tip ECs guide the emerging vessel sprout to vascularize oxygen-deprived tissue regions. They project filopodia at their front to sense proangiogenic signals such as VEGF in the microenvironment, and lamellipodia that allow their migration up the growth factor gradient [1]. Cell motility is a highly energy-demanding process, but tip ECs have limited access to nutrients and oxygen in avascular regions. To move, tip ECs must adapt their metabolism (Figure 3). Recent single-cell studies revealed that tip ECs can be resolved into migratory (former 'tip' ECs) and basement-membrane remodeling ('breach' ECs) phenotypes [5,6]. By expressing genes involved in podosome rosette formation, breach cells may pave the way for the tip cell to migrate [43]. Interestingly, tip cells constituted the only conserved EC phenotype that was congruent across diseases (tumor, choroid neovascularization), species (human, mouse), tissues (lung, eye), and models (freshly isolated vs cultured ECs) [5,6]. Further studies will be necessary to determine the metabolic specificities of each of these phenotypes. In the following we focus on the metabolic pathways that are relatively well characterized in tip ECs (Figure 3A and Table 1).

Glycolysis

Tip ECs exploit glycolysis to rapidly produce ATP without oxygen consumption. This is instrumental because oxygen levels drop faster than glucose levels over a similar distance from perfused blood vessels [44]. Because glycolysis generates ATP faster than OXPHOS, ECs can rapidly

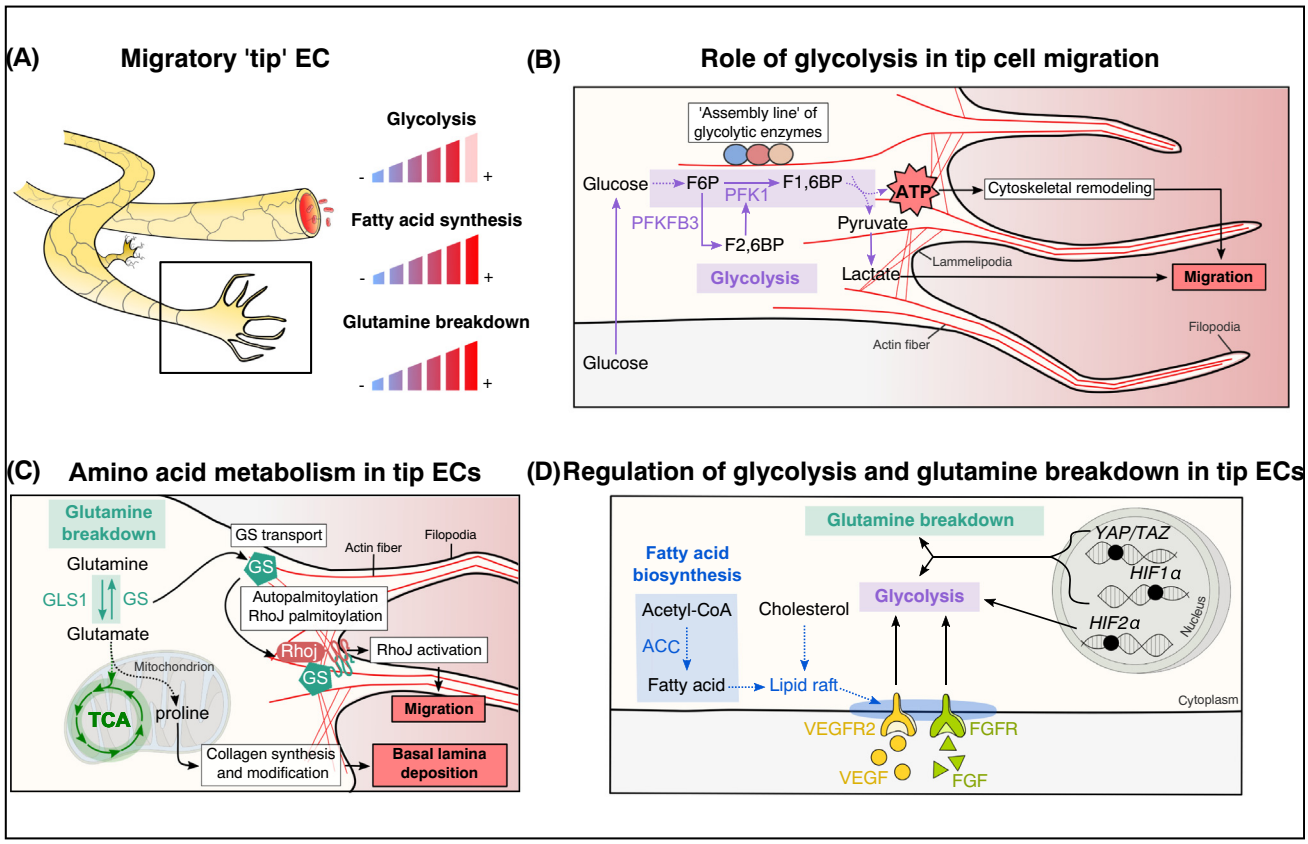


Figure 3. Metabolism of Migratory 'Tip' Endothelial Cells (ECs). (A) Migratory tip ECs exhibit a high rate of glycolysis, elevated fatty acid synthesis metabolism, and increased glutamine breakdown. (B) In tip cells, upregulation of 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) and phosphofructokinase 1 (PFK1) regulates the generation of ATP by glycolysis. The pool of ATP resulting from glycolysis is specifically present in regions of active cytoskeletal remodeling (filopodia and lamellipodia formation sites) during migration. The formation of a functional 'assembly line' of glycolytic enzymes along F-actin fibers allows efficient ATP production in these locations. Moreover, lactate, the end-product of anaerobic glycolysis, contributes to tip cell migration. (C) Glutamine metabolism, through glutaminase 1 (GLS1) and glutamine synthetase (GS), is also required for migration of tip ECs. The effect of GS is mediated independently of glutamine production: a pool of GS is transported to sites of active cytoskeletal remodeling, where it becomes autopalmitoylated and subsequently induces RhoJ palmitoylation and activation, ultimately promoting cell migration. Glutamate resulting from GLS1 activity, can enter the tricarboxylic acid (TCA) cycle or contribute to the biosynthesis of proline – an amino acid required for the production/modification of collagen that is involved in endothelial basal lamina deposition and mechanotransductive signaling during cell migration. (D) Acetyl-CoA carboxylase (ACC)-dependent fatty acid biosynthesis, as well as cholesterol, regulate membrane fluidity for filopodia formation as well as the formation of lipid rafts where vascular endothelial growth factor receptor 2 (VEGFR2) and fibroblast growth factor receptor (FGFR) are anchored. Other important players that stimulate glycolysis in migratory tip cells are transcription factors including hypoxia-inducible factor (HIF)1α, HIF2α, and Yes-associated protein (YAP)/TAZ coactivators. In addition, YAP/TAZ can promote glutaminase 1 (GLS1) expression and consequently glutamine breakdown. Abbreviations: F1,6BP, fructose-1,6-bisphosphate; F2,6BP, fructose-2,6-bisphosphate; F6P, fructose-6-phosphate.

vascularize ischemic tissues, thereby avoiding tissue necrosis [45]. The migratory process consumes a large amount of ATP for active remodeling of the cellular cytoskeleton. While QECs and PECs mainly concentrate ATP production in the perinuclear cytosol, tip ECs must generate high amounts of ATP locally at filopodial and lamellipodial protrusions to facilitate cell movement (Figure 3B) [46]. However, the small size of these structures (a diameter of 0.1–0.3 μm for filopodia) limits the access of bulky mitochondria, and thus precludes *in situ* ATP generation through OXPHOS [47]. Local glycolysis-derived ATP production in filopodia or lamellipodia regulates migratory speed and directionality (Figure 3B) [48]. It requires translocation of the glycolytic machinery from a perinuclear location to the actin filaments in the vicinity of the cell membrane

protrusions. Importantly, binding of glycolytic enzymes to actin stabilizes tetramerization, and this increases their enzymatic activity [49]. Moreover, construction of an 'assembly line' of glycolytic enzymes optimizes the local generation of large amounts of ATP (Figure 3B) [49]. This 'assembly line', whereby the product of one glycolytic enzyme becomes the substrate for the next glycolytic enzyme (i.e., metabolic channeling of glycolytic intermediates), includes phosphofructokinase (PFK), fructose 1,6-bisphosphate aldolase (aldolase), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) that are directly bound to F-actin, and other glycolytic enzymes such as the key glycolysis-stimulating enzyme, **6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3)**, may possibly associate with these glycolytic complexes [16,50]. Other glycolytic enzymes piggyback on these actin-bound glycolytic enzymes to further increase the assembly line [51]. Upon exposure to growth factors (VEGF, FGF) and/or hypoxia, PFKFB3 is upregulated and promotes EC differentiation into the tip cell phenotype [11,46,52]. Reduced VEGFR signaling together with increased Notch signaling results in reduced expression of PFKFB3 and downregulation of glycolysis [16]. Importantly, silencing of PFKFB3 expression in ECs alone sufficed to impair tip cell competitivity in mosaic EC spheroids *in vitro* and in zebrafish *in vivo* [53], whereas PFKFB3 overexpression overrules the pro-stalk activity of Notch [16], highlighting the primary role of EC metabolism in vessel sprouting.

Other glycolytic enzymes are also necessary for tip cell phenotype acquisition and function, for example, hexokinase 2 (HK2). Endothelial *Hk2* deletion in embryos and neonatal mice triggers defects in angiogenesis and impairs the development of the retinal vasculature, decreasing the number of both tip cells and branch points [11]. In addition to external glucose, ECs can also use internal glucose, stored as glycogen, that is synthesized from the glycolytic intermediate glucose-6-phosphate (G6P) and degraded by glycogen phosphorylase, when they are exposed to a microenvironment completely deprived of glucose and oxygen [54].

Amino Acid Metabolism

Sprouting angiogenesis is not only limited by glucose deprivation but also by glutamine removal from the extracellular milieu [12]. Inhibition of glutaminase 1 (GLS1), a crucial enzyme for glutamine catabolism, as well as glutamine starvation, both impair EC migration (Figure 3C) [12]. Conditional EC *Gls1* knockout in mouse pups leads to the formation of fewer distal sprouts with filopodia [12]. Furthermore, GLS1 promotes tip cell competitivity *in vitro* in a chimeric EC spheroid assay [12]. Glutamine is used as a precursor for the production of other nonessential amino acids, such as glutamate, asparagine, and aspartate, provides substrates for **anaplerosis**, and participates in redox homeostasis (through glutathione synthesis). Glutamine-derived asparagine is required for vessel sprouting, and a role for asparagine synthase (ASNS) has been highlighted. When ASNS is inhibited in ECs, fewer distal sprouts with filopodia are observed and vessel formation is impaired. Conversely, ECs may use asparagine when ASNS expression levels are augmented; although an effect of ASNS silencing on EC migration has not yet been reported, ASNS silencing impairs vessel sprouting *in vitro* [12] (see below for more information about the role of ASNS in angiogenic ECs). Although glutamine catabolism is also necessary for the migration of non-EC types [55–58], the precise mechanism for how glutamine may regulate EC motility independently of ATP production remains unknown [12,57–59], and the involvement of glutamine catabolism in EC migration requires further clarification [13].

Even though they are continuously exposed to abundant blood levels of glutamine, ECs express glutamine synthetase (GS), the enzyme that produces glutamine from glutamate and ammonia [60]. Nonetheless, in the absence of endothelial GS, vessel growth is defective because EC migration is impaired. Although ECs upregulate GS when the extracellular level of glutamine is decreased, this results in only modest glutamine production, suggesting an additional role for

GS that may be related to its newly discovered palmitoyl-acyltransferase activity (Figure 3C) [60]. During EC migration, submembrane GS interacts with RhoJ, an endothelial member of the Cdc42 small GTPase family, resulting in its palmitoylation and promoting its membrane localization and hence activation (Figure 3C). Activated RhoJ controls, among others, the activity of the RHOA–ROCK–MLC signaling pathway that is known to regulate EC motility by affecting stress-fiber formation (Figure 3C) [60–62]. Overall, GS controls EC migration and hence tip cell motility (Figure 3C).

Single-cell profiling studies have revealed that endothelial tip cells upregulate genes involved in collagen biosynthesis as well as collagen-modifying enzymes (Figure 3C) [5]. Proline biosynthesis is necessary for collagen biosynthesis and modification, which is involved in endothelial basal lamina deposition and mechanotransductive signaling during cell migration (Figure 3C) [5]. Knockdown of *ALDH18A1*, encoding Δ1-pyrroline-5-carboxylate synthetase (P5CS), a key enzyme for proline and collagen biosynthesis, inhibits tip cell phenotype acquisition and EC migration and proliferation [6]. Mechanistically, *ALDH18A1* knockdown lowers proline and hydroxyproline levels, the two major components of collagens, thus highlighting the role of proline biosynthesis in sprouting angiogenesis (Figure 3C) [6].

Fatty Acid Metabolism and Cholesterol Transport

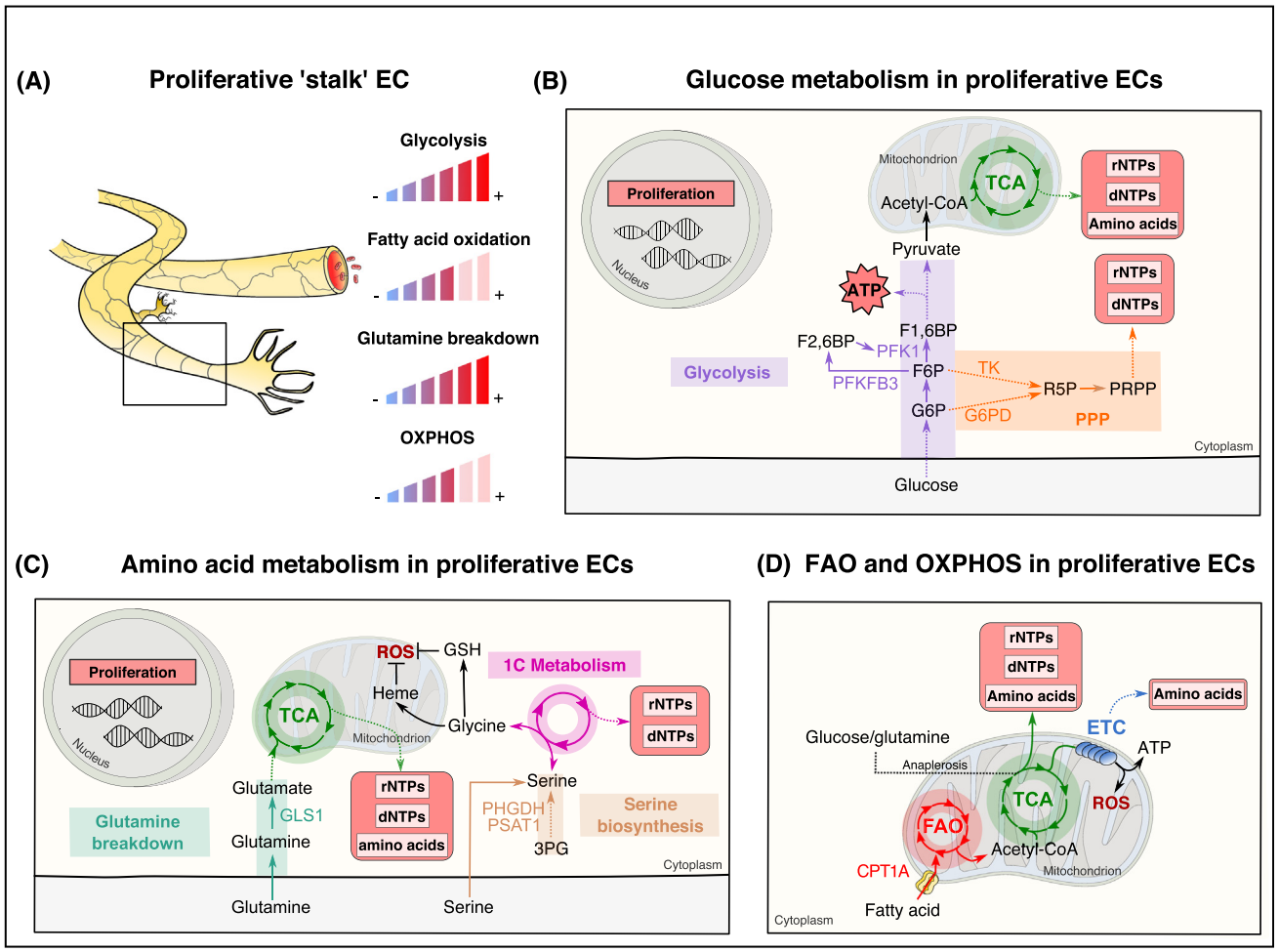
Lipids are the main components of cell membranes, and regulate their fluidity as well as the formation and maintenance of lipid rafts, signaling platforms for membrane receptors. ECs can produce and store fatty acids in triglyceride-rich lipid droplets, as well as catabolize these lipids into acetyl-CoA through FAO [63]. Inhibition of the acetyl-CoA carboxylase (ACC), the first and rate-limiting step in the fatty acid biosynthesis pathway, leads to alterations of the EC membrane composition (imbalance between polyunsaturated fatty acids and phosphatidylglycerol), resulting in decreased membrane fluidity, filopodia formation, and overall impaired EC migration [14]. Thus, lipid synthesis is essential for adequate tip cell motility (Figure 3D). In zebrafish embryos, tip cells exhibit a higher content of lipid rafts than stalk cells [15]. Cholesterol, a major constituent of lipid rafts, is exported from ECs through ATP-binding cassette (ABC) transporters to apolipoprotein A-I (ApoA-I) and ApoA-I-containing high-density lipoprotein (HDL), to maintain cholesterol homeostasis and EC function (Figure 3D) [15]. Cholesterol depletion by increasing its efflux induces lipid raft reduction, likely interfering with VEGFR2 membrane localization, dimerization, and signaling, and thereby impairing VEGFR2-induced angiogenesis *in vitro* and *in vivo* (Figure 3D) [15].

Proliferating 'Stalk' Endothelial Cells: Biomass Generators for Vessel Building

PECs in the stalk EC population are crucial for elongating the newly formed sprouts. This requires the production of high amounts of biomass such as nucleotides and amino acids, as well as ATP, that are necessary for cell division (Figure 4A and Table 1).

Glycolysis

Proliferative stalk ECs rely primarily on glycolysis for ATP production, more than many other cell types and, surprisingly, even more than some cancer cell types (Figure 4B) [16]. Notably, in situations of biomass and energy demand, ECs can raise their glycolytic flux by increasing PFKFB3 expression [16]. Pharmacological inhibition or genetic silencing of *PFKFB3* in ECs impairs angiogenesis [16,53]. Accordingly, freshly isolated mouse angiogenic ECs show upregulation of glycolytic genes in proliferating ECs [6]. Pyruvate kinase (PK) is a rate-limiting enzyme for glycolysis and catalyzes the conversion of phosphoenolpyruvate (PEP) to pyruvate. Although the M2 isoform (PKM2) is dispensable for energy homeostasis in ECs, it is involved in the maintenance of EC growth (via NF-kB/p53 suppression) and vascular barrier stability, independently of its enzymatic function [17,64].



Trends in Endocrinology & Metabolism

Figure 4. Metabolism of Proliferative 'Stalk' Endothelial Cells (ECs). (A) Proliferative 'stalk' ECs display high glycolysis, fatty acid oxidation, and glutamine breakdown, and moderate oxidative phosphorylation (OXPHOS). (B) Proliferative ECs show high glycolytic activity. A side-pathway of glycolysis, the pentose phosphate pathway (PPP), allows the production of ribose-5-phosphate (R5P) via both its oxidative (glucose-6-phosphate dehydrogenase-dependent, G6PD) and nonoxidative branches (transketolase-dependent, TK). R5P and 5-phospho-D-ribose α-1-pyrophosphate (PRPP) are precursors of ribonucleoside triphosphate (rNTP) and deoxynucleotide triphosphate (dNTP), the building blocks for RNA and DNA synthesis. Acetyl-CoA produced from the conversion of pyruvate generated by glycolysis enters the TCA cycle, ultimately generating rNTPs, dNTPs, and amino acids to sustain EC proliferation. (C) Proliferative ECs show high rates of glutamine breakdown. Synthesis of α-ketoglutarate from glutamate and glutamine via the glutaminolysis pathway, also sustains TCA cycle activity. In addition, proliferative ECs metabolize serine that can be either taken up from the extracellular milieu or produced from the glycolytic intermediate 3-phosphoglyceric acid (3PG) by phosphoglycerate dehydrogenase (PHGDH) and phosphoserine aminotransferase 1 (PSAT1). Serine is metabolized in the 1C metabolic pathway to generate rNTP and dNTP precursors. Glycine generated by the 1C metabolic pathway is further used as a precursor for heme and glutathione (GSH) synthesis, both serving to neutralize mitochondrial reactive oxygen species (ROS) that possibly leak from the electron transport chain (ETC). (D) In proliferative ECs, activation of fatty acid β-oxidation (FAO) provides acetyl-CoA to sustain the TCA cycle in conjunction with anaplerotic substrates, ultimately producing rNTPs, dNTPs, and amino acids, the key building blocks for DNA, proteins, and membrane lipids that are necessary for efficient EC proliferation and sprout elongation. Stalk cells produce extremely high amounts of biomass and ATP to sustain the proliferative process. This ATP is mainly produced by glycolysis and only minimally through the OXPHOS pathway, which plays another role in regenerating the pool of NAD^+ for the synthesis of amino acids. Abbreviations: 1C: one-carbon; F1,6BP, fructose-1,6-bisphosphate; F2,6BP, fructose-2,6-bisphosphate; F6P, fructose-6-phosphate; G6P, glucose-6-phosphate.

Amino Acid Metabolism

Amino acids are gaining increasing interest as a fuel in ECs. In this respect, glutamine is the amino acid that ECs metabolize the most [12]. The first reaction of glutamine catabolism, catalyzed by GLS1, not only contributes to anaplerotic replenishment of carbons into the TCA cycle but also

provides nitrogen for the biosynthesis of proteins and nucleotides (Figure 4C). An absence of glutamine induces an important loss of TCA intermediates that cannot be compensated by glucose-derived anaplerosis in ECs [12,13]. Therefore, it is not surprising that GLS1 inhibition or depletion of glutamine not only impairs EC migration, biomass synthesis, and EC proliferation, but also promotes EC senescence [65], reduces mTOR activity, and increases the expression of genes related to endoplasmic reticulum (ER) stress [12,13,66,67]. Furthermore, glutamine contributes to asparagine synthesis by providing nitrogen. Notably, asparagine was the only amino acid that could rescue the phenotype induced by glutamine deprivation, when used in combination with an anaplerotic substrate. Asparagine utilization was initially confined to protein synthesis, but cellular levels of asparagine are among the lowest of all nonessential amino acids in proliferating cells, and asparagine amination exclusively relies on glutamine, suggesting that asparagine synthesis plays a role as a rheostat in sensing the availability of TCA cycle intermediates and the supply of reduced nitrogen [12,67]. Asparagine can be taken up by ECs from the extracellular milieu, produced *de novo* by asparagine synthetase (ASNS) under specific conditions, or can be derived from macropinocytosis followed by lysosome-mediated protein breakdown [13]. Remarkably, however, even when asparagine is present in the medium, inhibition of ASNS impairs vessel formation, suggesting that the endogenous asparagine synthesis plays an essential role, independently of extracellular availability – a phenomenon that requires further study [12].

Serine is another important amino acid utilized by ECs. Similarly to asparagine, it can be taken up from the milieu or synthesized *de novo*. Notably, serine interconnects glucose and glutamine metabolism because it is synthesized from α-nitrogen of glutamate, and 3-phosphoglycerate from the glycolytic pathway under the control of the rate-limiting enzyme phosphoglycerate dehydrogenase, PHGDH. ECs use serine for the production of the pyrimidine nucleotide dTTP and the purine nucleotides dATP and dGTP (Figure 4C) [68]. Accordingly, *in vivo* proliferative ECs upregulate genes involved in one-carbon metabolism (Figure 4C) [6]. Unexpectedly, and unlike other non-EC cell types [69], PHGDH inhibition also indirectly impairs dCTP production as a result of reduced **mitochondrial respiration**. Indeed, *PHGDH* silencing impairs glycine production as a consequence of serine depletion. Glycine is required for heme synthesis in ECs (this has not been documented in other cell types) (Figure 4C). Because heme is crucial for the proper functioning of several enzymes, including the complexes II, III, and IV of OXPHOS, mitochondrial respiration is impaired [68]. As a result, the activity of mitochondrial dihydroorotate dehydrogenase (DHODH), an enzyme that catalyzes a key step in the production of dCTP and requires active mitochondrial respiration, is reduced, explaining impaired dCTP production upon *PHGDH* silencing [68]. Because of dysfunctional mitochondrial respiration, together with reduced glutathione production, both resulting from the reduced pool of glycine, *PHGDH* silencing leads to severe oxidative stress and mitochondriopathy (Figure 4C) [68].

Fatty Acid Metabolism

In addition to glucose and amino acids, fatty acids represent another fuel for proliferating stalk ECs (Figure 4D). Proliferating blood vascular and lymphatic ECs sustain the TCA cycle in a particular manner because a considerable fraction of acetyl-CoA entering the TCA cycle is acquired from fatty acids via FAO (Figure 4D) [18]. Sustaining the TCA cycle leads to the production of aspartate, a precursor of nucleotides that are essential for DNA replication during EC proliferation – a reliance seldom observed in other cell types [18,70]. The TCA cycle in ECs is not replenished by a net contribution of fatty acid-derived carbons in the classical connotation of anaplerosis. Indeed, even though ^{13}C-labeled acetyl-CoA acquired from palmitate enters the TCA cycle and labels oxaloacetate, there is no net formation of oxaloacetate from acetyl-CoA derived from fatty acids [18,70]. Instead, FAO provides acetyl-CoA in conjunction with an anaplerotic substrate to sustain the TCA cycle. In agreement, inhibition of CPT1a, the FAO rate-controlling enzyme,

reduces the pools of TCA cycle intermediates, nucleotide precursors, and dNTPs (Figure 4D), thereby impairing EC proliferation and vascular sprouting in vitro, and in mice lacking endothelial Cpt1a in vivo [18]. Notably, neither glucose nor glutamine metabolism compensated for the defect in nucleotide synthesis upon FAO blockade, suggesting that FAO plays a crucial role in EC nucleotide synthesis (Figure 4D) [18]. Other players such as fatty acid transport protein (FATP) and fatty acid binding protein (FABP) also control endothelial FAO [71,72]. Moreover, FAO inhibition in lymphatic ECs impairs lymphangiogenesis, a phenotype that can be rescued by acetate supplementation, supporting the importance of acetyl-CoA for lymphatic ECs as well [73]. Consistently, the use of ketone bodies, an alternative source of acetyl-CoA, promotes lymphatic EC proliferation, migration, and vessel sprouting [73]. Silencing of *OXCT1*, encoding the rate-controlling enzyme of the ketone body oxidation pathway, in lymphatic ECs lowers acetyl-CoA levels, TCA cycle metabolite pools, and nucleotide precursor and dNTP levels that are required for lymphatic EC proliferation [73]. Thus, ketone body supplementation could be used as a metabolic dietary approach for the treatment of diseases involving lymphatic dysfunction, a strategy currently being tested in a Phase II clinical trial for the treatment of lymphedema.

In the context of fatty acids, the role of the fatty acid synthase (FASN) in ECs is also noteworthy. This enzyme catalyzes the production of palmitate, using malonyl-CoA as substrate. Inhibition of FASN increases the levels of its substrate malonyl-CoA without substantially lowering cellular

Box 1. Transcription Factors Orchestrate EC Quiescence and Angiogenesis

Metabolic adaptations during EC proliferation, migration, and differentiation are controlled by transcription factors. Glycolysis is under the control of key transcription factors such as forkhead box protein O1 (FoxO1) and the growth and anabolic metabolism regulator MYC [10]. MYC blockade by FoxO1 decreases glycolysis and maintains EC quiescence (see Figure 5A in main text) [10]. FoxO1–MYC acts as a transcriptional regulator and gatekeeper of EC proliferation and growth [10]. FoxO1 decreases oxidative metabolism, and this may constitute a protective strategy against excessive ROS production in endothelial mitochondria (see Figure 5A in main text) [10]. Importantly, loss of endothelial FoxO1 promotes vessel enlargement and vascular hyperplasia as a result of increased EC proliferation [10].

Other transcription factors regulate glycolysis, such as Krüppel-like factor 2 (KLF2). Laminar shear stress induced by blood flow promotes binding of KLF2 to the promoter of *PFKFB3* (PFKFB3 catalyzes the production of F2,6BP, an allosteric activator of the rate-limiting glycolytic enzyme PFK1) to suppress its transcription, shifting ECs from a proliferative to a quiescent state, and reducing ATP usage to maintain energy homeostasis (see Figure 5B in main text) [92]. KLF2-dependent downregulation of *PFKFB3* favors shuttling of glycolytic intermediates to the hexosamine and glucuronic acid pathway, generating substrates of hyaluronan synthase 2 (HAS2), which is upregulated by KLF2 (see Figure 5B in main text) [93]. Hence, increased HAS2-dependent production of the EC **glycocalyx** reduces vascular permeability, thrombosis, inflammation, and angiogenesis (see Figure 5B in main text) [93].

Notch is another crucial signaling pathway that is upregulated when proliferating ECs switch to quiescence [22]. Notch is negatively regulated by the NAD^+-dependent deacetylase sirtuin 1 (SIRT1), which is activated by nutrient deprivation. SIRT1 functions as a deacetylase of the Notch intracellular domain (NICD, the transcriptionally active domain of Notch), which opposes acetylation-induced NICD stabilization. Consequently, ECs exhibit impaired growth and sprouting, and enhanced Notch target gene expression, in response to DLL4 stimulation in the absence of SIRT1 activity, thereby inducing a stalk phenotype [94]. Notch/Dll4 interaction also promotes CPT1a expression, thereby stimulating FAO while lowering glycolysis [22]. Notch signaling also regulates fatty acid transport in ECs by modulating the expression of endothelial lipase, CD36, and FABP4 [23,95].

The Hippo signaling transducer, Yes-associated protein (YAP1), is another example of transcription coactivator that regulates metabolism. YAP1 binds to the TEA domain (TEAD1) transcription factor and promotes mitochondrial biogenesis and oxygen consumption by activating the endothelial peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α) [96]. Notably, YAP/TAZ plays an important role in vascular barrier formation, sprouting angiogenesis, and coordination of endothelial proliferation and metabolism by MYC activation [97].

EC metabolism is also regulated by hypoxia, and multiple examples can be listed in this regard. For instance, hypoxia-inducible factor (HIF)-2α lowers MYC expression in pulmonary artery ECs (PAECs) and suppresses the mitochondrial transcription factor A (TFAM), whereas HIF-1α and HIF-2α suppress the transcriptional coactivator PGC-1β, an upstream regulator of TFAM [98]. In addition, accelerated glycolysis in hypoxic ECs is induced by ROS-driven HIF-1α accumulation as well as by protein kinase C (PKC) and PI3K signaling [99].

palmitate levels, likely because ECs can readily take up palmitate from the milieu [19]. Elevated levels of malonyl-CoA increase general protein malonylation, a recently discovered and poorly understood post-translational protein modification, including malonylation of mTOR, which reduces its activity and impairs EC growth [74]. In agreement, angiogenesis is impaired in mice lacking *FASN* in ECs, and treatment with a pharmacological FASN blocker inhibits pathological ocular neovascularization by reducing EC proliferation [19].

Mitochondrial Respiration

Inhibition of the respiratory chain complex III impairs mitochondrial respiration and EC proliferation *in vitro*, without affecting EC migration, owing to insufficient regeneration of NAD^+ that is necessary for the synthesis of amino acids (Figure 4D) [20]. *In vivo*, loss of the ubiquinone-binding protein QPC (a subunit of the mitochondrial complex III) in ECs diminishes EC proliferation and impairs retinal and tumor angiogenesis as a result of reduced levels of amino acids [20]. Consistently, genes encoding OXPHOS complexes are upregulated in proliferating ECs *in vivo* [6]. EC deletion of *Vegf in vivo* results in mitochondrial fragmentation and suppression of glucose metabolism, secondary to increased expression of the transcription factor FoxO1 [75].

Because metabolic adaptations during EC proliferation and migration, as well as in QECs, are controlled by transcription factors, we briefly summarize the transcriptional control of the metabolic pathways in ECs (Box 1), with special focus on the regulation of QECs by FoxO1/Myc (Figure 5A) and Klf2 (Figure 5B).

Perturbed EC Metabolism in Disease: Some Examples

Cancer and Ocular Neovascular Diseases

In cancer, excessive tumor vessels are formed, but these display structural and functional abnormalities characterized by, among others, a leaky barrier that facilitates extravasation and

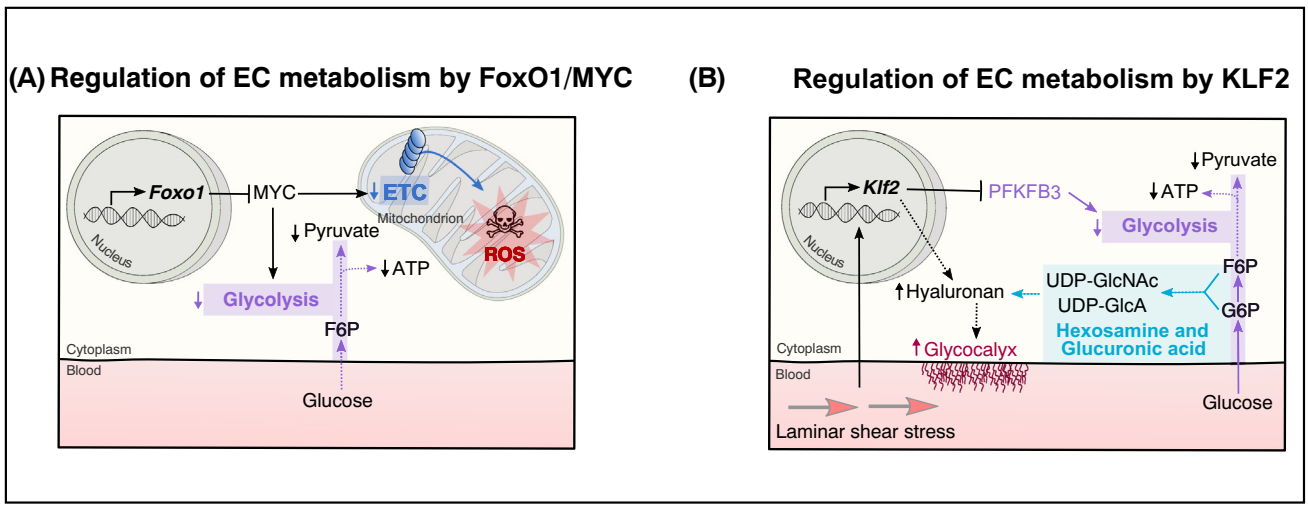


Figure 5. Transcriptional Regulation of the Metabolism of Quiescent 'Phalanx' Endothelial Cells (ECs). (A) FoxO1-dependent MYC repression inhibits glycolysis and the electron transport chain (ETC) of the oxidative phosphorylation (OXPHOS) pathway in quiescent ECs (QECs). (B) In QECs, the rate of glycolysis is maintained at a lower level than in angiogenic activated ECs, mainly because of the binding of the laminar shear stress-dependent KLF2 transcription factor to the *PFKFB3* promoter, thus suppressing 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) expression and consequently the formation of the fructose-2,6-bisphosphate (F2,6BP), a potent activator of phosphofructokinase (PFK)1. As a consequence, early glycolytic intermediates are shunted into side-pathways, such as the hexosamine and glucuronic acid pathways, which yield uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc) and uridine diphosphate glucuronic acid (UDP-GlcA), substrates for hyaluronan synthase 2 (HAS2). Moreover, KLF2 upregulates HAS2, leading to increased hyaluronan synthesis and glycocalyx production. Abbreviations: F6P, fructose-6-phosphate; G6P, glucose-6-phosphate.

dissemination of cancer cells and reduced perfusion [76,77]. Tumor ECs are characterized by increased glucose uptake, glycolysis, and diversion of glycolytic intermediates to the pentose phosphate and serine biosynthesis pathways, that all promote energy production and the synthesis of biomass (nucleotides) [77,78]. Lowering glycolysis by 15–20% in tumor ECs by treating tumor-bearing mice with the PFKFB3 inhibitor 3-(3-pyridinyl)-1-(4-pyridinyl)2-propen-1-one (3PO) sufficed to induce **tumor vessel normalization**, and secondarily reduced metastasis (by tightening the vascular barrier) and improved chemotherapy responses (by improving the delivery of chemotherapeutics) [77]. By contrast, treatment with a maximally tolerable dose of 3PO lowered EC glycolysis to the extent that it caused EC demise and vessel disintegration, in fact promoting rather than counteracting cancer cell extravasation and dissemination [79]. These results emphasize that, by contrast to cancer cell metabolism-targeted therapies, targeting EC metabolism should aim at restoring the perturbed metabolic pathways to their normal levels (as observed in healthy QECs) rather than eliminating them – because this will likely cause general toxicity and undesired effects. In addition to glycolysis, tumor ECs show alterations of fatty acid and serine biosynthesis pathways (upregulation of FASN, PHGDH, and phosphoserine aminotransferase 1, PSAT1), although further studies will be necessary to determine the potential of targeting these pathways for tumor vessel normalization [19,68]. Similarly, in neovascular ocular diseases such as retinopathy of prematurity (ROP), treatment with low-dose 3PO decreased retinal neovascularization [53]. Targeting FAO by inhibiting CPT1a with etomoxir also showed benefit in a model of retinal neovascularization [18].

Single-cell studies showed that tip and proliferating tumor ECs represent <10% of all tumor ECs in human lung tumors, and at least 13 tumor EC phenotypes have been identified [5]. Similarly, sprouting ECs from murine choroids display additional phenotypes beyond the traditional tip and proliferating EC phenotypes [6]. Thus, EC heterogeneity in tumors and in other diseases should be taken into consideration for the development of new effective antiangiogenic therapies.

Pulmonary Arterial Hypertension (PAH)

PAH is characterized by an increase in pulmonary arterial pressure and vascular resistance, ultimately leading to right ventricular failure and death [80]. PAH is associated with intense remodeling of pulmonary arterioles involving excessive EC proliferation, apoptosis resistance, and EC dysfunction [81]. PAH ECs display global metabolic reprogramming in which imbalance between increased glycolysis versus reduced OXPHOS secondary to upregulation of pyruvate dehydrogenase kinase (PDK), an inhibitor of pyruvate dehydrogenase (PDH), leads to impaired entry of glucose-derived carbons into the TCA cycle [82–84]. Pharmacological PDK inhibition by dichloroacetate reverts this glycolysis/TCA cycle uncoupling and improves PAH in a subset of patients [85]. Moreover, PAH ECs upregulate the expression of PFKFB3, a key regulator of glycolysis [86]. The resulting increase in glycolytic metabolites, such as pyruvate, stabilizes the hypoxia signaling molecule hypoxia-inducible factor (HIF)-2α in PAH-ECs, further stimulating the production of growth and inflammatory factors, and enhancing proliferation and inflammation of the pulmonary vessels [86]. Both pharmacological PFKFB3 blockade and EC-specific knockout of *Pfkfb3* reduce PAH development in rodent models [86]. In PAH ECs, downregulation of miR-124 and its target, splicing factor polypyrimidine tract binding protein (PTBP1), results in alternative splicing of pyruvate kinase muscle isoforms 1 and 2 (PKM1 and 2) leading to increased PKM2 expression and contributing to the metabolic and proliferation abnormalities of PAH ECs [87]. In addition, stiffening of the extracellular matrix in PAH leads to mechanoactivation of endothelial glycolysis and glutamine breakdown in a Yes-associated protein (YAP)/TAZ-dependent manner, resulting in higher EC proliferation and migration [88]. Glutamine metabolism is further affected in PAH ECs. Indeed, glutamine uptake in BMPR2-mutated PAH ECs is increased [89], whereas inhibition of GLS1 reduces PAH-EC proliferation and migration, and alleviates PAH in

a rat model [88]. Thus, the landscape of metabolic alterations in ECs, that promotes the remodeling of the pulmonary vessels in PAH, offers metabolic candidates for putative therapeutic interventions.

Concluding Remarks and Future Perspectives

Accumulating evidence over the past 10 years has highlighted the versatility of EC metabolism in health and disease. While ECs adapt their metabolism to fulfill particular functions [5,41], they often present a metabolic profile different from that of other cell types. This distinct (unique?) EC 'engine' may allow therapeutic targeting, and in particular, inhibition, or promotion of specific EC phenotypes (see Outstanding Questions). In cancer, preclinical studies have paved the way for low-dose metabolism-targeting agents that normalize tumor blood vessels [77]. Thus, combining antiangiogenic metabolic treatment with chemotherapeutic agents or others (i.e., anti-VEGF therapy, radiotherapy, immunotherapy) might warrant further exploration. Of note, to avoid toxic effects, antiangiogenic metabolic therapies should not completely abrogate key metabolic pathways in ECs [79]. Multiomic analyses in freshly isolated ECs, preferably at single-cell level, can also bring new insights into how EC metabolism is rewired in disease conditions (such as in cancer) and in specific EC phenotypes, thereby allowing the identification of new potential metabolic targets [5,6]. Genome-scale metabolic modeling, tuned from multiomic data, could prioritize metabolic candidates for targeting disease-specific EC subpopulations [6]. Single-cell profiling techniques have already revealed extensive EC heterogeneity in health and disease, but exhaustive analysis of all EC subpopulations and their congruency among tissues in health versus disease is warranted. A database grouping all published EC transcriptomic data should facilitate target discoveries [90]. In addition, manipulating EC metabolism to promote angiogenesis in the perspective of vascular regenerative medicine (organoid vascularization), or to limit tissue ischemia, also deserves further attention. In this respect, metabolic characterization of the recently identified tissue-resident endothelial stem cells that display self-renewal capacity and vascular regeneration potential would be valuable for identifying key metabolic pathways and would be applicable to regenerative medicine [91]. Studies analyzing the metabolism of single ECs in disease and in tissue regeneration would pave the way for the development of a new generation of EC subtype-specific metabolic therapies.

Outstanding Questions

ECs are highly glycolytic, even more than some cancer cells, and display an unusual profile of metabolic pathways that are not used by non-ECs. To what extent is EC metabolism distinct, or even unique? – which would make targeting EC metabolic pathways therapeutically attractive.

During sprouting angiogenesis, tip and stalk ECs rewire their metabolism, but there are other modes of vessel formation: which metabolic adaptations underlie intussusceptive angiogenesis, vessel cooption, and resident endothelial stem cell-mediated vessel repair or formation?

Can we target specific disease EC phenotypes, newly identified in single-cell RNA-seq studies, with metabolic strategies?

Single-cell RNA-seq studies yield atlases of innumerable marker genes, but the true challenge is to design strategies to select and prioritize functionally relevant angiogenic candidates. Will an integrated multiomic approach, combined with computational genome-scale metabolic modeling (GEM), as we recently developed, allow more accurate prediction of metabolic candidates that fuel EC growth?

Tuning down glycolysis with a PFKFB3 inhibitor induces tumor vessel normalization: could other metabolic pathways be targeted to achieve the same outcome?

Are EC metabolic pathways a targetable vulnerability of tumor ECs upon treatment with clinically approved antiangiogenic or anticancer therapies?

Can (dietary) metabolite supplementation stimulate or inhibit vessel growth in disease?

Acknowledgments

S.J.D. is supported by a Marie Skłodowska–Curie-individual fellowship. P.C. is supported by the Federal Government of Belgium (Belgian Policy Society grant IUAP P7/03), long-term structural Methusalem funding from the Flemish Government, and by the Research Foundation Flanders (FWO-Vlaanderen), the Foundation Against Cancer (grants 2012-175 and 2016-078), Kom op tegen Kanker (Stand up to Cancer) from the Flemish Cancer Society, an Advanced Grant from the European Research Council (ERC) (grant EU- ERC269073), and Regenerative Medicine Crossing Borders (RegMedXB).

Disclosure Statement

P.C. is named as an inventor on patent applications related to results discussed in this review.

References

1. Carmeliet, P. and Jain, R.K. (2011) Molecular mechanisms and clinical applications of angiogenesis. *Nature* 473, 298–307
2. Gimbrone Jr., M.A. and Garcia-Cardena, G. (2016) Endothelial cell dysfunction and the pathobiology of atherosclerosis. *Circ. Res.* 118, 620–636
3. Betz, C. *et al.* (2016) Cell behaviors and dynamics during angiogenesis. *Development* 143, 2249–2260
4. Jakobsson, L. *et al.* (2010) Endothelial cells dynamically compete for the tip cell position during angiogenic sprouting. *Nat. Cell Biol.* 12, 943–953
5. Goveia, J. *et al.* (2020) An integrated gene expression landscape profiling approach to identify lung tumor endothelial cell heterogeneity and angiogenic candidates. *Cancer Cell* 37, 21–36
6. Rohlenova, K. *et al.* (2020) Single-cell RNA sequencing maps endothelial metabolic plasticity in pathological angiogenesis. *Cell Metab.* 31, 862–877
7. Chung, A.S. and Ferrara, N. (2011) Developmental and pathological angiogenesis. *Annu. Rev. Cell Dev. Biol.* 27, 563–584
8. Ebos, J.M. and Kerbel, R.S. (2011) Antiangiogenic therapy: impact on invasion, disease progression, and metastasis. *Nat. Rev. Clin. Oncol.* 8, 210–221
9. Li, X. *et al.* (2019) Hallmarks of endothelial cell metabolism in health and disease. *Cell Metab.* 30, 414–433
10. Wilhelm, K. *et al.* (2016) FOXO1 couples metabolic activity and growth state in the vascular endothelium. *Nature* 529, 216–220

11. Yu, P. *et al.* (2017) FGF-dependent metabolic control of vascular development. *Nature* 545, 224–228
12. Huang, H. *et al.* (2017) Role of glutamine and interlinked asparagine metabolism in vessel formation. *EMBO J.* 36, 2334–2352
13. Kim, B. *et al.* (2017) Glutamine fuels proliferation but not migration of endothelial cells. *EMBO J.* 36, 2321–2333
14. Glatzel, D.K. *et al.* (2018) Acetyl-CoA carboxylase 1 regulates endothelial cell migration by shifting the phospholipid composition. *J. Lipid Res.* 59, 298–311
15. Fang, L. *et al.* (2013) Control of angiogenesis by AIBP-mediated cholesterol efflux. *Nature* 498, 118–122
16. De Bock, K. *et al.* (2013) Role of PFKFB3-driven glycolysis in vessel sprouting. *Cell* 154, 651–663
17. Kim, B. *et al.* (2018) Endothelial pyruvate kinase M2 maintains vascular integrity. *J. Clin. Invest.* 128, 4543–4556
18. Schoors, S. *et al.* (2015) Fatty acid carbon is essential for dNTP synthesis in endothelial cells. *Nature* 520, 192–197
19. Bruning, U. *et al.* (2018) Impairment of angiogenesis by fatty acid synthase inhibition involves mTOR malonylation. *Cell Metab.* 28, 866–880
20. Diebold, L.P. *et al.* (2019) Mitochondrial complex III is necessary for endothelial cell proliferation during angiogenesis. *Nat. Metab.* 1, 158–171
21. Li, X. *et al.* (2019) Metabolic pathways fueling the endothelial cell drive. *Annu. Rev. Physiol.* 81, 483–503
22. Kalucka, J. *et al.* (2018) Quiescent endothelial cells upregulate fatty acid beta-oxidation for vasculoprotection via redox homeostasis. *Cell Metab.* 28, 881–894 e13
23. Jabs, M. *et al.* (2018) Inhibition of endothelial Notch signaling impairs fatty acid transport and leads to metabolic and vascular remodeling of the adult heart. *Circulation* 137, 2592–2608
24. Knobloch, M. *et al.* (2017) A fatty acid oxidation-dependent metabolic shift regulates adult neural stem cell activity. *Cell Rep.* 20, 2144–2155
25. Heaster, T.M. *et al.* (2018) Autofluorescence imaging identifies tumor cell-cycle status on a single-cell level. *J. Biophotonics* 11, e201600276
26. Smolkova, K. and Jezek, P. (2012) The role of mitochondrial NADPH-dependent isocitrate dehydrogenase in cancer cells. *Int. J. Cell Biol.* 2012, 273947
27. Pike, L.S. *et al.* (2011) Inhibition of fatty acid oxidation by etomoxir impairs NADPH production and increases reactive oxygen species resulting in ATP depletion and cell death in human glioblastoma cells. *Biochim. Biophys. Acta* 1807, 726–734
28. Gorrini, C. *et al.* (2013) Modulation of oxidative stress as an anticancer strategy. *Nat. Rev. Drug Discov.* 12, 931–947
29. Di, A. *et al.* (2016) ROS-activated calcium signaling mechanisms regulating endothelial barrier function. *Cell Calcium* 60, 163–171
30. Patella, F. *et al.* (2015) Proteomics-based metabolic modeling reveals that fatty acid oxidation (FAO) controls endothelial cell (EC) permeability. *Mol. Cell. Proteomics* 14, 621–634
31. Xiong, J. *et al.* (2018) A metabolic basis for endothelial-to-mesenchymal transition. *Mol. Cell* 69, 689–698
32. Chen, P.Y. and Simons, M. (2018) Fibroblast growth factor-transforming growth factor beta dialogues, endothelial cell to mesenchymal transition, and atherosclerosis. *Curr. Opin. Lipidol.* 29, 397–403
33. Cho, J.G. *et al.* (2018) Endothelial to mesenchymal transition represents a key link in the interaction between inflammation and endothelial dysfunction. *Front. Immunol.* 9, 294
34. Platel, V. *et al.* (2019) Endothelial-to-mesenchymal transition (EndoMT): roles in tumorigenesis, metastatic extravasation and therapy resistance. *J. Oncol.* 2019, 8361945
35. Piera-Velazquez, S. *et al.* (2016) Endothelial to mesenchymal transition (EndoMT) in the pathogenesis of human fibrotic diseases. *J. Clin. Med.* 5, 45
36. Ranchoux, B. *et al.* (2015) Endothelial-to-mesenchymal transition in pulmonary hypertension. *Circulation* 131, 1006–1018
37. Eelen, G. *et al.* (2018) Endothelial cell metabolism. *Physiol. Rev.* 98, 3–58
38. Wong, B.W. *et al.* (2017) The role of fatty acid beta-oxidation in lymphangiogenesis. *Nature* 542, 49–54
39. Kalucka, J. *et al.* (2020) Single-cell transcriptome atlas of murine endothelial cells. *Cell* 180, 764–779
40. Lother, A. *et al.* (2018) Cardiac endothelial cell transcriptome. *Arterioscler. Thromb. Vasc. Biol.* 38, 566–574
41. Marcu, R. *et al.* (2018) Human organ-specific endothelial cell heterogeneity. *iScience* 4, 20–35
42. Dumas, S.J. *et al.* (2020) Single-cell RNA sequencing reveals renal endothelium heterogeneity and metabolic adaptation to water deprivation. *J. Am. Soc. Nephrol.* 31, 118–138
43. Seano, G. *et al.* (2014) Endothelial podosome rosettes regulate vascular branching in tumour angiogenesis. *Nat. Cell Biol.* 16, 931–941
44. Buchwald, P. (2011) A local glucose-and oxygen concentration-based insulin secretion model for pancreatic islets. *Theor. Biol. Med. Model.* 8, 20
45. Pfeiffer, T. *et al.* (2001) Cooperation and competition in the evolution of ATP-producing pathways. *Science* 292, 504–507
46. De Bock, K. *et al.* (2013) Role of endothelial cell metabolism in vessel sprouting. *Cell Metab.* 18, 634–647
47. Mattila, P.K. and Lappalainen, P. (2008) Filopodia: molecular architecture and cellular functions. *Nat. Rev. Mol. Cell Biol.* 9, 446–454
48. van Horssen, R. *et al.* (2009) Modulation of cell motility by spatial repositioning of enzymatic ATP/ADP exchange capacity. *J. Biol. Chem.* 284, 1620–1627
49. Real-Hohn, A. *et al.* (2010) Filamentous actin and its associated binding proteins are the stimulatory site for 6-phosphofructo-1-kinase association within the membrane of human erythrocytes. *Biochimie* 92, 538–544
50. Menard, L. *et al.* (2014) The structural and functional coordination of glycolytic enzymes in muscle: evidence of a metabolon? *Biology (Basel)* 3, 623–644
51. Waingeh, V.F. *et al.* (2006) Glycolytic enzyme interactions with yeast and skeletal muscle F-actin. *Biophys. J.* 90, 1371–1384
52. Xu, Y. *et al.* (2014) Endothelial PFKFB3 plays a critical role in angiogenesis. *Arterioscler. Thromb. Vasc. Biol.* 34, 1231–1239
53. Schoors, S. *et al.* (2014) Partial and transient reduction of glycolysis by PFKFB3 blockade reduces pathological angiogenesis. *Cell Metab.* 19, 37–48
54. Vizan, P. *et al.* (2009) Characterization of the metabolic changes underlying growth factor angiogenic activation: identification of new potential therapeutic targets. *Carcinogenesis* 30, 946–952
55. Dornier, E. *et al.* (2017) Glutaminolysis drives membrane trafficking to promote invasiveness of breast cancer cells. *Nat. Commun.* 8, 2255
56. Du, K. *et al.* (2018) Hedgehog–YAP signaling pathway regulates glutaminolysis to control activation of hepatic stellate cells. *Gastroenterology* 154, 1465–1479
57. Rodrigues, M.F. *et al.* (2016) Enhanced OXPHOS, glutaminolysis and beta-oxidation constitute the metastatic phenotype of melanoma cells. *Biochem. J.* 473, 703–715
58. Kim, D.S. *et al.* (2014) Effects of glutamine on proliferation, migration, and differentiation of human dental pulp cells. *J. Endod.* 40, 1087–1094
59. Han, T. *et al.* (2017) A novel glutaminase inhibitor-968 inhibits the migration and proliferation of non-small cell lung cancer cells by targeting EGFR/ERK signaling pathway. *Oncotarget* 8, 28063–28073
60. Eelen, G. *et al.* (2018) Role of glutamine synthetase in angiogenesis beyond glutamine synthesis. *Nature* 561, 63–69
61. Kim, C. *et al.* (2014) Vascular RhoJ is an effective and selective target for tumor angiogenesis and vascular disruption. *Cancer Cell* 25, 102–117
62. Kaur, S. *et al.* (2011) RhoJ/TCL regulates endothelial motility and tube formation and modulates actomyosin contractility and focal adhesion numbers. *Arterioscler. Thromb. Vasc. Biol.* 31, 657–664
63. Kuo, A. *et al.* (2017) Lipid droplet biogenesis and function in the endothelium. *Circ. Res.* 120, 1289–1297
64. Stone, O.A. *et al.* (2018) Loss of pyruvate kinase M2 limits growth and triggers innate immune signaling in endothelial cells. *Nat. Commun.* 9, 4077
65. Unterluggauer, H. *et al.* (2008) Premature senescence of human endothelial cells induced by inhibition of glutaminase. *Biogerontology* 9, 247–259
66. Jewell, J.L. *et al.* (2015) Differential regulation of mTORC1 by leucine and glutamine. *Science* 347, 194–198

67. Zhang, J. *et al.* (2014) Asparagine plays a critical role in regulating cellular adaptation to glutamine depletion. *Mol. Cell* 56, 205–218
68. Vandekeere, S. *et al.* (2018) Serine synthesis via PHGDH is essential for heme production in endothelial cells. *Cell Metab.* 28, 573–587 e13
69. Possemato, R. *et al.* (2011) Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. *Nature* 476, 346–350
70. Schoors, S. *et al.* (2015) Fatty acid carbon is essential for dNTP synthesis in endothelial cells. *Nature* 520, 192–197
71. Elmasri, H. *et al.* (2012) Endothelial cell-fatty acid binding protein 4 promotes angiogenesis: role of stem cell factor/c-kit pathway. *Angiogenesis* 15, 457–468
72. Elmasri, H. *et al.* (2009) Fatty acid binding protein 4 is a target of VEGF and a regulator of cell proliferation in endothelial cells. *FASEB J.* 23, 3865–3873
73. García-Caballero, M. *et al.* (2019) Role and therapeutic potential of dietary ketone bodies in lymph vessel growth. *Nat. Metab.* 1, 666–675
74. Saxton, R.A. and Sabatini, D.M. (2017) mTOR signaling in growth, metabolism, and disease. *Cell* 169, 361–371
75. Domigan, C.K. *et al.* (2015) Autocrine VEGF maintains endothelial survival through regulation of metabolism and autophagy. *J. Cell Sci.* 128, 2236–2248
76. Carmeliet, P. and Jain, R.K. (2011) Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. *Nat. Rev. Drug Discov.* 10, 417–427
77. Cantelmo, A.R. *et al.* (2016) Inhibition of the glycolytic activator PFKFB3 in endothelium induces tumor vessel normalization, impairs metastasis, and improves chemotherapy. *Cancer Cell* 30, 968–985
78. Zhang, L. *et al.* (2018) COX2 inhibition in the endothelium induces glucose metabolism normalization and impairs tumor progression. *Mol. Med. Rep.* 17, 2937–2944
79. Conradi, L.C. *et al.* (2017) Tumor vessel disintegration by maximum tolerable PFKFB3 blockade. *Angiogenesis* 20, 599–613
80. Thenappan, T. *et al.* (2018) Pulmonary arterial hypertension: pathogenesis and clinical management. *BMJ* 360, j5492
81. Xu, W. and Erzurum, S.C. (2011) Endothelial cell energy metabolism, proliferation, and apoptosis in pulmonary hypertension. *Compr. Physiol.* 1, 357–372
82. Diebold, I. *et al.* (2015) BMPR2 preserves mitochondrial function and DNA during reoxygenation to promote endothelial cell survival and reverse pulmonary hypertension. *Cell Metab.* 21, 596–608
83. Xu, W. *et al.* (2007) Alterations of cellular bioenergetics in pulmonary artery endothelial cells. *Proc. Natl. Acad. Sci. U. S. A.* 104, 1342–1347
84. Yu, Q. and Chan, S.Y. (2017) Mitochondrial and metabolic drivers of pulmonary vascular endothelial dysfunction in pulmonary hypertension. *Adv. Exp. Med. Biol.* 967, 373–383
85. Michelakis, E.D. *et al.* (2017) Inhibition of pyruvate dehydrogenase kinase improves pulmonary arterial hypertension in genetically susceptible patients. *Sci. Transl. Med.* 9, eaao4583
86. Cao, Y. *et al.* (2019) PFKFB3-mediated endothelial glycolysis promotes pulmonary hypertension. *Proc. Natl. Acad. Sci. U. S. A.* 116, 13394–13403
87. Caruso, P. *et al.* (2017) Identification of microRNA-124 as a major regulator of enhanced endothelial cell glycolysis in pulmonary arterial hypertension via PTBP1 (polypyrimidine tract binding protein) and pyruvate kinase M2. *Circulation* 136, 2451–2467
88. Bertero, T. *et al.* (2016) Vascular stiffness mechanoactivates YAP/TAZ-dependent glutaminolysis to drive pulmonary hypertension. *J. Clin. Invest.* 126, 3313–3335
89. Egnatchik, R.A. *et al.* (2017) Dysfunctional BMPR2 signaling drives an abnormal endothelial requirement for glutamine in pulmonary arterial hypertension. *Pulm. Circ.* 7, 186–199
90. Khan, S. *et al.* (2019) EndoDB: a database of endothelial cell transcriptomics data. *Nucleic Acids Res.* 47, D736–D744
91. Wakabayashi, T. *et al.* (2018) CD157 marks tissue-resident endothelial stem cells with homeostatic and regenerative properties. *Cell Stem Cell* 22, 384–397
92. Doddaballapur, A. *et al.* (2015) Laminar shear stress inhibits endothelial cell metabolism via KLF2-mediated repression of PFKFB3. *Arterioscler. Thromb. Vasc. Biol.* 35, 137–145
93. Wang, G. *et al.* (2020) Shear stress regulation of endothelial glycocalyx structure is determined by glucobiosynthesis. *Arterioscler. Thromb. Vasc. Biol.* 40, 350–364
94. Guarani, V. *et al.* (2011) Acetylation-dependent regulation of endothelial Notch signalling by the SIRT1 deacetylase. *Nature* 473, 234–238
95. Harjes, U. *et al.* (2014) Fatty acid-binding protein 4, a point of convergence for angiogenic and metabolic signaling pathways in endothelial cells. *J. Biol. Chem.* 289, 23168–23176
96. Mammoto, A. *et al.* (2018) YAP1–TEAD1 signaling controls angiogenesis and mitochondrial biogenesis through PGC1alpha. *Microvasc. Res.* 119, 73–83
97. Kim, J. *et al.* (2017) YAP/TAZ regulates sprouting angiogenesis and vascular barrier maturation. *J. Clin. Invest.* 127, 3441–3461
98. Zarrabi, A.J. *et al.* (2017) Hypoxia-induced suppression of c-Myc by HIF-2alpha in human pulmonary endothelial cells attenuates TFAM expression. *Cell. Signal.* 38, 230–237
99. Paik, J.Y. *et al.* (2017) Reactive oxygen species-driven HIF1alpha triggers accelerated glycolysis in endothelial cells exposed to low oxygen tension. *Nucl. Med. Biol.* 45, 8–14