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Review

# Endothelial Cell Metabolism in Health and Disease

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**The metabolism of endothelial cells (ECs) has only recently been recognized as a driving force of angiogenesis. Metabolic pathways, such as glycolysis, fatty acid oxidation, and glutamine metabolism, have distinct, essential roles during vessel formation. Moreover, EC metabolism is markedly perturbed in pathologies such as cancer and diabetes. For instance, because tumor ECs increase glycolysis, lowering hyperglycolysis in tumor ECs induces therapeutic benefits in preclinical tumor models. Expanding our knowledge of how ECs alter their metabolism in disease could pave the way for novel therapeutic opportunities. In this review, we discuss the most recent insights into EC metabolism in health and disease, with emphasis on the changes in metabolism in the tumor endothelium.**

## Trends

EC metabolism determines vessel sprouting; thus, targeting EC metabolism offers new therapeutic opportunities to inhibit or stimulate vessel formation.

Tumor vessel normalization by targeting endothelial metabolism improves combination chemotherapy and reduces metastasis.

Cooling the overheated metabolic engine of tumor ECs to normal levels may be an effective, yet safe strategy, and may be superior to completely eliminating the perturbed metabolic pathways.

## Angiogenesis: From Quiescent Cells to a New Vessel Sprout

ECs form the inner lining of blood and lymphatic vessels and are essential for normal functioning of the vascular system. The vascular network expands in response to changing metabolic demands during physiological organ growth to supply all tissues with sufficient oxygen and nutrients. Throughout adulthood, ECs stay mostly quiescent; however, they retain the capacity to rapidly initiate new vessel formation in response to injury or in pathological conditions. This tightly regulated process, called 'sprouting angiogenesis', starts with the protrusion of highly motile filopodia from a migratory tip cell and continues as proliferating stalk cells trail behind the leading tip cell as the sprout elongates [1] (Figure 1A). The phenotype of individual EC subtypes is not hard-wired. Instead, it is dynamically adjusted in response to cellular competition for growth factors, with vascular endothelial growth factor (VEGF) being a key regulator [2]. As the newly formed vessel matures, the restored perfusion contributes to the induction of a quiescent phalanx cell monolayer of tightly organized cobblestone-like cells. The three EC subtypes (tip, stalk, and phalanx cells) differ in their energy, biomass, and redox requirements, and, thus, in their metabolism [3,4]. A growing body of evidence indicates that these metabolic changes are not mere bystanders during the angiogenic switch. Instead, it is increasingly evident that growth factors and transcriptional signals converge on metabolic changes that drive phenotypic differentiation [3]. Recent evidence suggests that EC metabolism is perturbed in diseased tissues, such as in tumors and during diabetes. Therefore, deeper insight into metabolic alterations in ECs in these pathological conditions is required to exploit the translational potential of normalizing these metabolic perturbations.

## EC Metabolism in Health

### Glycolysis Is the Main Energy Source in the Endothelium

ECs are glycolysis addicts, since 85% of their ATP is produced glycolytically by converting glucose to lactate [3]. Compared with many other healthy cell types, ECs have higher rates of

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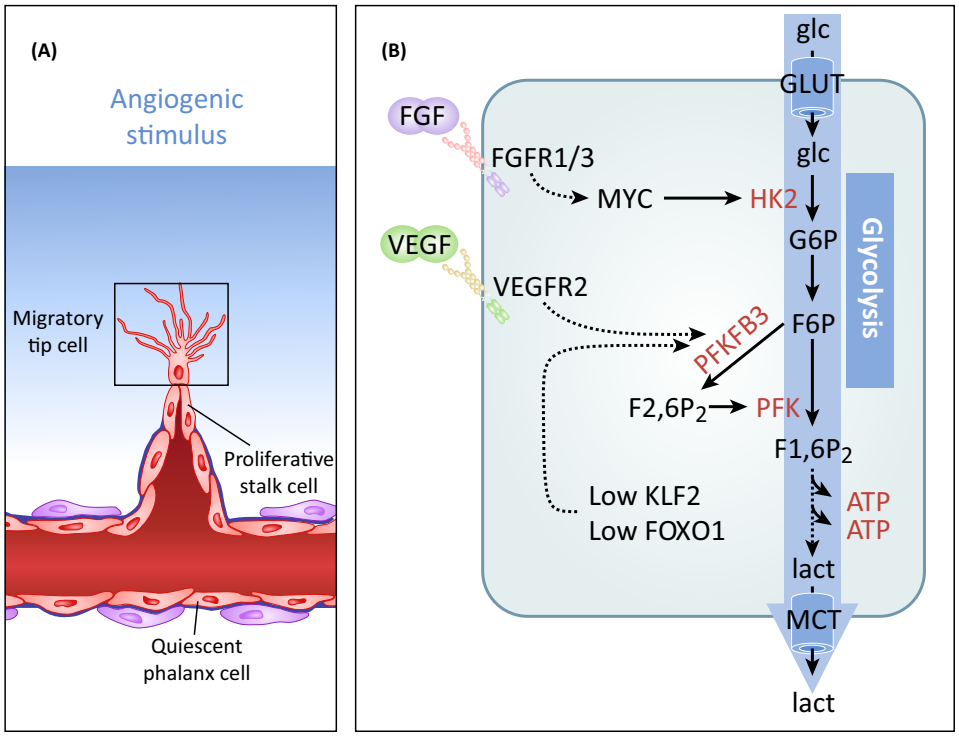



Figure 1. Glycolysis: The Main Energy Source in Sprouting Endothelium. (A) The migratory tip cell initiates the formation of a sprout from an existing quiescent vessel upon an angiogenic stimulus. Proliferative stalk cells follow the tip cell and eventually create a new vessel sprout. (B) VEGF and FGF promote glycolysis by induction of PFKFB3 and by MYC-mediated stimulation of HK2. High levels of glycolysis provide ATP to support the migration of the tip cell towards the angiogenic stimuli. At the same time, the levels of FOXO1 and KLF2, markers of quiescent endothelial cells (ECs), are repressed. Abbreviations: F1,6$P_2$, fructose-1,6-bisphosphate; F2,6$P_2$, fructose-2,6-bisphosphate; F6P, fructose-6-phosphate; FGF, fibroblast growth factor; FGFR1/3, fibroblast growth factor receptor 1/3; FOXO1, forkhead box O1; G6P, glucose-6-phosphate; glc, glucose; GLUT, glucose transporter; HK2, hexokinase 2; KLF2, Krüppel-like factor; lact, lactate; MCT, monocarboxylate transporter; MYC, c-MYC; PFKFB3, phosphofructokinase-2/fructose-2,6-bisphosphatase 3; VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor 2.

glycolysis and their glucose consumption is in the same range as that of many cancer cells [3]. Even though oxidative metabolism yields 34 extra molecules of ATP from one molecule of glucose, only a small fraction of pyruvate generated from glycolysis is metabolized oxidatively [3]. At first sight, it might seem enigmatic why quiescent ECs do not take full advantage of their easy access to oxygen. Phalanx cells bath in oxygen and, therefore, they might need to find ways to protect themselves from oxidative stress [5]. Preferentially utilizing glycolysis instead of oxidative metabolism would be one way of keeping reactive oxygen species (ROS) levels in check. At the same time, by relying on anaerobic metabolism, ECs save oxygen, which enhances the diffusion of oxygen to perivascular cells [5,6]. In addition, filopodia of tip cells explore and extend into hypoxic tissues, away from perfused blood vessels, where oxygen levels drop faster than glucose levels, making oxidative metabolism impractical. Also, glycolysis produces ATP with faster kinetics, necessary for the rapid revascularization of hypoxic tissues before their demise [3].

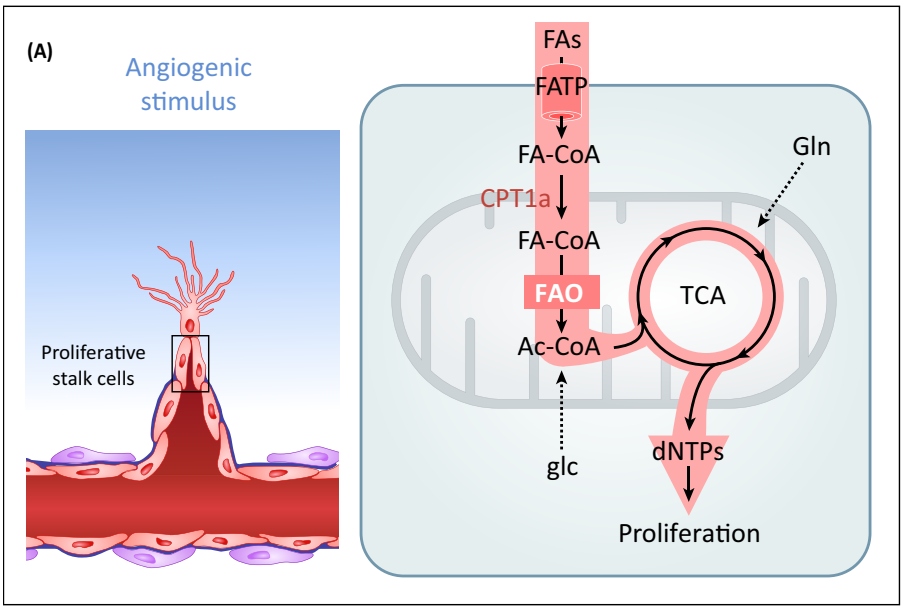
Glycolysis in ECs is stimulated by the regulator 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3). This enzyme produces fructose-2,6-bisphosphate (F2,6$P_2$), a

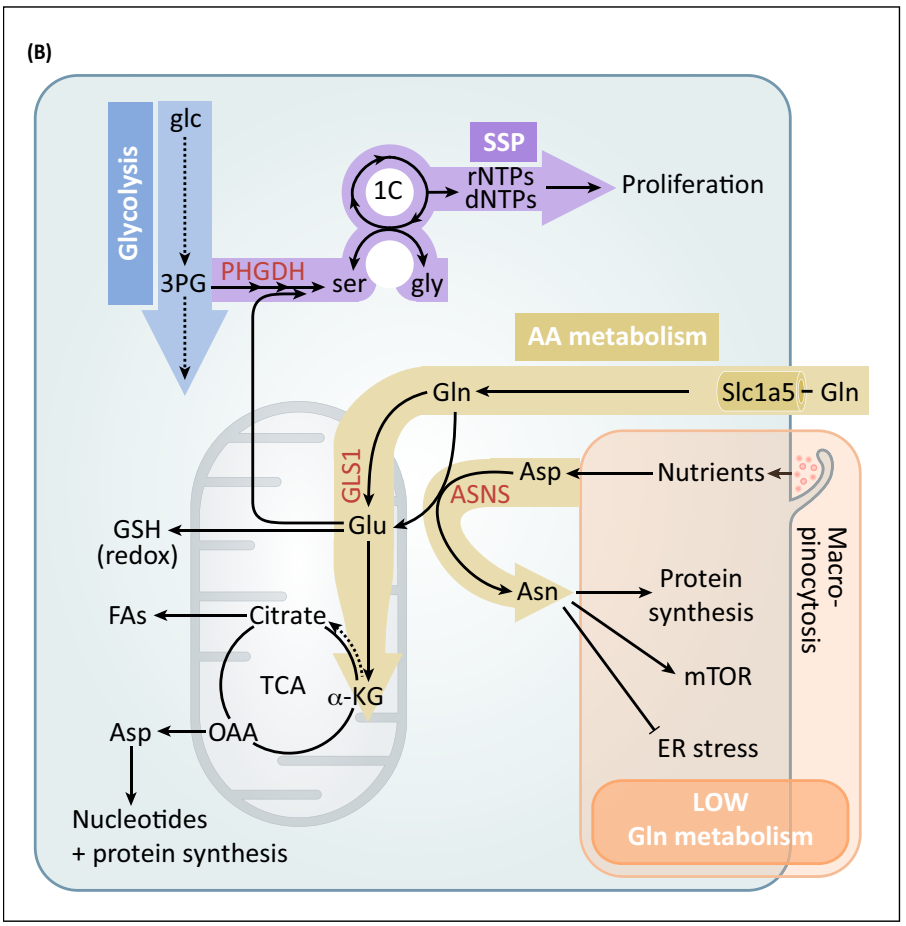
strong allosteric activator of phosphofructokinase-1 (PFK1), a rate-limiting enzyme of glycolysis [3]. Hexokinase 2 (HK2), the rate-limiting enzyme that phosphorylates glucose to glucose-6-phosphate, is another glycolytic regulator in ECs [7]. Blood flow keeps phalanx cells in a resting state, partly through inhibition of glycolytic metabolism. *PFKFB3*, *HK2*, and other glycolytic genes are suppressed by activation of the transcription factor KLF2 upon laminar shear stress [8]. Forkhead box O1 (FOXO1) is another transcription factor that indirectly reduces glycolysis by inhibiting the transcription factor MYC [9,10]. When ECs switch from to angiogenic tip or stalk cells, their glycolytic rate is increased [3]. Angiogenic ECs can interchange their position in the sprout, depending on their relative metabolic fitness. Indeed, genetically instructed stalk cells can overtake the tip cell position when they express higher levels of PFKFB3 (and, thus, increase glycolysis) [3]. In addition to the genetic control of vascular sprouting, growth factor-dependent EC metabolism also controls angiogenesis. For instance, VEGF stimulates PFKFB3 levels, but reduced FOXO1 or KLF2 levels might also contribute to higher PFKFB3 levels during sprouting (Figure 1B). Of note, besides VEGF signaling, FGF signaling promotes MYC expression, thereby stimulating HK2 levels and glycolysis [7]. Hence, several other growth factor signaling pathways control metabolic pathways in ECs, such as glycolysis.

Interestingly, PFKFB3 and other glycolytic enzymes are compartmentalized in lamellipodia and filopodia to generate glycolytic ATP at so-called 'ATP hot-spots', colocalizing with F-actin in membrane ruffles of lamellipodia [3]. Reducing glycolysis by either pharmacological inhibition (with the small molecule 3PO) or genetic silencing of PFKFB3 decreased glycolytic rates *in vitro*, impairing EC proliferation and migration. Moreover, *in vivo* silencing of PFKFB3 (both pharmacologically and genetically) impaired vessel outgrowth and branching in the mouse retina and zebrafish [3]. Similarly, reducing glycolysis by silencing of HK2 reduced arterial development and branching in the mouse skin, as well as proliferation and number of filopodia in the retinal vasculature [7]. Altogether, these data underline the importance of glycolysis in angiogenesis and in the maintenance of EC specialization.

### Fatty Acids Support Biomass Synthesis

In many cell types, mitochondria are the main site of ATP production, functioning as the energy powerhouse of the cell [11]. Acetyl-CoA molecules derived from fatty acids can be used in the TCA cycle to produce reducing equivalents to sustain ATP production via oxidative phosphorylation (OXPHOS). For example, cardiomyocytes primarily burn fatty acids to supply ATP for contractile purposes [12,13]. However, in ECs, mitochondria serve as a biosynthetic hub, rather than as an energy powerhouse [4]. Fatty acid oxidation (FAO)-derived carbons help to sustain the TCA cycle in conjunction with an anaplerotic substrate, resulting in the production of the precursors aspartate and glutamate from its intermediates oxaloacetate (OAA) and α-ketoglutarate (α-KG) for deoxynucleotide (dNTP) synthesis during the proliferation of stalk cells [4] (Figure 2A). This is remarkable, because, in most other cells, acetyl-CoA is derived from glucose and/or glutamine to fuel the TCA cycle for biomass synthesis [11]. Carnitine palmitoyltransferase 1a (CPT1a) is a rate-controlling enzyme of FAO that imports fatty acids into the mitochondria, where they are metabolized through β-oxidation into acetyl-CoA molecules that enter the TCA cycle [4]. CPT1a silencing causes a decrease in the dNTP pool, which perturbs EC sprouting *in vitro* (Figure 2B). Even though FAO is linked to redox homeostasis and ATP production in other cell types, the proliferation defect is not caused by redox imbalance or ATP depletion in ECs. However, a recent study documented that intracellular lipid droplets, formed upon loading with oleic acid, release fatty acids for energy production through FAO in ECs [14]. *In vivo*, genetic loss or pharmacological blockade of endothelial CPT1a reduced the number of branch points and diminished the radial expansion of the retinal vascular network in mice [4]. In

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accordance with the *in vitro* data, migratory properties were not affected, as evidenced by the normal number of filopodia [4]. Unlike PFKFB3-driven glycolysis, which promotes EC proliferation and migration, CPT1a-controlled FAO selectively stimulates EC proliferation [3,4].

Besides its importance in blood vascular ECs, CPT1a is also essential for lymphatic development [15]. Lymphatic ECs also rely on FAO to produce dNTPs to proliferate, similar to vascular ECs [15]. Notably, lymphatic EC metabolism, more specifically FAO, was shown to control the differentiation of lymphatic ECs through epigenetic mechanisms [15]. While it was known that the transcription factor PROX1 is essential to induce vascular to lymphatic EC differentiation, it was recently documented that PROX1 additionally stimulates FAO through binding to the *CPT1a* promoter, with a subsequent increase in *CPT1a* gene expression [15]. In fact, FAO-derived acetyl-CoA is used by the histone acetyltransferase p300 for histone acetylation at *PROX1*-binding sites, preferentially at lymphatic genes (e.g., *VEGFR3*), thereby inducing the proliferation, migration, and differentiation of lymphatic ECs. As such, PROX1 exploits FAO for epigenetic changes at lymphatic genes, making them more accessible to enhance its own transcriptional activity [15]. It is tempting to speculate that not only lymphatic ECs, but also vascular ECs use metabolic intermediates for the epigenetic modulation of angiogenesis, although this requires further study.

### Glutamine Metabolism Is Linked to Asparagine

ECs sustain proliferation and vascular expansion through the use of glutamine [16,17], the most abundant circulating nonessential amino acid (NEAA) [18]. In fact, of all amino acids, glutamine is the one consumed the most by ECs [16]. In proliferating ECs, glutamine serves as an anaplerotic source of carbons to replenish the TCA cycle via glutaminase 1 (GLS1) to support protein and nucleotide synthesis [16,17]. Accordingly, both protein and nucleotide synthesis were impaired upon glutamine depletion in ECs, mTOR activity was decreased, and endoplasmic reticulum (ER) stress marker genes were increased [16]. Furthermore, glutamine is used to produce glutathione (GSH) for redox homeostasis, and glutamine depletion made ECs more susceptible to ROS-induced damage [16,19]. However, reports differ on whether ECs utilize glutamine for ATP synthesis [16,17]. Also, ECs metabolize glutamine for citrate production by means of reductive carboxylation. In *in vitro* and *in vivo* models, interference with glutamine metabolism restrains angiogenesis by affecting EC proliferation [16,17] and migration [16]. The link between α-KG and asparagine appears to be particularly crucial, because angiogenesis, impaired by limitation of glutamine (or its metabolism), can be rescued by supplementation with α-KG plus asparagine [16], or with α-KG alone when asparagine is

Figure 2. Fatty Acids and Amino Acids Support Nucleotide and Protein Synthesis. (A) Proliferating endothelial cells (ECs) metabolize fatty acids to sustain the TCA cycle for nucleotide synthesis. Upon fatty acid uptake by FATPs, CPT1a transports fatty acids into mitochondria, where they are oxidized to acetyl-CoA, which is then used to sustain the TCA cycle, in conjunction with an anaplerotic substrate, to generate precursors of nucleotide synthesis.(B) ECs use the serine synthesis pathway (SSP), fueled by the glycolytic intermediate 3PG, to produce nucleotides. Serine is synthesized by PHGDH, converted to glycine, and contributes to one-carbon metabolism. Glutamine, a source of nitrogen for serine synthesis, replenishes the TCA cycle at the level of α-KG, which is used for fatty acid or nucleotide biosynthesis. Glutamine is also used for the production of glutathione for redox homeostasis. Impaired EC proliferation upon glutamine depletion is rescued by supplementation with α-KG plus asparagine, or with α-KG when asparagine is acquired via macropinocytosis. Given a role in suppressing ER stress and reactivation of mTOR signaling in other cell types, asparagine might also function as a metabolic regulator in ECs. Abbreviations: 1C, one-carbon; 3PG, 3-phosphoglycerate; α-KG, α-ketoglutarate; AA, amino acid; Ac-CoA, acetyl CoA; Asn, asparagine; ASNS, asparagine synthetase; Asp, aspartate; CPT1a, carnitine palmitoyltransferase 1a; dNTPs, deoxyribonucleotides; ER, endoplasmic reticulum; FA-CoA, fatty acid-CoA; FAO, fatty acid β-oxidation; FAs, fatty acids; FATP, fatty acid transporter; glc, glucose; GLS1, glutaminase 1; gly, glycine; mTOR, mammalian target of rapamycin; OAA, oxaloacetate; PHGDH, phosphoglycerate dehydrogenase; rNTPs, ribonucleotides; ser, serine; Slc1a5, solute carrier family 1 (neutral amino acid transporter), member 5; TCA, tricarboxylic acid cycle.

acquired from the extracellular milieu through macropinocytosis [17]. Notably, silencing of asparagine synthetase (ASNS), the enzyme producing asparagine from glutamine-derived nitrogen and aspartate, also impairs EC proliferation [16], highlighting the importance of asparagine, regardless of whether it is taken up from the culture medium or *de novo* synthesized. Interestingly, increasing evidence suggests a role of asparagine as a signaling metabolite that senses metabolic fuel reserves and availability, and accordingly coordinates cellular homeostatic responses [20] (Figure 2C). Studies with conditional knockout mice lacking the endothelial *ASNS* gene or *RAC1* gene (an essential component of the macropinocytosis pathway) will help to prove the relevance of these *in vitro* findings.

### Serine Metabolism

Serine can be not only taken up by ECs, but also *de novo* synthesized from the glycolytic intermediate 3-phosphoglycerate (3PG) in a three-step enzymatic reaction, controlled by the rate-limiting enzyme phosphoglycerate dehydrogenase (PHGDH) [21]. Of note, glutamine metabolism allies with glycolysis for serine synthesis, since the latter process uses nitrogen from glutamate [21]. Serine can be interconverted with glycine and is thereby connected to one-carbon metabolism for redox homeostasis and nucleotide synthesis [22] (Figure 2C). PHGDH is critical for ECs, because endothelial loss of PHGDH causes severe lethal vascular defects, due to impaired EC proliferation and survival, in part resulting from defective heme synthesis (S. Vandekeere and P. Carmeliet, unpublished).

### Alternative Metabolism of Glucose

ECs use additional metabolic pathways; however, their exact role is understudied in the context of angiogenesis, especially *in vivo*. For instance, glucose is not exclusively destined for glycolysis once phosphorylated by hexokinase (HK) to glucose-6-phosphate (G6P). Instead, ECs can use part of the G6P pool for glycogen storage through the use of glycogenic enzymes [23–25]. Glycogen could be particularly useful as an endogenous source of glucose when ECs are sprouting into glucose-deprived milieus. The fact that inhibition of glycogen phosphorylase (PYG), a rate-limiting enzyme for glycogen breakdown, impairs EC migration and viability supports this hypothesis [24]. However, the role of glycogenolysis for angiogenesis is still largely speculative.

G6P can also enter the oxidative branch of the pentose phosphate pathway (oxPPP) to generate ribulose-5-phosphate (Ru5P) and NADPH [26]. Ru5P is a precursor for ribose-5-phosphate (R5P), which is used for nucleotide biosynthesis [26]. In addition to other functions, NADPH is essential for restoring glutathione from its oxidized form, GSSG, to its reduced form, GSH, a key antioxidant [26]. The non-oxPPP branch connects to two intermediates of glycolysis (fructose-6-phosphate, F6P and glyceraldehyde-3-phosphate, G3P) and yields R5P, but not NADPH [26]. The inhibition of oxPPP or non-oxPPP, controlled by the rate-limiting enzymes G6P dehydrogenase (G6PD) and transketolase (TKT), respectively, leads to compromised EC viability and migration [24,27].

Another side branch of glycolysis, the hexosamine biosynthesis pathway (HBP), originates from F6P and produces UDP-*N*-acetylglucosamine (UDP-GlcNAc) for protein *N*- and *O*-glycosylation, using acetyl-CoA, ATP, glucose, glutamine, and uridine in the process [28]. Therefore, the HBP was suggested to function as a nutrient sensor that modulates angiogenesis, since the glycosylation status co-determines the functionality of angiogenic pathways, such as VEGFR2 and NOTCH1 [29,30].

## Metabolism of Tumor Endothelium

Blood vessels promote multiple inflammatory and malignant diseases. Emerging evidence suggests that the metabolism of ECs in these disorders is perturbed. For reasons of brevity, we focus here on cancer, and refer readers to Box 1 for other vascular disorders (including diabetes). Cancer cells stimulate angiogenesis to supply oxygen and nutrients. However, unlike in healthy tissue, blood vessels in tumors are disorganized, excessive in number, and dysfunctional [31]. Indeed, due to its abnormal development, the tumor vasculature lacks the organized hierarchy of a normal vascular bed and instead comprises a chaotic network of structurally and functionally defective vessels [32]. In addition, tumor vessels are leaky, tortuous, and have an uneven vessel lumen. Therefore, blood flow is arbitrary and sluggish, and the poor perfusion renders the tumor hypoxic, which, together with a leaky and sticky endothelial barrier, promotes metastatic escape of cancer cells [33,34] (Figure 3A). Initial peculiarities of tumor EC metabolism have begun to emerge.

### Tumor ECs Are Hyperglycolytic, yet Retain Functional Mitochondria

ECs in the tumor vasculature rely, even more than healthy ECs, on glycolysis for ATP production and display a hyperglycolytic phenotype, evidenced by enhanced expression of glucose transporter GLUT1 and the glycolytic activator PFKFB3 [35,36]. In addition, compared with healthy ECs, tumor ECs hyperactivate the pentose phosphate and serine biosynthesis pathways, used for nucleotide and biomass synthesis [35] (Figure 3B). Several signals in the tumor microenvironment (TME), including hypoxia and proinflammatory cytokines [35] or hormonal signaling [37], upregulate PKFBF3 [3]. These observations might have implications for cancer treatment. Indeed, lowering glycolysis in tumor ECs by restraining PFKFB3 function arrests their proliferation [3,38], decreases EC leakiness, tightens the vascular barrier, and restores perfusion, all signs of tumor vessel normalization [35] (Box 2). Importantly, the aim of such a therapeutic strategy is to lower glycolysis to the level detected in normal ECs, not to decrease it too much or completely eliminate glycolytic flux, because this induces EC death and tumor vessel disintegration, thereby facilitating cancer cell escape and metastasis [39]; thus, dosing PFKFB3-blocker therapy is essential.

### Box 1. Metabolic Changes in EC Metabolism in Other Diseases

In pulmonary arterial hypertension, pulmonary arterial ECs (PAECs) are hyperproliferative, similar to tumor ECs [76]. PAEC metabolism is characterized by increased glycolysis, fatty acid biosynthesis, and glutamine metabolism [76,77]. In other vascular diseases (such as atherosclerosis), nitric oxide (NO)-mediated vasoprotection is impaired due to its decreased production. NO is produced through conversion of arginine to citrulline by endothelial nitric oxide synthase (eNOS). Upregulation of arginase and asymmetric dimethylarginine (AMDA) in atherosclerosis can impair NO synthesis in ECs. Indeed, arginase metabolizes arginine to urea and ornithine, thereby depleting the arginine pool [78,79], while AMDA is a competitive inhibitor of eNOS [80]. In diabetes, the elevated circulating glucose levels increase ROS production in ECs through auto-oxidation of glucose, hyperglycemia-activated NADPH oxidases, and mitochondrial malfunctioning. ROS also cause eNOS uncoupling, thereby exacerbating oxidative stress due to the production of superoxide and $ONOO^-$. The oxidative stress induces oxidative damage in DNA, activation of polyADP-ribose polymerase-1 (PARP1) [81,82] and ultimately inhibition of the glycolytic enzyme GAPDH through ribosylation. Eventually, glycolytic intermediates accumulate upstream of GAPDH, triggering an increase in the polyol pathway flux, and in hexosamine biosynthetic and glycation pathways as an alternative to process the glucose overload. The polyol pathway consumes NADPH and produces sorbitol [83], thereby impairing ROS scavenging and increasing oxidative stress; sorbitol conversion to fructose yields 3-deoxyglucosone as its side product, which can also be formed from glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (accumulated glycolytic intermediates) together with methylglyoxal and glyoxal. These three aldehydes are highly reactive and disrupt the integrity of the endothelium through DNA and protein glycation [84–86], which leads to the formation of toxic advanced glycation end products. The negative impact of ROS in EC metabolism was also documented in ischemic heart disease, embolic events, aneurysms, and aorta dissection. ROS production results from compromised mitochondrial respiration in ischemia and embolic disease [87], and from the inflammatory process in aneurysm and aortic dissection [88].

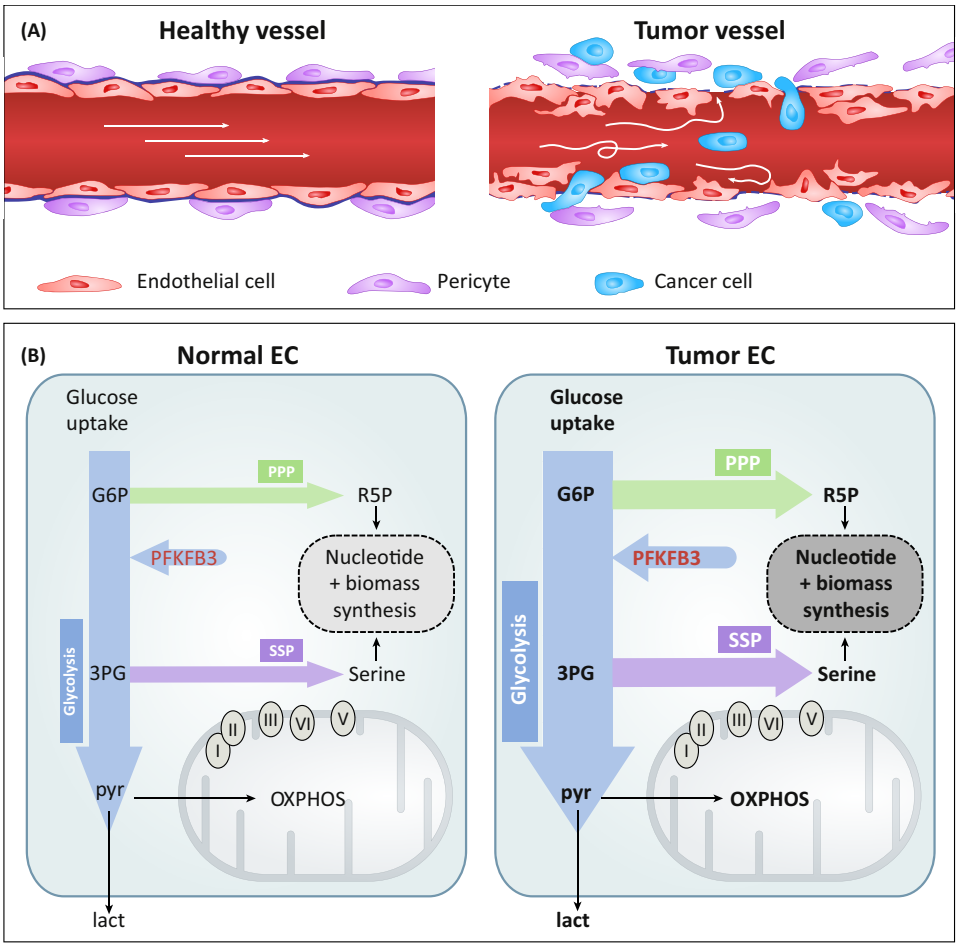


Figure 3. Metabolism of Tumor Endothelial Cells (ECs). (A) Normal endothelium contains tightly adhering and interconnected ECs and an intact basement membrane supported by perivascular pericytes. Tumor endothelium lacks this organized hierarchy and comprises structurally and functionally defective vessels with leaky ECs, disrupted basement membrane, and poor pericyte coverage, leading to perturbed blood flow and facilitating the infiltration of cancer cells. (B) Normal proliferating ECs exhibit a high glycolytic flux triggered by activation of PFKFB3. The pentose phosphate pathway (PPP) and serine synthesis pathway (SSP) provide R5P and serine, respectively, as precursors for nucleotide and biomass synthesis. Tumor ECs are hyperglycolytic and present hyperactivity of the PPP and SSP, thereby sustaining the proliferative phenotype. Both healthy and tumor ECs retain functional OXPHOS. Abbreviations: 3PG, 3-phosphoglycerate; G6P, glucose-6-phosphate; lact, lactate; OXPHOS, oxidative phosphorylation; PFKFB3, phosphofructokinase-2/fructose-2,6-bisphosphatase 3; pyr, pyruvate; R5P, ribose-5-phosphate.

While glycolysis is crucial, tumor ECs retain functional mitochondria [40] (Figure 3B). Active OXPHOS not only adds to the flexibility of utilizing other substrates for energy production, but also provides metabolites for biomass synthesis to support proliferation, which may be of particular importance for the hyperproliferative tumor ECs. Accordingly, inhibition of mitochondrial respiration induced death of proliferating tumor ECs, more than of quiescent ECs [41–45]. The proposed mechanisms of cell death upon inhibition of mitochondrial respiration relate to the generation of ROS and uncoupling of the mitochondrial membrane potential. Therefore, the role of mitochondria in tumor ECs may be underestimated at present and warrants further investigation.

Box 2. Tumor Vessel Normalization

Traditional antiangiogenic therapy focuses primarily on the inhibition of VEGF signaling to prevent new vessel formation, thereby starving cancer cells. This strategy, although promising in preclinical trials [89], suffers from resistance and insufficient efficacy in patients with cancer [90]. An emerging alternative paradigm, tumor vessel normalization [91], aims to heal the structurally and functionally abnormal tumor vessels, thereby improving perfusion (and, hence, drug delivery), reducing hypoxia (which improves immune function), and tightening the vascular barrier (which impairs metastasis) [92]. Normalization of tumor vessels can be achieved by multiple approaches, including combined Tie2 receptor activation/inhibition of angiopoietin 2 [93], chloroquine-induced activation of Notch1 signaling [94], and inhibition of angiotensin signaling [95]. This can also be achieved by inhibition of PFKFB3, which improves the efficacy of chemotherapy and reduces metastasis [96].

### Glutamine and Fatty Acids in Tumor ECs

As discussed above, glutamine is essential for proliferation of ECs *in vitro* and *in vivo* [16]. Glutamine metabolism in ECs is induced by TGF-β1 and RAF/MEK/ERK-signaling, pathways implicated in cancer progression [46]. Also, glutaminolysis is enhanced upon infection of ECs by Kaposi's sarcoma virus during the development of transformed lesions, and infected ECs become dependent on glutamine catabolism for their survival [47]. Such reports also suggest a role of glutamine metabolism in tumor ECs. Moreover, in contrast to cancer cells [48,49], the proliferation defect of ECs upon glutamine starvation cannot be rescued by supplementation with antioxidants or replenishment of the TCA cycle alone, implying that ECs rely differently and perhaps also more prominently on glutamine, and additionally asparagine [16]. However, the exact role of glutamine/asparagine metabolism in tumor ECs is unknown.

Fatty acids are used by proliferating ECs to sustain the TCA cycle for *de novo* nucleotide synthesis during proliferation. These findings could also have therapeutic implications. Indeed, blockade of FAO inhibits injury-induced (lymph)-angiogenesis [15]. While these data suggest a therapeutic potential for FAO blockers to suppress tumor growth and metastasis, such an effect remains to be studied. Moreover, inhibitors of fatty acid synthase (FASN) decrease the viability, proliferation, and migration of lymphatic ECs, thereby impairing cancer cell spread [50].

## Metabolic Crosstalk between Endothelium and Other Cell Types

While metabolism of individual cell types in the TME are a focus of intense research, how these various cell types communicate with each other via nutrient and metabolite exchange remains understudied. Cells in the TME experience selective pressure, in part due to the lack of nutrients and increased accumulation of metabolic waste products, caused to some extent by inefficient blood perfusion. These conditions alter the secretome of the cell, excreted directly or via exosomes [51].

ECs engage in metabolic crosstalk with both cancer and immune cells. An important factor in these interactions is local ischemia, determined by the position of a cell relative to the vasculature. This has been shown to affect the secretome of cancer cells, and to co-determine the differentiation of tumor-associated macrophages (TAMs), immune T cells, and ECs [52]. Also, cancer cells can prime ECs to proliferate by stimulating their glucose uptake [36], or by secreting metabolites, such as the TCA cycle intermediate succinate [53]. Another inducer of tumor EC proliferation is extracellular lactate, secreted in part by cancer cells [54] and cancer-associated fibroblasts [55]. Lactate acts as a signaling molecule and stimulates angiogenesis via enhancing VEGF signaling through the activation of HIF-1α [56,57] and the PI3K/AKT pathway [58]. Moreover, ECs can take up lactate for oxidation upon conversion to pyruvate, which stimulates angiogenesis via NF-κB/IL-8 signaling [59]. Lactate accumulation creates an acidic microenvironment, which causes ER stress in ECs and induces an inflammatory response [60].

ECs in the TME interact with several immune cell types, which may evoke both pro- or antiangiogenic responses. For instance, type 1 T helper cells enhance tumor vessel normalization by changing the cytokine milieu and enhancing pericyte recruitment [61]. By contrast, macrophages have a dual role in the TME; although they can eliminate tumor cells, TAMs also act as drivers of tumor progression by promoting the proliferation of cancer cells and angiogenesis, eliciting an immunosuppressive environment [62]. In the TME, TAMs undergo changes in the expression of several metabolic genes to cope with hypoxia [63]. REDD1, a negative regulator of the key nutrient sensor mTOR [64], was shown to regulate the shared use of glucose between TAMs and ECs [65]. Indeed, inhibition of mTOR by REDD1 downregulates glycolysis in TAMs, which results in increased TME levels of glucose that can then be utilized by tumor ECs and promotes angiogenesis. Conversely, deletion of REDD1 induces glycolysis in TAMs, resulting in metabolic competition for glucose between TAMs and tumor ECs, which leads to decreased angiogenesis, normalization of tumor vessels, and reduced metastatic spread [65] (Figure 4).

The metabolism of immune cells relies on the subtype and activation status [66]. Hence, the lack of certain nutrients in the TME influences immune cell metabolism, which subsequently promotes the angiogenic phenotype of ECs but impairs immune cell activity [67,68]. For instance, TAMs in the TME express high levels of not only VEGF (which supports the proliferation of ECs) but also arginase 1, which reduces the levels of arginine in the TME [63], thereby impairing the function of T cells [69]. The proangiogenic properties of TAMs are further dependent on glutamine synthetase. Inhibition of glutamine synthesis in TAMs decreases glutamine in the TME, leading to tumor vessel normalization and metastasis reduction [70]. By contrast, low glutamine levels in the TME, caused by cancer cells that switch to glutamine metabolism in response to acidification [71], negatively impact activated lymphocytes in the TME, which rely on glutamine as an energy substrate [72].

The network of metabolic interactions in a tumor becomes even more complex upon anticancer treatment, leading to compensatory mechanisms. For instance, glycolytic cancer cells in

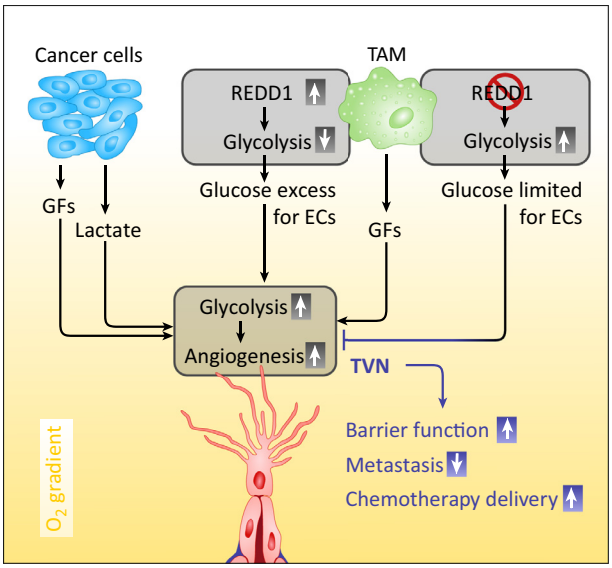


Figure 4. Metabolic Crosstalk of Endothelial Cells (ECs) in the Tumor Microenvironment. ECs and tumor blood vessels have a central role in establishing the tumor microenvironment and metabolic crosstalk of individual cell types. Accelerated angiogenesis of tumor ECs is stimulated by growth factors and lactate produced by cancer cells in poorly oxygenated areas. Tumor-associated macrophages (TAMs) in hypoxic regions upregulate the expression of REDD1, which hinders glycolysis in TAMs, thereby providing glucose for ECs. By contrast, blocking REDD1 increases glucose consumption by TAMs, leading to competition with ECs for glucose. Lower glucose availability for tumor ECs results in a less-hyperglycolytic phenotype and tumor vessel normalization. Abbreviations: GFs, growth factors; REDD1, regulated in development and DNA damage response 1; TVN, tumor vessel normalization.

hypoxic regions consume glucose and produce lactate, which is then used as substrate to fuel oxidative metabolism of neighboring cancer cells in the vicinity of blood vessels [73]. This metabolic symbiosis may cause resistance to antiangiogenic therapy, whereby cancer cells in more hypoxic regions feed cancer cells in more oxidative areas [74,75]. As another example, the antitumor effect of mTOR inhibitors is partially hindered by their effect on TAMs, as discussed above. Indeed, while inhibition of mTOR has antitumoral effects on cancer cells, it enhances the protumorigenic properties of TAMs by stimulating angiogenesis. Thus, simultaneous targeting of complementary pathways may improve the overall efficacy of therapeutic targeting of metabolism, but concerns about safety for such combination strategies will need to be addressed.

## Concluding Remarks

EC metabolism is a key regulator of angiogenesis, and represents an attractive therapeutic target. Although overlooked for decades, substantial perturbations of the metabolism of tumor ECs and of the endothelium in other vascular diseases were recently documented. These differences between normal and diseased ECs might offer novel therapeutic opportunities for improved antiangiogenesis medication. Another level of opportunity relates to targeting metabolic crosstalk between ECs, cancer cells, and other stromal cells. The variety of metabolites in the environment, originating from several cell types, their integration for signaling, and functional outcome are yet to be fully determined. State-of-the-art technologies, such as single cell transcriptomics and metabolomics profiling, promise to yield exciting insights for future therapeutic approaches (see Outstanding Questions).

### Author Contributions

All authors prepared and revised the manuscript, and approved the final version; K.R. and K.V. prepared the figures.

### Disclaimer Statement

P.C. is named as an inventor on patent applications related to the results described in this manuscript.

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## Outstanding Questions

Which other metabolic pathways are important for vessel sprouting?

In addition to vessel sprouting, other modes of vessel growth exist; are the same or different metabolic pathways involved?

ECs are a heterogeneous population of tip, stalk, and phalanx cells: does their metabolic signature differ, and how?

Arterial, venous, capillary, and lymphatic ECs in different organs differ from each other phenotypically, functionally, and morphologically; does their metabolism also differ?

Tumor ECs are hyperglycolytic, but which other metabolic pathways are perturbed?

Tumor ECs are heterogeneous; can their metabolic signature be profiled by single cell RNA sequencing and analysis of metabolic genes?

ECs become dysfunctional in diabetes; which other metabolic perturbations underlie EC dysfunction and can they be targeted?

Computational genome-scale metabolic modeling (GEM) allows one to predict metabolic candidates driving EC biology; will the development of EC-tailored GEMs accelerate the discovery of EC metabolic targets?

Will it be feasible to develop effective, yet safe antiangiogenic therapies based on targeting metabolic candidates, and will adequate dosing be possible?

Can other metabolism targeting-based strategies be developed to induce tumor vessel normalization?

Can the manipulation of metabolism or delivery of metabolites be used to stimulate (lymph)-angiogenesis for regenerative medicine or treatment of ischemic disease?

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