The Link Between Angiogenesis and Endothelial Metabolism

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Abstract

Angiogenesis has traditionally been viewed from the perspective of how endothelial cells (ECs) coordinate migration and proliferation in response to growth factor activation to form new vessel branches. However, ECs must also coordinate their metabolism and adapt metabolic fluxes to the rising energy and biomass demands of branching vessels. Recent studies have highlighted the importance of such metabolic regulation in the endothelium and uncovered core metabolic pathways and mechanisms of regulation that drive the angiogenic process. In this review, we discuss our current understanding of EC metabolism, how it intersects with angiogenic signal transduction, and how alterations in metabolic pathways affect vessel morphogenesis. Understanding EC metabolism promises to reveal new perspectives on disease mechanisms in the vascular system with therapeutic implications for disorders with aberrant vessel growth and function.

INTRODUCTION

EC: endothelial cell

Tip cell: a motile EC at the tip of a growing sprout that extends sensory filopodia toward the source of the angiogenic signal

Stalk cell: a proliferating EC that follows the tip cell and forms the base of the angiogenic sprout

The vascular system is a highly branched network lined by endothelial cells (ECs) that supplies tissues with oxygen (O_2) and nutrients. The ability to expand this network (angiogenesis) in response to changing metabolic demands is vital for organ growth and function in health and disease. A reduction in O_2 tension or nutrient availability is a primary stimulus for angiogenesis, which prompts ECs to break out of their quiescent lining and form new connections. A main focus of research has been the understanding of mechanisms that induce such proangiogenic behavior, work that has led to the identification of signaling pathways controlling EC migration and proliferation (1–3).

However, the adoption of an angiogenic phenotype has significant consequences for EC metabolism, a link that is widely overlooked. This notion stems from the premise that ECs have to meet the bioenergetic and biomass demands of cell migration and proliferation when they switch from quiescence to growth (4, 5). Angiogenic ECs require nutrients and energy not only for their motile behavior but also for biosynthesis of building blocks (proteins, nucleotides, and lipids) for cell duplication. Hence, during angiogenesis, ECs must increase their metabolic activity to generate energy quickly and to facilitate the incorporation of nutrients into biomass. A heightened metabolic state, however, poses a challenge for angiogenic ECs, as it builds vessels in tissues that are hypoxic and nutrient starved. It is therefore fundamental for ECs to have a metabolic machinery that supports vessel formation under varying environmental conditions. In this review, we focus on the metabolism of ECs and highlight its importance for normal vessel growth and function. We consider EC metabolism from a cell biological perspective by explaining the metabolic requirements of branching vessels and by discussing the cellular consequences of altering metabolic pathways in ECs. We also discuss how ECs sense changes in their metabolic microenvironment and how changes in EC metabolism can contribute to disease.

ANGIOGENESIS AND ITS METABOLIC REQUIREMENTS

Vessel growth can occur via different mechanisms, dependent on the vascular bed, physiological context, or whether it is driven by an aberrant tissue response in disease (1–3). In the embryo, the first vessels form by the differentiation and assembly of mesoderm-derived precursor cells (angioblasts). Subsequent vessel sprouting and splitting expand this primitive plexus and create a network of arteries, capillaries, and veins (1–3). Angiogenesis is often used as an umbrella term for these different types of vessel growth but refers in its classic meaning to the sprouting of vessel branches from existing ones.

Angiogenic Sprouting and Branching: The Model

Vessel growth by sprouting is a critical mechanism for vascular expansion in development and tissue regeneration and repair but also in disease states such as cancer or inflammation (3, 6). This dynamic process involves multiple interactions between ECs and their (metabolic) microenvironment (**Figure 1*a***). Indeed, tissues in need of O_2 and nutrients secrete proangiogenic molecules, which trigger ECs to become invasive and protrude filopodia (**Figure 1*b***). These tip cells lead the sprouts and extend their filopodia toward the source of the angiogenic signal (7). Tip cells are followed by stalk cells, which proliferate to elongate the sprout (8, 9). Specification in migratory tip and proliferating stalk cells is dynamic, and ECs continuously compete for the lead position (10). Eventually, tip cells connect with tip cells from adjacent sprouts to establish new vessel circuits (**Figure 1*c***). The sprouting process continues until nutrient and O_2 supply meets

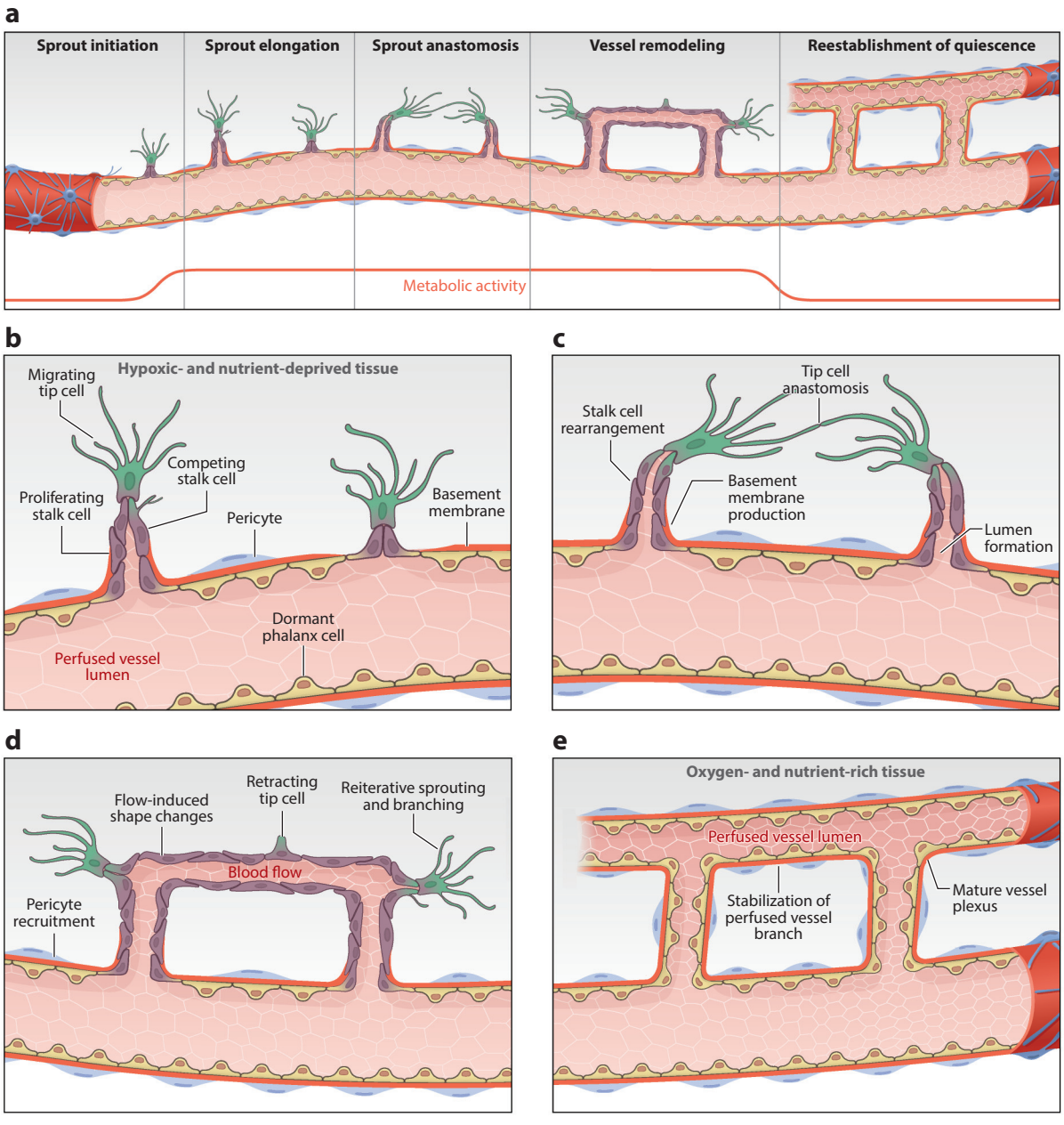


Figure 1

The cellular steps of angiogenic sprouting and branching. (*a*) The consecutive steps of sprouting angiogenesis conceptualized as sprout initiation, sprout elongation, sprout anastomosis, vessel remodeling, and the reestablishment of vascular quiescence, which are separated by a fine gray line. The red line at the bottom of the scheme indicates the relative metabolic activity of endothelial cells when they switch from quiescence to angiogenic growth and back again. (*b–e*) High-magnification illustrations of the cellular events taking place during (*b*) sprout initiation and elongation, (*c*) sprout anastomosis, (*d*) vessel remodeling, and (*e*) the reestablishment of vessel quiescence.

tissue demand, proangiogenic factors are silenced, and ECs become quiescent again (**Figure 1*d*,*e***). The establishment of a basement matrix, the recruitment of pericytes, and the onset of blood flow consolidate the quiescent endothelial phenotype, which has a cobblestone shape and tight monolayer organization (**Figure 1*e***). Because of its resemblance to a Spartan military formation, this phenotype has been named a phalanx cell (11).

A principal driver of sprouting is vascular endothelial growth factor (VEGF). VEGF is released by hypoxic tissues and binds to VEGF receptor 2 (VEGFR2) expressed by ECs (12, 13). VEGFR2 activation induces a myriad of signaling cascades that promote migration, proliferation, and tip cell formation. ECs with high VEGFR2 signaling become tip cells and instruct neighboring ECs to adopt a stalk cell phenotype by upregulating the Notch ligand Delta-like 4 (DLL4). DLL4 induces stalk cell behavior by activating the NOTCH1 receptor, which in turn leads to suppression of VEGFR2 and the concomitant induction of VEGFR1, a VEGF trap. The reciprocal regulation of VEGFR expression by Notch signaling reduces sensitivity to VEGF and thereby enforces stalk cell specification (14). The levels of VEGFRs, DLL4, and NOTCH1 are, however, constantly changing as ECs meet new neighbors. As a result, stalk cells can be relieved from tip cell inhibition and overtake the lead position, resulting in a dynamic position shuffle in the growing sprout (14). The integrated regulation of VEGF and Notch is a prime example of a mechanism that allows ECs to sprout reiteratively in a concerted action, thereby ensuring robust network formation (14). Besides these core pathways, other signaling cascades contribute to angiogenic sprouting; this topic is reviewed elsewhere (1, 2, 6).

Phalanx cell: quiescent EC that aligns in a tight cobblestone monolayer, similar to the phalanx formation of ancient Greek soldiers

ATP: adenosine triphosphate

NADPH/NADP$^+$: oxidized and reduced form of nicotinamide adenine dinucleotide phosphate

Glycolysis: the metabolic pathway that converts glucose into pyruvate to produce ATP (adenosine triphosphate) and NADH (reduced nicotinamide adenine dinucleotide)

OXPHOS: oxidative phosphorylation

Metabolic Demands of Angiogenic Growth

Endothelial sprouting requires not only a coordination of morphogenetic behaviors but also an adjustment of metabolic activities. This is because the switch from quiescence to growth is metabolically demanding (4, 5) (**Figure 1*a***). Unlike phalanx cells, which rest in the vessels' interior as dormant cells, tip cells require energy for migration and their continuous battle to stay at the tip (**Figure 2*a***). In contrast, stalk cells need to synthesize all cellular components for proliferation and growth, including DNA, RNA, proteins, and lipids (4, 5). In addition to adenosine triphosphate (ATP), these cells need carbons (e.g., from glucose, fatty acids, or amino acids), nitrogens (e.g., from glutamine), and reducing agents (e.g., NADPH) to fuel macromolecular biosynthesis (15) (**Figure 2*a***). To meet these demands, ECs must shift from a metabolic state that maintains basal functions to a state of increased energy and biomass production. However, this shift presents a challenge for ECs as they form new vessels in conditions of tissue hypoxia and starvation. Most parenchymal cells become inactive or undergo demise in such environments, but ECs still manage to grow, divide, and migrate. This implies that ECs wire their metabolism differently from that of the tissue they supply.

ENDOTHELIAL METABOLISM

Metabolic activities in ECs are indeed different from those in other differentiated cells, whose cellular bioenergetics center around oxidative mitochondrial metabolism (4, 5). Neurons, for instance, break down glucose to pyruvate via glycolysis and then direct most of the pyruvate into mitochondria to generate ATP via oxidative phosphorylation (OXPHOS) (16). Because O_2 is required as an electron acceptor during OXPHOS, O_2 is essential for this process. Other cell types, such as cardiomyocytes, primarily rely on fatty acids (FAs), which they oxidatively metabolize in mitochondria to produce ATP (17). It is only under anaerobic conditions that these cells switch to glycolysis as the primary source of ATP production (18). In contrast, ECs are highly glycolytic

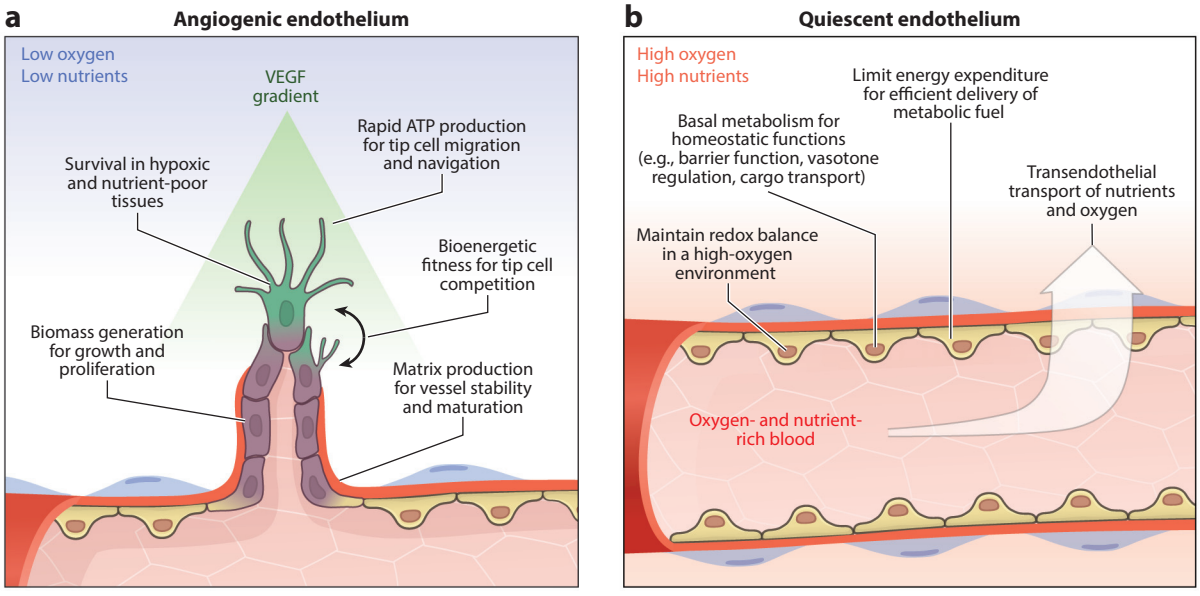


Figure 2

Metabolic challenges in the growing and resting vasculature. (*a*) Angiogenic ECs are confronted with numerous metabolic challenges when they sprout into O_2- and nutrient-depleted tissues. Angiogenic ECs follow a gradient of VEGF (*light green*) that is built up in the malnourished tissue. Activated by VEGF, ECs migrate, shuffle positions, and proliferate, all of which are energy (ATP) demanding. Angiogenic ECs also need substantial biosynthetic activity to produce the biomass (RNA, DNA, proteins, and lipids) required for sprout elongation, expansion, and matrix production. (*b*) Quiescent ECs rest in well-perfused vessels and deliver O_2 and nutrients for the oxidative metabolism of the perivascular tissue. These metabolic fuels are transported across the endothelium. To sustain its homeostatic functions, the quiescent endothelium needs basal metabolic activity. The basal metabolism must be kept in check to prevent excessive nutrient and O_2 consumption that would make the delivery process less efficient. The high O_2 levels in the blood stream also force ECs to maintain redox balance. Abbreviations: ATP, adenosine triphosphate; EC, endothelial cell; O_2, molecular oxygen; VEGF, vascular endothelial growth factor.

and produce large amounts of lactate even in the presence of ample O_2 (sufficient for oxidative glucose metabolism), a phenomenon called aerobic glycolysis (18).

Endothelial Cells Are Highly Glycolytic

Aerobic glycolysis is an inefficient way of generating ATP (2 mol of ATP per mole of glucose versus 36 mol of ATP upon glucose oxidation), yet ECs generate up to 85% of their ATP through this pathway (19–21) (**Figure 3**). When ECs switch from quiescence to vessel branching, glycolysis is further accelerated (21). Proangiogenic molecules such as VEGF enhance glycolysis by increasing glucose uptake and driving expression of glycolysis activators such as phosphofructokinase-2/fructose-2,6-bisphosphatase 3 (PFKFB3) (21) (**Figure 3**). As a result, ECs rely on glucose as a substrate for ATP generation and die when deprived of glucose or when treated with the glucose analog 2-deoxy-D-glucose (22–24). In this regard, ECs resemble cancer cells, which thrive on high rates of aerobic glycolysis and expend large amounts of glucose to satisfy their bioenergetic needs (18, 25, 26). Interestingly, the rate of glycolysis differs in EC subtypes (arterial, microvascular, and venous). Arterial ECs are more oxidative, whereas microvascular ECs are more glycolytic and more proliferative (21, 27). Glycolysis levels in ECs are also affected by disease. In Kaposi's sarcoma, a highly vascularized tumor of ECs, the ECs are even more glycolytic (28).

PFKFB3: bifunctional enzyme involved in both the synthesis and degradation of fructose-2, 6-bisphosphate, a regulatory molecule that controls glycolysis

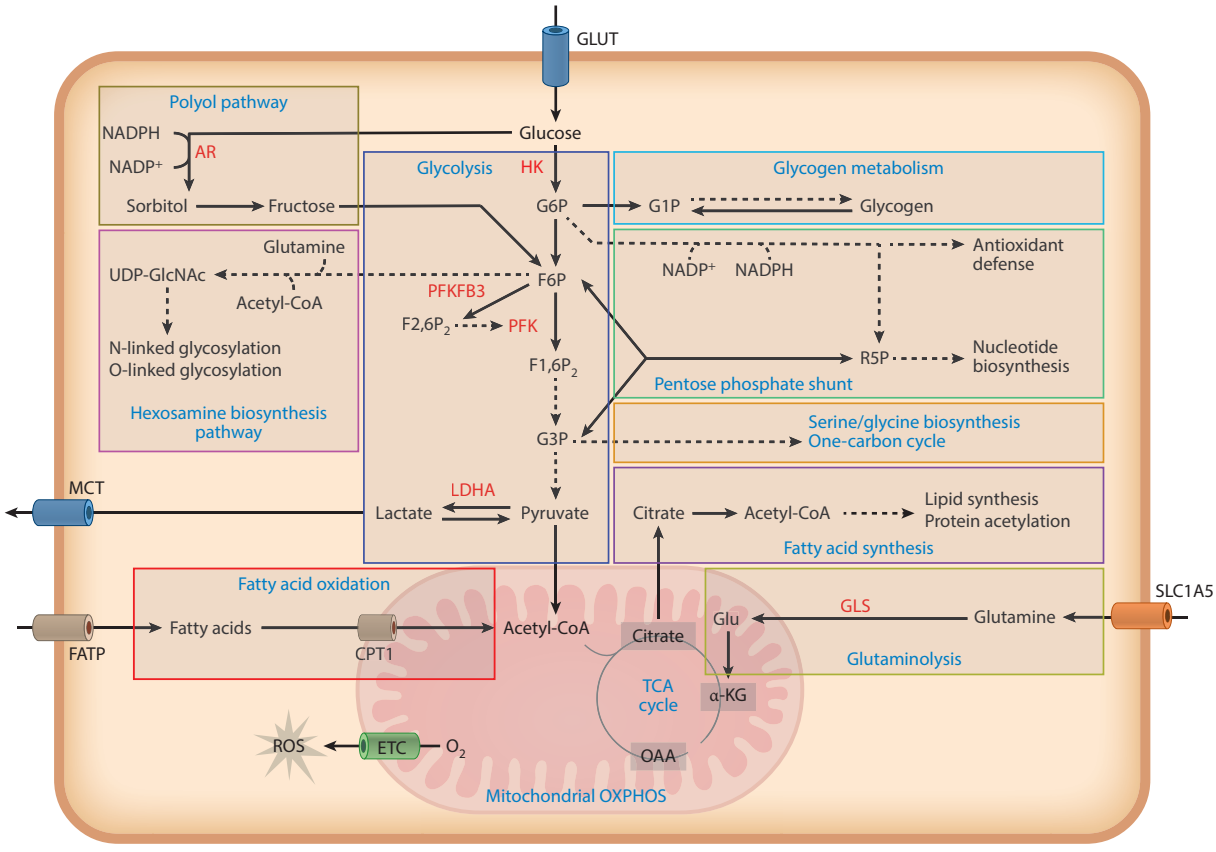


Figure 3

A conceptualized overview of endothelial metabolism, which highlights metabolic pathways (*boxes*) and enzymes (*red characters*) discussed in this review. Names of the individual pathways are highlighted in blue characters. Dashed arrows indicate that additional intermediate reactions are involved in a metabolic pathway. For clarity, these intermediate steps are not depicted. Abbreviations: α-KG, α-ketoglutarate; AR, aldolase reductase; CPT1, carnitine palmitoyltransferase 1; EC, endothelial cell; ETC, electron transport chain; $F1,6P_2$, fructose-1,6-bisphosphate; F6P, fructose-6-phosphate; FATP, fatty acid transport protein; G1P, glucose-1-phosphate; G3P, 3-phosphoglycerate; G6P, glucose-6-phosphate; GLS, glutaminase; Glu, glutamate, GLUT, glucose transporter; HK, hexokinase; LDHA, lactate dehydrogenase A; MCT, monocarboxylate transporter; $NADP^+$, oxidized nicotinamide adenine dinucleotide phosphate; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; O_2, molecular oxygen; OXPHOS, oxidative phosphorylation; PFK, phosphofructokinase; PFKFB3, fructose-2,6-bisphosphate isoform 3; R5P, ribose-5-phosphate; ROS, reactive oxygen species; SLC1A5, amino acid transporter; TCA, tricarboxylic acid cycle; UDP-GlcNAc, uridine diphosphate-N-acetylglucosamine.

Overall, aerobic glycolysis is dynamically regulated, and tissue environment and function are key determinants of endothelial metabolic activity.

Why Glycolysis?

Considering that ECs have immediate access to O_2 in the blood stream, the preferential use of glycolysis appears wasteful. This raises the question of why ECs use this less efficient metabolism. One explanation is that glycolysis allows ECs to use the most abundant extracellular nutrient, glucose, to produce ATP (15, 29). Although the energetic yield per mole is low, glycolysis can

produce ATP faster and at greater quantities if glucose uptake and glycolytic flux are accelerated (15, 18, 29). The fast kinetics of glycolysis is also beneficial for the sprouting behavior of ECs, as they need a rapid ATP supply when competing for the tip cell position. Glycolysis also makes ECs more hypoxia resistant. This is because ECs can use glycolysis anaerobically as long as glucose is available. ECs become sensitive to O_2 deprivation only when glucose is limiting. The use of glycolysis as a primary energy source thus seems like a bona fide adaptation for a cell that forms new vessels in hypoxic tissues. Interestingly, ECs can store glucose intracellularly as glycogen (30, 31) (**Figure 3**), raising the possibility that ECs use this endogenous source to sprout into avascular tissues. However, the relevance of glycogen breakdown for sprouting is unclear.

Another possible explanation is that glycolysis facilitates EC functions that go beyond energy production. For instance, a key task is to supply O_2 to energy-consuming tissues (e.g., brain, heart). Glycolysis might aid this function, as O_2 delivery can be maximized when ECs use little O_2 for their homeostatic needs. Crucially, glycolysis is also a source of metabolites that are needed for biosynthetic pathways (**Figure 3**). For instance, diverting glucose into the oxidative or nonoxidative arms of the pentose phosphate pathway (PPP) produces ribose-5-phosphate (R5P), a key intermediate in nucleotide biosynthesis (15, 18). The oxidative PPP arm also generates NADPH, which supplies reducing equivalents for nucleotide and FA biosynthesis and redox control (15, 18). The PPP is relevant for ECs because inhibition of its rate-limiting enzymes reduces EC viability (31, 32). Glycolytic intermediates are also channeled into the hexosamine biosynthesis pathway (HBP), another side branch of glycolysis, which generates N-acetylglucosamine for O- and N-glycosylation of proteins. Interestingly, the functionality of VEGFR2 and NOTCH1 depends on their glycosylation status, suggesting that glycolysis codirects angiogenic signal transduction (33, 34). Finally, the glycolytic intermediate 3-phosphoglycerate (G3P) is metabolized to produce serine and glycine, which in turn can contribute to pools of nonessential amino acids to fuel protein synthesis and supply one-carbon metabolism for nucleotide synthesis and other metabolic reactions (35) (**Figure 3**). Thus, glycolysis provides a metabolic platform that allows ECs to perform diverse roles in the growing and resting vasculature.

PPP: pentose phosphate pathway, a side branch of glycolysis that generates NADPH and precursors for nucleotide synthesis

HBP: hexosamine biosynthetic pathway, a side path of glycolysis that produces substrates for protein glycosylation and the synthesis of glycolipids and proteoglycans

TCA cycle: tricarboxylic acid (Krebs) cycle; a series of chemical reactions used by aerobic organisms to generate energy through the oxidation of acetyl-CoA

Mitochondrial Metabolism in the Endothelium

The observation that ECs do not maximize ATP production via OXPHOS (20, 21) suggests that ECs do not depend on mitochondrial function for energy generation. Indeed, ECs have fewer mitochondria and consume lower amounts of O_2 than other cell types (36). In the presence of physiological glucose levels, only a minor fraction of pyruvate enters the tricarboxylic acid (TCA) cycle (21). Nonetheless, ECs retain their capacity for oxidative metabolism when glycolysis is compromised or under conditions of stress (19, 37). The relevance of OXPHOS for ECs may also depend on tissue specialization and function. For instance, ECs of the blood-brain barrier have more mitochondria (38) and upregulate TCA cycle genes in response to flow (39), likely to enable the energy-consuming transfer of cargo across the blood-brain barrier. Importantly, mitochondria have functions other than the generation of ATP, such as biomass generation and signaling.

Mitochondria As Biosynthetic Hubs

The TCA cycle is a source of precursors for the synthesis of lipids, proteins, and nucleotides. The consumption of TCA cycle intermediates for biosynthesis is important for highly proliferating cells, which generate the majority of these macromolecular components de novo rather than scavenging them from the environment (15). Hence, proliferating cells shunt much of the carbon that enters mitochondria into pathways that consume rather than produce ATP. For instance,

the fraction of pyruvate not converted to lactate enters the TCA cycle to generate a source of acetyl-CoA, which is required for lipid synthesis and protein acetylation (**Figure 3**). This process is called cataplerosis and also applies to other TCA cycle intermediates, such as oxaloacetate (OAA) and α-ketoglutarate (α-KG), which supply intracellular pools of nonessential amino acids for nucleotide and protein synthesis. Using the TCA cycle for biomass generation therefore allows cells to meet the biosynthetic demands of cell growth and proliferation.

Cataplerosis: extrusion of intermediate metabolites from the citric acid cycle for biosynthetic reactions

CPT1: carnitine palmitoyltransferase 1, a key enzyme in the carnitine-dependent movement of fatty acids into the mitochondrial matrix

Anaplerosis: the process of replenishing TCA cycle intermediates that have been extracted for biosynthetic reaction

Glutaminolysis: a series of biochemical reactions by which the amino acid glutamine is catabolized to glutamate, aspartate, CO_2, pyruvate, lactate, alanine, and citrate

In ECs, mitochondria are also used for biosynthetic purposes. Carbons derived from fatty acid β-oxidation (FAO) enter the TCA cycle and are used for the de novo synthesis of nucleotides during DNA replication (40). Carnitine palmitoyltransferase 1 (CPT1), a rate-limiting enzyme of FAO, shuttles long-chain FAs into mitochondria where they become metabolized to acetyl-CoA (**Figure 3**). The acetyl-CoA-derived carbons are taken up into the TCA cycle and are incorporated into aspartate (a nucleotide precursor), uridine monophosphate (a pyrimidine nucleoside triphosphate precursor), and DNA. FAs do not anaplerotically replenish carbons to the TCA cycle, as for every two carbons of FA-derived acetyl-CoA that enter the TCA cycle, two carbons exit the cycle as CO_2; thus, ECs rely on other carbon sources for continued TCA cycling (see below). Nevertheless, FAO inhibition depletes deoxyribonucleoside triphosphate stores in ECs and inhibits their proliferation (40). The use of FA-derived carbons for nucleotide biosynthesis distinguishes ECs from other proliferating cells (including cancer cells), which use glucose and glutamine for this purpose (41). However, given the higher carbon content of FAs, the breakdown of FAs for biomass production might prove more efficient. It might also allow ECs to maintain nucleotide synthesis in nutrient-poor environments because they can catabolize FAs from endogenous lipids, though this hypothesis remains speculative.

To grow and divide, ECs also must synthesize FAs and other lipids, which also involves TCA cycle-derived intermediates. The production of cholesterol, isoprenoids, and FAs is crucial for the formation of new membranes, the generation of signaling molecules, and the modulation of cellular signaling (42). Lipid synthesis requires the transfer of TCA cycle-derived citrate to the cytosol where it is converted to oxaloacetate and acetyl-CoA (**Figure 3**). Acetyl-CoA is used for FA production by fatty acid synthetase (FASN), but it can also be used for cholesterol synthesis. FASN ablation impairs capillary formation and pathological angiogenesis (43–45).

However, the use of mitochondria for biosynthesis brings about the risk of losing mitochondrial integrity as TCA cycle intermediates get exhausted. To prevent mitochondrial disintegration and cell death, these intermediates must be replenished, a phenomenon called anaplerosis (15). The nonessential amino acid glutamine is a major contributor to anaplerosis. Glutamine can be converted to glutamate (Glu) and α-KG, thereby contributing its carbons to the TCA cycle (glutaminolysis) (46) (**Figure 3**). Moreover, glutamine is an important source of nitrogen required for nucleotide biosynthesis (46). ECs also consume glutamine and have high glutaminase (GLS1) activity (47, 48). GLS1 catalyzes the conversion of glutamine to glutamate, the rate-limiting step of glutaminolysis. GLS1 is upregulated in sprouting ECs in vivo (49), and its blockade leads to a senescence-like state in ECs in vitro (50). Besides the replenishment of the TCA cycle, glutamine catabolism can also be used for ATP production, biosynthesis of nonessential amino acids and glutathione (GSH), and reductive carboxylation for lipid synthesis (35, 46). Whether and how these different routes of glutamine metabolism contribute to vessel growth remain to be determined.

Endothelial Mitochondria and Redox Signaling

Endothelial mitochondria are also a source of reactive oxygen species (ROS), produced at a low level by the electron transport chain (ETC) as a byproduct of oxidative metabolism (**Figure 3**). ROS are formed during a one-electron reduction of O_2 resulting in the formation of superoxide

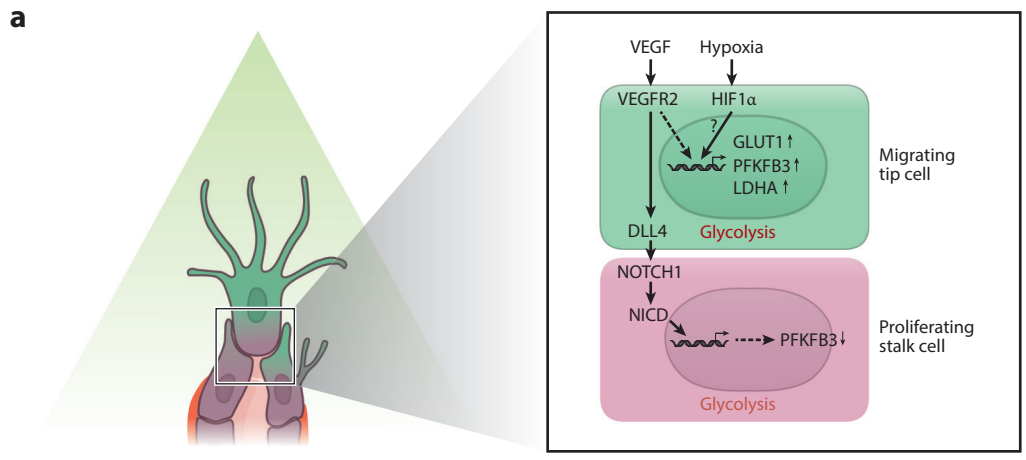
anion ($O2\bullet^-$), the primary ROS produced in mitochondria. Superoxide is converted to hydrogen peroxide (H_2O_2) by superoxide dismutases in mitochondria and cytosol (51). These low-level ROS play a physiological role in the regulation of cellular signaling (51) and were shown to promote proangiogenic signal transduction. Apart from mechanical forces (52), VEGF stimulates mitochondrial metabolism in cultured ECs and leads to an increase in ROS levels (53). Mitochondrial ROS also promote EC migration in response to proangiogenic stimulation (54, 55). At the molecular level, ROS enhance VEGFR2 activation (56) while blocking O_2-sensing prolyl hydroxylase domain proteins (PHDs). The ROS-dependent inhibition of PHDs results in the activation of hypoxia-inducible factors (HIFs), mediating a transcriptional program that promotes angiogenesis (57). However, ROS levels can rise as a consequence of changes in nutrient and O_2 availability or other stresses causing cellular damage and death (51, 58). The threshold at which ROS become pathological is less clear (58). Sensitivity to ROS varies depending on the environmental context and location of production, and different species of ROS can lead to different cellular outcomes. For instance, ROS generated by hyperglycemia in diabetes promotes ligand-independent but SRC-dependent phosphorylation of VEGFR2. VEGFR2 phosphorylation dampens sensitivity to VEGF and weakens its proangiogenic activity (59). Increased ROS in diabetes also induce endothelial expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α), rendering ECs unresponsive to growth signals (60). Interestingly, these effects are independent of the well-established role of PGC1-α in mitochondrial biogenesis but involve the regulation of Notch and other signaling pathways. Finally, mitochondria are a pivotal component of the apoptosis machinery (3, 61, 62), which contributes to vessel injury and can reduce vessel density or provoke regression in certain conditions (63). An example is the inhibition of autocrine VEGF signaling in ECs, necessary for endothelial survival. Depletion of endothelial VEGF causes mitochondrial fragmentation and metabolic collapse, leading to excessive autophagy and cell death (64). Together, these considerations highlight the important biosynthetic and signaling functions of mitochondria in ECs. However, more work is needed to understand their specific roles in ECs.

REGULATION OF ANGIOGENESIS BY ENDOTHELIAL METABOLISM

In the adult, most ECs are quiescent for extended periods but rapidly switch to growth upon proangiogenic stimulation. Signaling pathways that control this angiogenic switch also control EC metabolism, eliciting a metabolic state permissive for growth and proliferation (4, 21). Interfering with these metabolic adaptions renders ECs less sensitive to angiogenic signals (4, 5, 65).

Metabolic Regulation of Tip and Stalk Cell Behavior

VEGF signaling rewires metabolic activities in ECs: Both the uptake and breakdown of glucose are stimulated by VEGF, resulting in a doubling of glycolysis. At the molecular level, VEGF enhances the expression of their primary glucose transporter, glucose transporter type 1 (GLUT1), and of glycolytic enzymes, such as lactate dehydrogenase A (LDHA) and PFKFB3 (21, 27, 66, 67) (**Figure 4*a***). A similar induction of glycolysis genes is evoked by hypoxia and involves activation of HIF1α (68–70). An example is the O_2-dependent regulation of PFKFB3, whose promoter is activated by HIF1α binding (21, 69, 70) (**Figure 4**). PFKFB3 activates glycolysis by producing fructose-2,6-bisphosphate, which allosterically activates phosphofructokinase 1 (PFK1), itself a rate-limiting enzyme of glycolysis, thereby fueling glycolytic flux. Inhibition of PFKFB3 moderately reduces glycolysis in ECs (by 35–40%). Nevertheless, this is sufficient to compromise vessel sprouting, highlighting the sensitivity of angiogenic ECs toward changes in glycolysis (21). At the cellular level, PFKFB3-driven glycolysis is particularly important for tip cell migration and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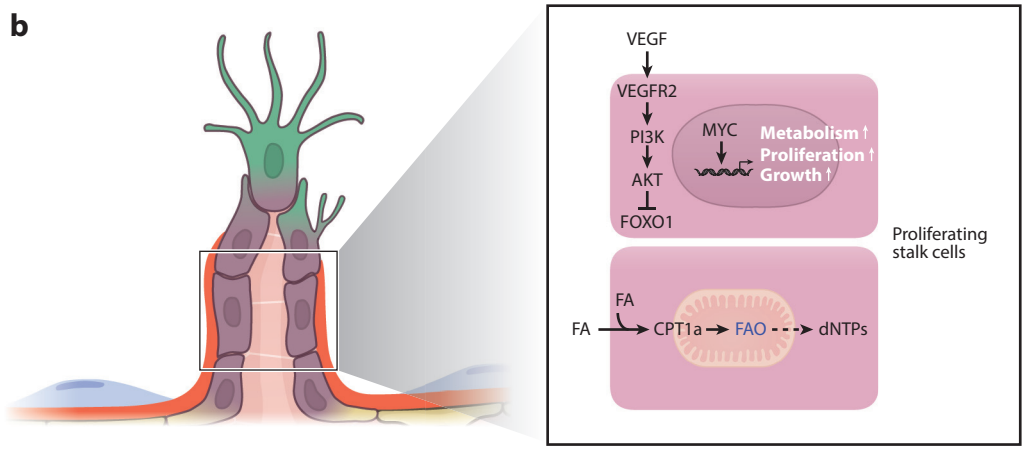
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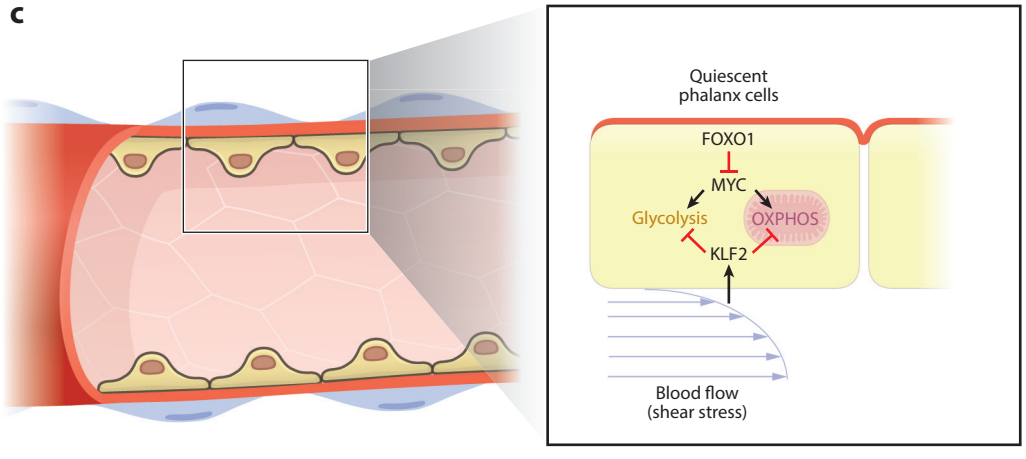
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Quiescent phalanx cells
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Glycolysis
OXPHOS
KLF2
Blood flow (shear stress)

competition by providing energy for cytoskeletal remodeling during filopodia and lamellipodia formation. PFKFB3 blockade reduces the propensity of ECs to become or remain tip cells when competing against ECs with normal PFKFB3 function. Conversely, PFKFB3 overexpression enhances the competitive advantage to stay at the tip, showing that bioenergetic fitness codetermines tip cell potential (21). PFKFB3-driven glycolysis is also important for stalk cells. PFKFB3 inactivation reduces stalk cell proliferation, and its expression is lowered, but not extinguished, by DLL4-NOTCH1 (**Figure 4*a***). Notably, PFKFB3 activation even induces tip cell-behavior in ECs that are genetically forced to become stalk cells, i.e., in cells with constitutive NOTCH1 signaling (21). These findings illustrate that metabolic adaptation is not simply a bystander of angiogenic signal transduction and suggest that the endothelial metabolic state can exert control over their hardwired genetic circuits.

Despite the avidity of ECs to consume glucose, they also need other metabolic resources to sustain vessel growth. As described above, FAs are an important fuel for ECs during sprouting (40) and are catabolized by FAO (**Figure 4*b***) (21, 40, 71, 72). Mitochondrial FAO breaks down FAs in a cyclical series of reactions that produce acetyl-CoA (for use in the TCA cycle and elsewhere), NADH, and $FADH_2$ (for use in OXPHOS). It is typically carried out in energy-demanding tissues, as it produces high amounts of ATP. Indeed, oxidation of one mole of palmitate, a long-chain FA, can yield up to 129 mol of ATP equivalents compared to oxidation of one mole of glucose, which generates a maximum of 38 mol of ATP. However, ECs do not use FAs for energy requirements or for redox control similar to some stressed cancer cells (73, 74). Instead, they take advantage of the high carbon content of FAs to fuel the de novo synthesis of nucleotides required for DNA replication and cell proliferation. The inhibition of endothelial FAO-depleted nucleotide stores, reduced incorporation of FA-derived carbons into DNA, and compromised EC proliferation together leading to reduced vascular density (40) (**Figure 4*b***). These cellular defects are rescued with nucleotide supplements, highlighting the selective requirement of FA catabolism for nucleotide biosynthesis. Together, these findings predict that stalk cells rely on FAO for normal function and reaffirm the concept that tip and stalk cells have distinct metabolic needs.

The precise mechanisms whereby proangiogenic signals set the metabolic state in ECs are under investigation. However, the transcription factor MYC appears to be a critical nodal point

←

Figure 4

Molecular regulation of EC metabolism during growth and quiescence. (*a*) VEGF (presumably through VEGFR2 activation) enhances glycolysis in ECs by inducing the expression of GLUT1, PFKFB3, and LDHA (and others), which increase flux through this metabolic pathway. Glycolysis supports tip cell migration but also stalk cell proliferation. The hypoxia-induced transcription factor HIF1α also promotes glycolysis, which involves a similar set of glycolytic target genes (as demonstrated in non-ECs). In stalk cells, expression of the key glycolytic regulator PFKFB3 is lowered (but not extinguished) by the activated NOTCH1 receptor (NICD), which is released upon NOTCH1 receptor activation by DLL4. The precise molecular mechanism for the Notch-dependent regulation of PFKFB3 is not yet known. (*b*) In proliferating (stalk) ECs, the activated VEGFR2 stimulates signaling through the PI3K/AKT pathway, which leads to suppression of the growth-inhibiting transcription factor FOXO1. The growth-enhancing transcription factor MYC resides in the nuclei of ECs to drive the expression of genes that cooperatively promote anabolic metabolism, growth and proliferation. Besides glucose, proliferating ECs also consume FAs, which are transferred into mitochondria via CPT1a. FA-derived carbons are essential for nucleotide synthesis (dNTPs), which are required for cell proliferation. (*c*) In phalanx ECs, FOXO1 represses MYC signaling, thereby lowering glycolysis and mitochondrial metabolism, overall favoring quiescence. The transcription factor KLF2 is activated by blood flow, a proquiescent signal, which leads to a similar reduction in metabolic rate. Abbreviations: AKT, serine/threonine kinase; CPT1a, carnitine palmitoyltransferase 1a; DLL4, Notch ligand Delta-like 4; dNTP, deoxynucleoside triphosphate; EC, endothelial cell; FA, fatty acid; FAO, fatty acid β-oxidation; HIF1α, hypoxia-inducible factor 1α; LDHA, lactate dehydrogenase A; MYC, V-Myc avian myelocytomatosis viral oncogene homolog; NICD, NOTCH1 intracellular domain; OXPHOS, oxidative phosphorylation; PI3K, phosphatidylinositol 3-kinase; PFKFB3, phospho-fructokinase-2/fructose-2,6-bisphosphatase 3; VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor 2.

between growth factor signaling and endothelial metabolism (75). MYC is a driver of cell growth, proliferation, and anabolic metabolism, functioning downstream of growth factor signaling cascades such as extracellular-regulated kinase (ERK) (76). Upon stimulation, ERK phosphorylates MYC, which inhibits the proteasomal degradation of MYC protein. As a result, MYC accumulates and amplifies transcription of genes boosting glycolysis, mitochondrial function, and cell cycle progression (77). Among the canonical targets of MYC is hexokinase (HK), the first rate-limiting enzyme of glycolysis, as well as LDHA, which converts pyruvate to lactate to generate nicotinamide adenine dinucleotide (NAD^+) for sustained glycolysis. Other targets of MYC include GLS1 and serine hydroxymethyltransferases involved in serine metabolism and nucleotide biosynthesis (77). In ECs, MYC governs a similar set of target genes to coordinate biomass generation with cell cycle progression and growth (75, 78) (**Figure 4*b***). MYC protein is highly expressed in sprouting vessels, and its depletion reduces glycolysis, mitochondrial activity, and proliferation (75). Forced expression of MYC in ECs evokes opposite effects, underscoring the key function of MYC in the expanding vasculature. Determining which endothelial growth factors and pathways signal to MYC requires further investigation.

NAD^+/NADH: oxidized and reduced form of nicotinamide adenine dinucleotide

FOXO1: member of the evolutionarily conserved family of forkhead box O transcription factors that suppress growth and anabolic metabolism

Metabolism of Quiescent Phalanx Cells

Although the metabolism of angiogenic ECs has gained much attention, less is known about the metabolism of quiescent ECs. This is surprising given the critical role of endothelial quiescence for normal vessel function and that endothelial homeostasis is often compromised by nutrient excess in diabetes and hyperlipidemia (65). The transcription factor FOXO1 regulates the metabolic activity of quiescent ECs (75). FOXO1 belongs to the forkhead box O (FOXO) family of transcriptional regulators, which restrict growth and anabolic metabolism in a wide range of tissues (79, 80). FOXO1 activity is inhibited by phosphatidylinositol-3-OH kinase [PI(3)K]/AKT, which induces FOXO1 nuclear exclusion upon AKT-mediated phosphorylation (79, 80) (**Figure 4*b***). ECs are exquisitely sensitive to changes in FOXO1 activity, as EC-specific deletion leads to early embryonic lethality and uncoordinated endothelial overgrowth. FOXO1 drives endothelial quiescence, in part by reducing the metabolic rate of ECs (75). Rather than switching from glycolysis to mitochondrial OXPHOS, FOXO1 lowers the activity of both (**Figure 4*b***). This reduction in overall metabolic activity is not a consequence of FOXO1-induced autophagy, senescence, or cell death but relies on the suppression of MYC, which fuels glycolysis and mitochondrial metabolism in ECs (75). Notably, a similar metabolic state is caused by blood flow, a known instigator of EC quiescence, which reduces endothelial glucose uptake, glycolysis, and mitochondrial respiration (81). These effects of flow are mediated by the transcription factor Krüppel-like factor 2 (KLF2), which suppresses transcription of glycolytic genes, including HK1, PFKFB3, and PFK1 (81) (**Figure 4*b***). ECs consistently reduce glucose consumption and glycolysis when integrated into a stable network (72). Interestingly, at the same time, these ECs increased FAO (72), raising the possibility that (quiescent) ECs in stable vessels switch from glucose to FA utilization. The reasons for such a change in nutrient utilization and whether it contributes to the slower metabolic rate in quiescent ECs remain to be studied.

Why, then, do quiescent ECs reduce their metabolic rate despite having immediate access to nutrients and O_2 in the blood stream? A possible explanation is that reduced metabolic activity is beneficial for the homeostasis of the long-lived endothelium. For instance, by reducing anabolic metabolism, ECs limit their proliferative capacity and thus enforce quiescence. By lowering metabolic activity, ECs also consume less energetic fuel for their homeostatic needs, thereby ensuring efficient nutrient and O_2 delivery to perivascular tissues. Reducing mitochondrial metabolism might also contribute to EC redox balance. Indeed, ECs need to protect themselves

against oxidative damage exerted by high O_2 levels in the bloodstream. Minimizing oxidative metabolism is thus a conceivable mechanism to lower the production of mitochondria-derived ROS, thereby conferring protection against their high-O_2 environment. Altogether, these considerations support the concept that the reduced metabolic rate is not a secondary stress response but rather a primary adaptation of the quiescent endothelium.

Sirtuins: a family of lysine deacetylases that require the metabolic cofactor NAD^+ for their catalytic activity

REGULATION OF ANGIOGENIC SIGNAL TRANSDUCTION BY METABOLITES

The impact of endothelial metabolism on angiogenesis is exerted through both the supply of energy and building blocks as well as through substrates for protein modifications that modulate protein activity, stability, localization, and gene expression. These modifications regulate signaling in a metabolism-responsive manner because metabolites are needed to generate these post-translational modifications (82, 83).

Acetylation

Acetylation describes the addition of an acetyl group to a protein and requires acetyl-CoA as an acetyl-group donor. Acetylation on histones determines epigenetic gene regulation, but other proteins are also acetylated, including transcriptional regulators and metabolic enzymes (84). Metabolism itself is a pivotal regulator of acetylation because of flux through glycolysis and the pyruvate dehydrogenase reaction supply acetyl-CoA for acetylation (84). The abundance of acetyl-CoA also regulates the activity of acetyltransferases, the enzymes that catalyze acetylation. When nutrient levels are high, the cellular concentration of acetyl-CoA rises, leading to an overall increase in acetyltransferase activity (82). The removal of acetyl-groups (deacetylation) from proteins is also metabolically regulated. Deacetylases of the sirtuin family are sensitive to metabolic state, as they require NAD^+ for their activity (85–87). NAD^+ levels increase in response to nutrient deprivation, resulting in higher sirtuin activity and the subsequent deacetylation of their targets (85–87). Regulation of protein acetylation levels thus offers an avenue to couple protein function to the metabolic state of the cell.

Whether endothelial metabolism impacts histone acetylation and epigenetics remains uninvestigated, but mounting evidence indicates that key regulators of angiogenic signaling are controlled by reversible acetylation. An example is VEGFR2, which becomes acetylated on its intracellular part (88). VEGFR2 acetylation positively regulates VEGFR2 phosphorylation and maintains receptor activity upon ligand stimulation. The NOTCH1 intracellular domain (NICD) is another highly acetylated protein in ECs (89). NICD is the short-lived protein fragment of the membrane-bound NOTCH1 receptor that is released upon ligand binding. It translocates to the nucleus where it forms a transcriptional complex that includes the acetylatransferase p300 to regulate Notch target genes. p300 acetylates NICD, which stabilizes it (89, 90). Deacetylation is mediated by the sirtuin family member SIRT1, which primes NICD for proteasomal degradation (89). Notably, several other components of the Notch pathway are acetylated and targeted by SIRT1, including all components of the Notch transcription-regulatory complex (91). This pervasive control of the Notch pathway by acetylation suggests that Notch signaling is particularly sensitive to the metabolic state of the cell and predicts that the sprouting behavior of ECs is regulated in tune with their intracellular metabolic resources.

A growing body of evidence also suggests that acetylation plays a crucial role in the cellular response to hypoxia by targeting HIF1α and HIF2α (57). SIRT1 activates HIF2α signaling through deacetylation (92) while inhibiting HIF1α (93). This differential regulation may be rationalized

by the nonoverlapping biological functions of these HIFs, which regulate different target genes and often do not compensate for each other's function (57). Other sirtuins also reduce HIF1α signaling but through indirect mechanisms. The mitochondrial SIRT3 reduces HIF1α levels by lowering intracellular ROS (94), whereas chromatin-bound SIRT6 represses HIF1α target genes by histone deacetylation (95). Investigations on the role of these sirtuins on HIFs in ECs have the potential to expand our knowledge about how nutrient and O_2 availability shape the vascular network.

Glycosylation

Glycosylation is another nutrient-sensitive protein modification (82). It is frequently found on growth factor receptors and nutrient transporters and regulates their membrane localization and function. Levels of glycosylation are modulated through the HBP, which diverges from glycolysis at fructose-6-phosphate and produces the metabolite UDP-N-acetylglucosamine (UDP-GlcNAc), critical for N- and O-linked glycosylation reactions (96). As the HBP requires glucose, glutamine, acetyl-CoA, and ATP to generate UDP-GlcNAc, protein glycosylation is sensitive to the availability of these nutrients. Glycosylation affects the signaling activity of VEGF and Notch. VEGFR2 is a glycosylated protein, and glycosylation modulates both its retention at the cell membrane (33) and VEGF-independent activation (97). Glycosylation also changes the responsiveness of NOTCH1 to ligands DLL4 and Jagged1 (34). Whether NOTCH1 glycosylation is regulated by the HBP and whether it changes in response to nutrient availability needs to be investigated.

α-Ketoglutarate-Dependent Signaling

α-KG is a central metabolite of the TCA cycle that influences cell signaling and epigenetic gene regulation. It is produced from isocitrate by isocitrate dehydrogenases in mitochondria and cytoplasm (35, 98). Besides its role in the TCA cycle, this metabolite regulates the activity of α-KG-dependent dioxygenases, which require α-KG as a cosubstrate and catalyze a variety of post-translational modifications. Among the α-KG-dependent dioxygenases are the HIF-regulating PHD enzymes and ten-eleven translocation DNA demethylases, which influence epigenetic gene expression but whose roles in ECs are unknown. Jumonji C domain-containing (JmjC) histone demethylases also require α-KG for their catalytic activity, and silencing JmjD6 compromises EC sprouting in vitro by altering VEGFR1 splicing (99). The enzymatic reaction carried out by these dioxygenases involves decarboxylation of α-KG to a succinate; it also requires iron and O_2 and is inhibited by its end product succinate (35, 98). As such, the activity of α-KG-dependent dioxygenases is sensitive to metabolism. A subset of these enzymes is inhibited by (D)-2-hydroxyglutarate (D-2-HG), an oncometabolite produced by cancer-associated mutations in IDH enzymes (35, 98). IDH1 and IDH2 mutations occur in certain tumors and leukemias, in which the D-2-HG-mediated inhibition of α-ketoglutarate-dependent enzymes promotes malignancy (35, 98). Of note, hypoxic ECs were recently found to selectively generate high levels of the L enantiomer 2-HG (L-2-HG) (100), which has similar but nonidentical biological properties (35). L-2-HG levels increase in response to O_2 limitation via the enzymatic reduction of α-KG. The hypoxia-induced increase in L-2-HG is not mediated by IDH enzymes but involves promiscuous substrate usage by LDHA and malate dehydrogenase (100, 101). Whether L-2-HG is just a marker of hypoxia or whether it transmits a biological function in ECs merit investigation.

Metabolites can also affect endothelial functions in a paracrine fashion. 3-Hydroxybutyrate (3-HIB) is a catabolic intermediate of valine metabolism produced by skeletal muscle in response

to PGC1-α activation. 3-HIB enhances the flux of circulating FAs across the endothelium to stimulate their uptake in myofibers (102). Via paracrine metabolite signaling, PGC1-α instructs vessels to increase transendothelial FA transport for catabolism in skeletal muscle. Another example for paracrine metabolite signaling is lactate, which is extruded by cancer cells. ECs can take up lactate via monocarboxylate transporter 1 (MCT1), which leads to PHD2 inhibition and subsequent HIF1α activation (103). ECs exposed to lactate increase the expression of VEGF and other receptor tyrosine kinase ligands, together promoting angiogenic behavior (104, 105).

SENSING METABOLISM IN THE ENDOTHELIUM

For ECs to respond dynamically to changing environmental conditions, they must possess signaling mechanisms that sense changes in cellular energetics and (re)balance energy supply with demand. One such mechanism is the protein kinase AMPK, which is activated by adenosine monophosphate (AMP), whose levels increase in conditions of energy shortage (106). By phosphorylating metabolic targets, AMPK promotes catabolic pathways that generate ATP while inhibiting anabolic pathways that consume ATP. Regulation of this serine-threonine kinase thus allows cells to restore their energy balance. In ECs, AMPK is activated by glucose deprivation, hypoxia, and shear stress (blood flow), all of which can affect EC energy charge (107). AMPK activation enhances endothelial FAO and maintains ATP levels when glucose is limiting (71, 108). AMPK is functionally relevant, as its inhibition impairs EC migration and angiogenesis in response to hypoxia (109).

Among the targets of AMPK is mammalian target of rapamycin complex 1 (mTORC1), which itself functions as a sensor of metabolic signals. mTORC1 is a protein complex that includes the serine-threonine kinase mTOR. It integrates inputs from intra- and extracellular cues, including growth factors, energy state, O_2, and amino acids to control anabolic processes, such as protein and lipid synthesis (110–112). AMPK negatively regulates mTORC1 by phosphorylating TSC2, an upstream regulator of mTORC1, and RAPTOR, a regulatory protein of the mTORC1 complex. mTORC1 is activated by growth factors but also by amino acids, which promote mTORC1 translocation to the outer surface of lysosomes where its activator RHEB resides (113). Because RHEB is required for mTORC1 activation, and amino acids are critical for lysosomal recruitment, growth factors can only stimulate mTORC1 signaling if amino acids are abundant (113). Hence, mTORC1 relays information from both systemic and cellular nutrient levels to control anabolic metabolism and growth. Although the regulation of endothelial mTORC1 by metabolism requires further investigation, it is clear that ECs are sensitive to mTORC1 signaling, as mice with constitutively active mTORC1 develop vascular malformations and lymphangiosarcomas (114). Sustained mTORC1 signaling has also been involved in Kaposi's sarcoma (115), an endothelial-derived tumor with increased glycolytic activity (28).

Another potential sensor of the endothelial metabolic state is SIRT1, whose activity increases in conditions of nutrient restriction. Notably, mice lacking SIRT1 in ECs are unable to revascularize ischemic tissues (116). SIRT1 deficiency impairs EC branching and proliferation, which is partly due to an unrestrained activity of FOXO1, an acetylated protein targeted by SIRT1 (80).

ENDOTHELIAL METABOLISM IN DISEASE

A dysfunctional endothelium contributes to more diseases than any other tissue in the body (6, 117). Perturbation of EC metabolism is best characterized for diabetes, a public health problem of

epidemic proportions (118). Diabetes is characterized by hyperglycemia (elevated plasma glucose levels) due to a deficiency (type I) or resistance to (type II) insulin and is associated with micro- and macroangiopathies (119–121). EC dysfunction is an early event in hyperglycemia-induced pathologies and a consequence of enhanced oxidative stress (122). Whereas diabetes-induced EC dysfunction is closely linked to the development of these vascular disorders in clinical studies, many mechanistic studies discussed below are deduced from in vitro experiments for which in vivo evidence is still lacking.

Hyperglycemia Diverts Glycolytic Intermediates to Other Metabolic Pathways

In hyperglycemic conditions, ECs cannot handle the surplus of glucose adequately and deviate glycolytic intermediates to side pathways, overall increasing oxidative stress. One reason for the impaired glucose handling is the inactivation of the key glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH). GAPDH is polyADP-ribosylated by polyADP-ribose polymerase (PARP1), which occurs in response to oxidative stress-induced DNA damage (123–125). This polyADP-ribosylation inactivates GAPDH and stalls glycolysis, causing an accumulation of glycolytic metabolites, which are diverted into three metabolic pathways: the HBP, the polyol pathway, and the glycation pathway (123). Because PARP1 inhibition abrogates the hyperglycemia-induced increase in activity of each of these pathways in ECs (123), oxidative stress is likely an early event in EC dysfunction.

In diabetic conditions, excess glucose enters the HBP, which generates UDP-GlcNAc, essential for protein glycosylation (see above); however, in hyperglycemic conditions, protein glycosylation is deregulated, evoking aberrant protein expression, localization, or activity (126). Excess glucose is also diverted into the polyol pathway, in which aldose reductase (ALR2) reduces glucose to sorbitol in an NADPH-consuming reaction (127). The resulting NADPH depletion impedes regeneration of the cellular antioxidant glutathion (GSSG to GSH), thereby impairing superoxide scavenging and enhancing oxidative stress. Sorbitol dehydrogenase converts sorbitol to fructose, which is metabolized to 3-deoxyglucosone (3-DG), a highly reactive dicarbonyl compound that contributes to nonenzymatic production of noxious advanced glycation end products (AGEs) (128). ALR2 is implicated in the early stages of diabetic retinopathy (129), and the inhibition of endothelial ALR2 reduces ROS production and EC proliferation (130–132).

Glycation, the process of nonenzymatic glycosylation, contributes to the post-translational modification of proteins. Through a set of spontaneous fragmentation reactions, glucose and the glycolytic triosephosphates glyceraldehyde-3-phosphate and dihydroxyacetone phosphate are converted to three α-oxoaldehydes, i.e., methylglyoxal, glyoxal, and 3-DG. These highly reactive dicarbonyl compounds can modify DNA (through glycation) and react with lysine and arginine residues in proteins to nonenzymatically form toxic AGEs. In ECs, methylglyoxal is a key reactive aldehyde (133, 134), and elevated methylglyoxal levels are implicated in diabetic-associated angiopathies (135, 136).

Hyperglycemia Increases ROS Levels

Hyperglycemia-induced elevations of ROS levels can stem from the activation of xanthine or NADPH oxidases (NOXs) (137) or auto-oxidation of glucose (138). Heightened ROS levels, produced by NOX enzymes, can uncouple endothelial nitric oxide synthase (eNOS) [a process whereby eNOS no longer produces nitric oxide (NO) and citrulline but instead generates superoxide anion ($O2\bullet^-$)]. This ROS type reacts with NO to yield peroxynitrite ($ONOO^-$),

ultimately lowering NO levels in a vicious cycle (139). In normal conditions, eNOS-derived NO induces endothelium-dependent vasodilation, required for normal vascular homeostasis, and inhibits events promoting atherosclerosis. Not surprisingly, diabetic patients display impaired endothelium-dependent vasodilation. eNOS uncoupling also contributes to EC dysfunction in diabetic mouse models (140). Additional mechanisms causing eNOS uncoupling include insufficient availability of the NO-precursor L-arginine and cofactor tetrahydrobiopterin (BH_4), abnormal O-glycosylation of eNOS (126), and elevated levels of AGEs (141, 142).

Hyperglycemia also evokes endothelial mitochondriopathy, evidenced by changes in mitochondrial biogenesis, dysfunction, and fragmentation, as well as mitophagy (143, 144). In dysfunctional mitochondria, ROS production is excessive, and the Ca^{2+} load rises, resulting in the exacerbation of oxidative stress, aggravated EC dysfunction, and even cell death (145). The vascular injury in diabetes is linked to the increased ROS production in the mitochondrial ETC in hyperglycemic ECs (145, 146).

Because glycolytic intermediates feed into the PPP, it was hypothesized that increased diversion of these metabolites into the PPP (away from glycolysis) might be protective in hyperglycemic conditions. Increased PPP flux would not only divert the excess glycolytic metabolites away from the aforementioned three alternative pathways and thus decrease levels of damaging metabolites, but it would also increase NADPH production, thereby improving the antioxidant capacity of ECs. However, hyperglycemia reduces the glucose-6-phosphate dehydrogenase-mediated entry of glucose into the PPP (32), thereby increasing oxidative stress and decreasing NO bioavailability in ECs (147). The accumulation of AGEs in hyperglycemic conditions also causes elevated oxidative stress (148, 149) and contributes to impaired angiogenesis, apoptosis, and activation of inflammatory and tissue-damage-provoking molecules. These effects are mediated at least in part via an interaction of AGEs with the receptor for advanced glycation end products (150).

CONCLUSIONS AND PERSPECTIVES

The aforementioned studies illustrate how metabolism pervades many aspects of angiogenesis, affecting energetics, proliferation and growth, signaling, and gene expression of ECs. Changes in EC metabolism can thus have wide-ranging effects and even override genetically hard-wired signals. Still, our understanding of EC metabolism is incomplete, and numerous questions remain. To date, only a handful of metabolic pathways and enzymes were functionally analyzed, and we are only beginning to understand how they affect endothelial differentiation and function. The characterization of additional pathways and analysis of their functions promise to reveal new insights into the mechanisms of vascular development and disease and uncover new therapeutic targets. Examples of such mechanisms include the metabolism of amino acids, to which glutamine and serine belong; the various side branches of glycolysis, to which the PPP, HBP, and polyol pathways belong; and the metabolism of acetyl-CoA, a central metabolite with functions in energy generation, signaling, and epigenetics. It will also be interesting to study how these metabolic pathways are regulated and how they integrate with core angiogenic signaling cascades. Characterizing regulators of EC metabolism will provide valuable insights into how ECs rewire their metabolism in tune with angiogenic stimulation. Finally, it will be important to assess the dynamics of EC metabolism in vivo, as almost all studies until now were performed in vitro. With the current pace of technology development, we foresee new possibilities of analysis that will undoubtedly advance our understanding of the physiological and pathophysiological roles of vascular metabolism in the near future.

SUMMARY POINTS

1. ECs are highly glycolytic and produce most of their energy through this pathway.
2. Glycolysis supports endothelial functions and allows sprouting into O_2-deprived tissues.
3. Proangiogenic growth factors boost glycolytic flux by increasing the expression of key glycolytic enzymes.
4. The endothelial metabolic state can exert control over hard-wired genetic signal transduction, such as the DLL4/NOTCH1 pathway.
5. FAO is required for endothelial proliferation during angiogenic sprouting.
6. Quiescent ECs lower their metabolic rate by reducing glycolysis and mitochondrial respiration.
7. Metabolites regulate endothelial signaling, gene expression, and transendothelial transport.
8. Deregulated endothelial metabolism contributes to abnormal vascular growth and function in disease.

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