Manipulating Angiogenesis by Targeting Endothelial Metabolism: Hitting the Engine Rather than the Drivers—A New Perspective?

Lucas Treps, Lena-Christin Conradi, Ulrike Harjes, and Peter Carmeliet

Laboratory of Angiogenesis and Vascular Metabolism, Department of Oncology, University of Leuven, and Laboratory of Angiogenesis and Vascular Metabolism, Vesalius Research Center, Vlaams Instituut voor Biotechnologie, Leuven, Belgium

Abstract

—Excessive angiogenesis (i.e., the formation of new blood vessels) contributes to different pathologies, among them cancer and ocular disorders. Conversely, dysfunction of endothelial cells (ECs) contributes to cardiovascular complications, as is the case in diabetes. Inhibition of pathologic angiogenesis in blinding eye disease and cancer by targeting growth factors such as vascular endothelial growth factor has become an accepted therapeutic strategy. However, recent studies also unveiled the emerging importance of EC metabolism in controlling angiogenesis. In this overview, we will discuss recent insights in the metabolic regulation of angiogenesis, focusing on the best-characterized metabolic pathways, and highlight deregulation of EC metabolism in cancer and diabetes. We will give an outlook on how targeting EC metabolism can be used for blocking pathologic angiogenesis and for normalizing EC dysfunction.

Address correspondence to: Dr. P. Carmeliet, Laboratory of Angiogenesis and Vascular Metabolism, Vesalius Research Center, Vlaams Instituut voor Biotechnologie, KU Leuven, Campus Gasthuisberg O&N4, Herestraat 49-912, B-3000, Leuven, Belgium. E-mail: peter.carmeliet@vib-kuleuven.be

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I. Introduction

The vascular system is required for nutrient and oxygen delivery, metabolite waste removal, and immune surveillance. Endothelial cells (ECs) line the blood vessel tree. In healthy adults, most ECs remain quiescent. Angiogenesis, the formation of new vessels from a preexisting vascular network, is activated in the adult only during menstruation and pregnancy, and pathologic conditions, including stress, injury, inflammation, cancer, and other pathologies (Carmeliet and Jain, 2011). Angiogenesis is under control of multiple (epi)-genetic mechanisms. Given the key importance of vascular endothelial growth factor (VEGF) in angiogenesis in health and disease, most current antiangiogenic therapies targeting VEGF have been clinically approved for the treatment of various types of cancer and ocular diseases (Table 1). However, the success of targeting VEGF is limited because of insufficient efficacy and resistance (Welti et al., 2013; Jayson et al., 2016). This necessitates the development of new antiangiogenic strategies based on entirely different mechanisms.

Recently, a series of papers shed light on a newly emerging field, postulating that EC metabolism is instrumental for angiogenesis. We put forward a new concept in which EC metabolism represents the engine of ECs, onto which proangiogenic signals (such as VEGF and others) converge. Above all, activated ECs in the proangiogenic tumor or eye milieu require energy and biomass, generated by cellular metabolism, to form new blood vessels. We thus hypothesized that targeting EC metabolism might paralyze ECs despite exposure to multiple pro-angiogenic stimuli.

In a simplified comparison (see graphical abstract), a driver (VEGF) steers a car (stimulates ECs to form vessels). When removing the driver (blocking VEGF), the car can no longer ride (angiogenesis is halted). However, as long as there is a new driver (another angiogenic factor), this new driver will still be able to steer the car (form new vessels). Precisely this is what happens with traditional anti-VEGF therapy in cancer, i.e., when blocking VEGF, other angiogenic factors can take over. In contrast, by hitting the engine of the car (EC metabolism), the car will no longer be able to ride (ECs can no longer sprout), regardless of how many drivers (angiogenic factors) are still present.

In this overview, we will discuss the role of EC metabolism (Fig. 1) in normal vessel sprouting and how EC metabolism becomes deregulated in diseases such as cancer and diabetes. We will also illustrate the therapeutic potential of manipulating EC metabolism for inhibiting pathologic neovascularization in cancer and eye disease or for normalizing EC dysfunction in diabetes.

II. Genetic Control of Angiogenesis

In normal health, ECs are quiescent “phalanx” cells. When proangiogenic cues are upregulated, dormant ECs quickly switch to an angiogenic phenotype and differentiate to migratory “tip” and proliferating “stalk” cells (Potente et al., 2011). Among proangiogenic players, VEGF is of vital importance, as indicated by the early embryonic lethality in VEGF heterozygous deficient embryos (Carmeliet et al., 1996; Ferrara et al., 1996). In tip cells, activation of VEGF receptor 2 (VEGFR-2) by VEGF induces the expression of Delta-like ligand 4 (Dll4), a Notch receptor ligand (Fig. 2A). Binding of Dll4 to the Notch receptor on adjacent ECs induces cleavage of the Notch intracellular domain (NICD), which then acts as a transcription factor, leading to downregulation of VEGF receptors VEGFR-2 and VEGFR-3 and the VEGF coreceptor neuropilin-1, while increasing VEGFR-1 expression (Fig. 2A) (Geudens and Gerhardt, 2011). This feedback loop laterally inhibits the tip cell phenotype in adjacent cells and instead promotes the proliferative stalk cell phenotype, which permits the extension of the sprout toward the proangiogenic gradient (Carmeliet and Jain, 2011). During migration, VEGF promotes endocytosis of the junctional molecule VE-cadherin thereby favoring EC migration, whereas activation of VEGF-dependent Notch signaling inhibits membrane protrusions and promotes cell adhesion (Bentley et al., 2014).

The nascent vascular sprout fuses with a nearby sprout. This process is regulated by VEGF-C (secreted by macrophages), which stimulates Notch signaling, thereby decreasing the tip cell sensitivity to VEGF and permitting fusion (Tammela et al., 2011). After angiogenesis, EC lumen formation takes place and the vessel becomes perfused, enabling tissue oxygenation (Potente et al., 2011). Another growth factor, platelet-derived growth factor β, stimulates the recruitment of pericytes, whereas transforming growth factor-β promotes the differentiation of these mural cells and the deposition of a basement membrane, all processes that stabilize and mature the nascent vessel sprout (Carmeliet and Jain, 2011). Angiopoietin/Tie2 signaling is also necessary for the development of new antiangiogenic therapies in diseases (Table 1). However, the success of targeting VEGF is limited because of insufficient efficacy and resistance (Welti et al., 2013; Jayson et al., 2016). This necessitates the development of new antiangiogenic strategies based on entirely different mechanisms.

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<tr>
<td>Bevacizumab (Avastin)</td>
<td>Genentech/Roche</td>
<td>monoclonal antibody</td>
<td>VEGF</td>
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<td>chimeric soluble receptor</td>
<td>VEGF, VEGF-B, PIGF</td>
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<td>tyrosine kinase inhibitor</td>
<td>VEGFRs, FGFR, PDGFR</td>
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CRVO, central retinal vein occlusion; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; FLT-3, fms-related tyrosine kinase 3; GEJ, gastroesophageal junction; KIT, v-kit feline sarcoma viral oncogene homolog; MET, met protooncogene; PDGFR, platelet derived growth factor receptor; PIGF, placental growth factor; RAF, v-raf-1 murine leukemia viral oncogene homolog 1; RET, ret proto-oncogene; TIE, TEK tyrosine kinase endothelial; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.
instrumental in vessel sprouting and regulates maturation of the new vessel and EC quiescence (Potente et al., 2011).

### III. Metabolic Control of Angiogenesis

#### A. Glycolysis Addiction of Endothelial Cells

Because of their privileged position, ECs are in direct contact with high oxygen levels in the blood. One would therefore expect that ECs generate energy primarily through mitochondrial respiration, because the yield of adenosine triphosphate (ATP) per mole of glucose from oxidative metabolism is much higher than from anaerobic metabolism. Surprisingly, however, ECs do not rely on mitochondrial respiration, but rather on glycolysis (Murphy, 1960; Feiden et al., 2007; Koppenol et al., 2011; De Bock et al., 2013). Indeed, ECs from macro- and microvessels exhibit high glycolytic rates (Dobrina and Rossi, 1983; Krutzfeldt et al., 1990; Mertens et al., 1990; Culic et al., 1997) and generate 85% of their total ATP content via glycolysis, whereas mitochondrial respiration is low in ECs relative to other cell types (Peters et al., 2009; De Bock et al., 2013).

ECs are plastic cells that rapidly switch from quiescence to angiogenic sprouting. Given that these distinct growth states greatly differ in their bioenergetic and biosynthetic needs, ECs must be able to adjust their metabolism accordingly and couple metabolic activity to growth state. Although the molecular mechanisms underlying this dynamic switch are not fully understood, recent evidence indicates that the transcription factor Forkhead box protein O1 (FOXO1), by suppressing c-MYC, acts as a gatekeeper of endothelial quiescence to decelerate metabolic activity by reducing glycolysis and mitochondrial respiration (Wilhelm et al., 2016). These findings identified FOXO1 as a critical rheostat of vascular expansion and defined the FOXO1-MYC transcriptional network as a novel metabolic checkpoint during endothelial growth and proliferation.
1. Glycolysis and Sprouting Angiogenesis. Glucose enters ECs via facilitated diffusion catalyzed by glucose transporters (GLUTs) (Fig. 2B) (Uldry and Thorens, 2004). Glucose is then converted to pyruvate via glycolysis, generating 2 molecules of ATP. 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3), which has kinase as well as bisphosphatase activity, stimulates glycolysis by generating fructose-2,6-bisphosphate, a potent allosteric activator of the glycolytic rate-limiting enzyme phosphofructokinase-1 (PFK-1) (Fig. 2B) (Van Schaftingen et al., 1982; De Bock et al., 2013). The kinase enzymatic activity of PFKFB3 is 700-fold higher than its bisphosphatase activity, thus favoring glycolysis (Sakakibara et al., 1997; Yalcin et al., 2009). Interestingly, PFKFB3 null mice are embryonically lethal during early embryogenesis (Chesney et al., 2005). Furthermore, loss of PFKFB3 in ECs causes vascular defects in vivo (De Bock et al., 2013). Proangiogenic signals like VEGF and fibroblast growth factor-2 (FGF2) signaling enhance PFKFB3-driven glycolysis (Fig. 2B) (De Bock et al., 2013). PFKFB3-driven glycolysis is essential for adequate EC motility. Indeed, ECs lacking PFKFB3 are less competitive to take the tip cell position during vessel sprouting (De Bock et al., 2013). PFKFB3 overexpression drives tip cell formation and even overcomes genetic hard-wired stalk cell induction by Notch signaling (De Bock et al., 2013). Moreover, in contact-inhibited (quiescent) ECs, PFKFB3 and other glycolytic enzymes are present in the perinuclear cytosol, whereas in migrating ECs, they are relocated to the membrane ruffles in lamellipodia and filopodia.
filopodia of migrating ECs (Fig. 2B) (Real-Hohn et al., 2010; De Bock et al., 2013). Here, they are bound to the dense filamentous actin network and generate spatially focused, high levels of ATP needed for actin-myosin contraction during the remodeling of the actin cytoskeleton (De Bock et al., 2013; Phng et al., 2013). In contrast, bulky mitochondria are excluded from the thin filopodia. Hence, glycolytic ATP production in lamellipodia and filopodia enables ECs to migrate when forming vessel sprouts.

2. Regulation of Endothelial Cell Metabolism by Oxygen and Flow. Unlike most other cell types, ECs proliferate and migrate to form new blood vessels when oxygen levels drop to revascularize and improve oxygen supply in the tissue. Of note, several genes of the glycolytic pathway are upregulated in hypoxia through the activity of hypoxia-inducible factor HIF-1α: GLUT1, GLUT3, PFK-1, aldolase, pyruvate kinase, lactate dehydrogenase A (LDH-A), hexokinase, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and phosphoglycerate kinase (Denko, 2008; Semenza et al., 1994; Ebert et al., 1996; Fukasawa et al., 2004; Obach et al., 2004). Interestingly, also PFKFB3 harbors hypoxia response elements and is a target of HIF-1α (Fukasawa et al., 2004; Obach et al., 2004). Among PFKFB isoenzymes, PFKFB3 is the most abundant isoenzyme in ECs and also the most upregulated form in ECs during hypoxia (De Bock et al., 2013; Xu et al., 2014).

Of note, miR-206, which inhibits angiogenesis in zebrafish embryos by downregulating VEGF levels (Stahlhut et al., 2012; Lin et al., 2013), reduces glycolysis by targeting PFKFB3 mRNA levels (Ge et al., 2015; Tang et al., 2015). When becoming assembled into a stable network, ECs decrease glucose consumption (Patella et al., 2015). Moreover, upon establishment of blood flow in the newly formed vessel network, ECs experience shear stress. This further promotes EC quiescence also through reduction of glycolytic metabolism, via kruppel-like factor 2 dependent transcriptional suppression of PFKFB3, hexokinase 2, and PFK-1 (Galie et al., 2014; Doddaballapur et al., 2015). These data illustrate the pivotal role of PFKFB3-driven glycolysis during active sprouting angiogenesis and its regulation at different levels (transcription, mRNA stability, and cellular compartmentalization).

3. Why do Endothelial Cells Rely on Glycolysis? The ATP yield of glycolysis is much lower than that of mitochondrial respiration (2 versus 36 moles of ATP per mole of glucose, respectively). Nonetheless, ECs generate more than 85% of their cellular energy via glycolysis (Peters et al., 2009; De Bock et al., 2013), raising the question of why ECs rely so much on this pathway. We speculate that this reliance on glycolysis may be due to several reasons. First, if ECs would rely primarily on oxidative metabolism, they would not be able to sprout into avascular oxygen-deprived tissues; instead, by relying on anaerobic glycolysis, they can generate energy in the absence of oxygen. Second, because the primary function of blood vessels is to deliver oxygen, glycolysis helps to preserve oxygen for transfer to perivascular cells. Third, nonoxidative metabolism generates less reactive oxygen species (ROS) than oxidative metabolism, and the pentose phosphate pathway, or PPP, a glycolytic side pathway, synthesizes NADPH (nicotinamide adenine dinucleotide phosphate), used by glutathione reductase to convert oxidized glutathione to reduced glutathione (GSH), a major antioxidant (Dobrina and Rossi, 1983; Krutzfeldt et al., 1990; Peters et al., 2009).

Fourth, glycolysis produces ATP faster than oxidative metabolism and can generate similar quantities of ATP as long as there is a sufficient supply of glucose (Vander Heiden et al., 2009; Locasale and Cantley, 2011). This enables ECs to compete for the tip position and to rapidly revascularize ischemic tissues. Fifth, glycolytic side pathways such as the PPP and the serine biosynthesis pathway are used for biomass production and, as mentioned above, for redox homeostasis (Lunt and Vander Heiden, 2011; Goveia et al., 2014). Last, glycolysis also regulates protein glycosylation via the hexosamine biosynthesis pathway (HBP), of importance for proper VEGFR-2 and Notch signaling (Haines and Irvine, 2003; Benedito et al., 2009; Croci et al., 2014).

B. Fatty Acid Metabolism and de novo Nucleotide Synthesis

Even though fatty acid (FA) oxidation (FAO) is able to generate 129 moles of ATP equivalent per mole of palmitate (a common saturated FA), i.e., thus much higher than the 36 molecules of ATP produced by glucose oxidation, FAO contributes only minimally to ATP synthesis in ECs, but rather provides carbons for de novo nucleotide synthesis via the tricarboxylic acid (TCA) cycle, thereby promoting EC proliferation and sprouting angiogenesis (Schoors et al., 2015).

1. Fatty Acid Uptake and Use. FAs enter the cell across the plasma membrane via a mechanism known as “flip-flop,” or via dedicated transport proteins such as fatty acid transporter/CD36. The uptake of FAs is also regulated by the activity of membranal or membrane-associated FA transport proteins (FATPs), which transport FAs, and may also have the ability of activating FAs and cytosolic FA binding proteins (FABPs), which chaperone FAs inside the cell (Glatz et al., 2010). Within the cell, FAs are activated by acyl-CoA synthetase to form FA-CoA, which can be imported into mitochondria (Fig. 1) (Glatz et al., 2010; Harjes et al., 2016). Carnitine palmitoyltransferases (CPTs), more precisely CPT1, catalyze the transesterification of long-chain FA-CoA to acyl-l-carnitine and its transport into the intramitochondrial matrix where acyl-l-carnitine is then converted back to acyl-CoA by CPT2 (Kopec and Fritz, 1973; Kerner and Hoppel, 2000).
2. The Role of Fatty Acid Metabolism in Angiogenesis.
The expression of FATPs and FABPs has been shown to be under the control of angiogenic signaling and to regulate EC function. For example, proangiogenic signals such as VEGF and FGF2 upregulate the expression of FABP4, which is required for EC proliferation and sprouting (Elmasri et al., 2009, 2012). FABP4 is also a target of NICD, which together with FOXO1 activates FABP4 transcription (Harjes et al., 2014). FATP3 and FATP4 levels may also be upregulated by VEGF-B in ECs and mediate transendothelial FA transport (Hagberg et al., 2010), but this is debated (Dijkstra et al., 2014; Kivela et al., 2014).

By controlling the shuttling of FA-CoA in mitochondria, CPT1 is a rate-determining enzyme of FAO (Fig. 2C) (McGarry and Brown, 1997). CPT1A is the most abundant isoform in ECs, and silencing of CPT1A impairs vessel sprouting (Schoors et al., 2015) and weakens the EC barrier (Patella et al., 2015). In vivo angiogenic sprouting is also impaired in mice lacking CPT1A in ECs (Schoors et al., 2015).

CPT1A deficiency in the endothelial compartment reduces EC proliferation (not migration) without, however, altering ATP levels and barely affecting oxygen consumption (Schoors et al., 2015). Indeed, FAO only contributes to less than 5% of the total amount of ATP generated (De Bock et al., 2013). In other cell types, FAO is important to ensure proper redox homeostasis via production of NADPH (cofactor for GSH synthesis), but CPT1A knockdown in ECs elevates ROS production (De Bock et al., 2013). In other cell types, ROS contribute to ROS accumulation (Goldin et al., 2006). Glucose-6-phosphate dehydrogenase overexpression in ECs restores the redox balance in conditions of high glucose (Leopold et al., 2003; Zhang and Forman, 2006). Glucose-6-phosphate dehydrogenase overexpression in ECs restores the redox balance in conditions of high glucose (Leopold et al., 2003; Zhang and Forman, 2012).

A third mechanism of ROS production relates to a malfunctioning of endothelial nitric oxide synthase (eNOS), the enzyme that produces nitric oxide (NO).
As a vasodilator with antithrombotic, anti-inflammatory, and proangiogenic activities, NO is important to maintain vessel wall function and health. In diabetic ECs, production of NO by eNOS is perturbed, in part because of the reduced availability of NADPH (a critical cofactor of eNOS) from the impaired PPP flux. In addition, metabolic perturbations in diabetic ECs lead to uncoupling of eNOS, which now starts to produce superoxide anion (O$_2^-$) instead of NO that then can react with NO to yield peroxynitrite (ONOO$^-$), a highly reactive ROS (Guzik et al., 2002; Battault et al., 2016). This not only reduces NO bioavailability, but peroxynitrite nitrosylates eNOS and converts the eNOS cofactor tetrahydrobiopterin (BH$_4$) to the biologically inactive dihydrobiopterin (BH$_2$), overall resulting in further uncoupling of eNOS in a vicious cycle. This is further aggravated by high glucose-activated PKC, which will stimulate O$_2^-$ production. Thus, uncoupling of eNOS initiates and aggravates EC dysfunction and promotes atherosclerosis in the further disease course (Forstermann and Munzel, 2006; Thum et al., 2007).

In mitochondria, exposure to high glucose evokes endothelial mitochondriopathy, evidenced by changes in mitochondrial biogenesis, dysfunction, and fragmentation, leading to accumulation of damaged mitochondria and removal via mitophagy (Shenouda et al., 2011; Pangare and Makino, 2012). In dysfunctional mitochondria, excessive ROS production is observed and Ca$^{2+}$ overload exacerbates the oxidative stress, thus promoting EC dysfunction and even causing cell demise (Tang et al., 2014). Elevated ROS production in the mitochondrial electron transport chain has been linked to vascular damage in hyperglycemic ECs (Kakimoto et al., 2002; Tang et al., 2014).

b. Diversion of glycolytic intermediates to side pathways. Central to many aspects of the deregulated EC metabolism in diabetes is the finding that ECs exposed to high glucose cannot handle the glucose overload properly and deviate glycolytic intermediates to side pathways, which overall results in enhanced oxidative stress. However, one of the reasons why glycolysis in ECs is reduced relates to the inactivation of the key glycolytic enzyme GAPDH by elevated ROS (Fig. 3). Indeed, ROS induces DNA breaks, thereby activating polyADP-ribose polymerase-1 (Du et al.,
2000, 2003; Nishikawa et al., 2000; Giacco and Brownlee, 2010; Blake and Trounce, 2014). PolyADP-ribosylation of GAPDH by polyADP-ribose polymerase 1 inactivates this enzyme and stalls glycolysis, resulting in the accumulation of glycolytic metabolites (Fig. 3) (Du et al., 2003).

Accumulation of the glycolytic intermediate fructose-6-phosphate (F6P) increases the flux through the HBP, which generates UDP-GlcNac, a precursor of glycosylation reactions (Brownlee, 2001). Although glycosylation is important for physiologic EC performance, hyperglycemia-induced protein glycosylation can inhibit angiogenesis, although its effects are contextual (Du et al., 2001; Federici et al., 2002; Luo et al., 2008). For instance, in hyperglycemia, glycosylation contributes to EC dysfunction by exacerbating oxidative stress and interfering with NO synthesis (Fig. 3) (Luo et al., 2008; Rajapakse et al., 2009; Belezni and Bagi, 2012).

Other glycolytic intermediates are diverted into the polyol and methylglyoxal pathways that ultimately lead to the production of damaging agents such as ROS and AGEs (Goldin et al., 2006). AGEs render ECs dysfunctional by altering extracellular matrix protein and deregulating cytokine expression (Vlassara et al., 1995; Yan et al., 2008). In addition, activation of the receptor of AGE by AGEs in vascular cells evokes oxidative stress and inflammation and reduces NO availability associated with vascular complications in diabetic patients (Bucala et al., 1991; Min et al., 1999; Waatier and Schmidt, 2004; Goldin et al., 2006; Manigrasso et al., 2014).

Excess glucose that cannot be metabolized by glycolysis enters the polyol pathway where it is converted into sorbitol by aldose reductase (AR) at the expense of NADPH, thereby increasing ROS levels (Fig. 3). Intracellular accumulation of the cell membrane impermeable sorbitol in retinal pericytes, but not ECs, increases leakiness and cell death, possibly by causing osmotic stress (Kador et al., 2009; Zhang et al., 2014). Sorbitol is subsequently converted into fructose and thereafter into the highly reactive 3-deoxyglucosone, which promotes the formation of AGEs (Kashiwagi et al., 1994; Schalkwijk et al., 2004; Oyama et al., 2006; Giacco and Brownlee, 2010; Sena et al., 2012; Yoshida et al., 2012; Mapanga and Essop, 2016). Overexpression of an AR transgene in ECs of diabetic mice accelerates atherosclerosis and inhibition of endothelial AR reduces ROS production and EC proliferation (Obrosova et al., 2003; Tammali et al., 2011; Vedenantham et al., 2011; Yadav et al., 2012).

Accumulating glycolytic intermediates can also take part in glycation (nonenzymatic glycosylation) of proteins. Indeed, glucose and the triosephosphates glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (DHAP), accumulating because of GAPDH inactivation, can undergo spontaneous fragmentation into the α-oxoaldehydes methylglyoxal, glyoxal, and 3-deoxyglucosone. These highly reactive dicarbonyl compounds contribute to nonenzymatic production of noxious advanced glycation end products (AGEs) (Waatier and Schmidt, 2004). Methylglyoxal is a high-affinity substrate of AR (Vander Jagt et al., 2001; Dhar et al., 2010). In addition to increased substrate availability causing increased flux through AR, its activity is also increased due to lack of NO-mediated suppression (Mapanga and Essop, 2016). Methylglyoxal is detoxified by conversion into pyruvate via the multienzyme glyoxalase system, of which glyoxalase-I is rate limiting (Fig. 3) (Thornalley, 1993). Overexpression of glyoxalase-I normalizes hyperglycemia-induced angiogenesis impairment in vitro and transgenic overexpression of glyoxalase-I reduces vascular AGE formation and improves vasoactivity (Ahmed et al., 2008; Brouwers et al., 2010, 2014). Also, the accumulating DHAP levels may lead to increased production of DAG, which, through activation of PKC, activates NOX and promotes ROS production and vascular perturbation (Inoguchi et al., 1994; Jiang et al., 2011; Mapanga and Essop, 2016). Together, targeting AR and glyoxalase might confer a therapeutic benefit in diabetic conditions. However, apart from these maladaptations, the global metabolic changes of diabetic ECs remain largely unexplored.

2. Excessive Endothelial Cell Growth in Diabetic Retinopathy. Vessel overgrowth in diabetic retinopathy is enhanced by ischemia, a potent angiogenic stimulus (Vinores et al., 2006; Ramsey and Arden, 2015). Retinal ischemia is caused by vessel hypoperfusion due to multiple vessel abnormalities, including vessel disintegration due to vascular cell death, leakage due to impaired junctional integrity and bleeding due to rupture of microaneurysms (Rask-Madsen and King, 2013). Death of these vascular cells occurs as a result of excessive ROS production by an activated polyol pathway and mitochondrial dysfunction (Shah et al., 2013). Increased glucose flux through the HBP may also be relevant, possibly via O-linked glycosylation of Akt (Luo et al., 2008; Shah et al., 2013; Ghesquiere et al., 2014).

In the resultant low oxygen conditions evoked by vessel hypoperfusion, stabilization of HIF-1α activates a transcriptional program (including VEGF) that enhances angiogenesis. In PDR patients, levels of VEGF and its homolog placental growth factor (PIGF) are highly elevated in the eye, evoking excessive retinal angiogenesis and vascular leakage (Aiello et al., 1994; Khalil et al., 1998; Miyamoto et al., 2007; Kowalczyk et al., 2011; Simo et al., 2014). Overstimulation of retinal angiogenesis in addition to hyperglycemia-induced damage of existing vessels then leads to the formation of abnormally shaped and functioning vessels, leakage, hemorrhaging, and eventual retinal edema and detachment, and blindness. Current antiangiogenic therapeutic strategies are in part based on blocking VEGF, while genetic studies in PIGF-deficient mice, showing protection against vascular abnormalities, suggest that PIGF blockade might also be useful.
Angiogenesis and Endothelial Metabolism

(Freitas-Andrade et al., 2012; d’Audigier et al., 2014; Huang et al., 2015; Simunovic and Maberley, 2015). It is worth noting that glycemic control reduces clinical micro- but not macrovascular complications, suggesting a distinctive role of EC metabolism in different vascular beds. However, whether such EC metabolic heterogeneity exists, is currently unknown.

B. Tumor Angiogenesis

1. Proangiogenic Tumor Microenvironment Driven by Metabolism. Multiple angiogenic signals, including members of the VEGF, angiopoietin, FGFR, and other families fuel excessive angiogenesis in tumors (see previous reviews, Carmeliet and Jain, 2000; Carmeliet, 2003; Jain, 2014). Activation of HIF-1α by low oxygen conditions in tumors induces the expression of several of these proangiogenic factors (Krock et al., 2011; McIntyre and Harris, 2015).

Depletion of nutrients is also a stimulus for upregulating VEGF expression. Indeed, because of the intense cancer cell metabolism, glucose concentrations in tumor tissue can be as low as 0.1 mM, i.e., 50-fold lower than the normal plasma glucose levels (Hirayama et al., 2009; Nardo et al., 2011; Urasaki et al., 2012). Glucose deprivation leads to induction of VEGF mRNA and protein release (Zhang et al., 2002; Yun et al., 2005). Some tumor types rely heavily on glutamine, a mitochondrial substrate that helps maintaining NADPH production for redox homeostasis and energy production (Wise and Thompson, 2010). Upon deprivation of glutamine, cancer cells increase the production and secretion of the proangiogenic signal interleukin 8 (IL-8)/chemokine (C-X-C motif) ligand 8. This relies on reduced anaerobic replenishment of glutamine carbons into the TCA cycle, because addition of dimethyl a-ketoglutarate restored the TCA cycle flux, and reduced IL-8 secretion (Shanware et al., 2014).

Of note, other metabolites that are abundant in the tumor microenvironment can also be proangiogenic. For instance, cancer cells secrete high amounts of lactate. This glycolytic end product can be taken up by tumor ECs via monocarboxylate transporter 1 (MCT1) and activate HIF-1α as well as angiogenic IL-8/chemokine ligand 8 signaling (Vegran et al., 2011; Sonveaux et al., 2012; Choi et al., 2014). The tumor microenvironment is acidic, because of the corelease of lactate/proton and because of the synthesis of bicarbonate/proton and excretion of protons by cancer cells. Acidosis elevates VEGF levels by increasing transcription and mRNA stability via a yet undetermined mechanism (Shi et al., 2001). Acidosis also increases the interaction of VEGF with fibronectin, which could serve to stabilize VEGF stores in the extracellular matrix (Goerges and Nugent, 2004). Thus, the tumor metabolite profile and changes in the tumor microenvironment promote tumor angiogenesis.

2. Tumor Endothelial Cell Characteristics and Metabolism. Tumor ECs differ from healthy ECs in several ways and express a subset of genes specific to tumor ECs across several tumor types (St Croix et al., 2000). In fact, even in baseline conditions, tumor ECs express an array of proangiogenic genes, have higher proliferation rates, and are resistant to serum starvation-induced apoptosis (Bussolati et al., 2003; Lu et al., 2007; Kurosu et al., 2011). A hallmark of cancer is genetic instability, which drives tumor initiation and progression (Hanahan and Weinberg, 2011). Tumor ECs have also been reported to exhibit genomic aberrations with higher rates of aneuploidy, and the severity of the tumor EC irregularities is dependent on the tumor stage, with angiogenic capacity and rate of aneuploidy being amplified during tumor progression (Akino et al., 2009; Hida et al., 2013, 2016).

Studies if and how metabolism is altered in tumor ECs are lacking and genes fueling tumor EC metabolism have not been documented extensively. One study, reporting tumor EC gene expression profiling, documented an upregulation of lactate dehydrogenase B (LDH-B), although without functional validation (van Beijnum et al., 2006). LDH-B catalyzes the conversion from lactate to pyruvate. Because lactate is actively taken up by ECs via MCT1 (Vegran et al., 2011), it may therefore contribute to tumor EC metabolism via conversion to pyruvate and subsequent entry into the TCA cycle. However, although MCT1 silencing inhibited tumor angiogenesis (Vegran et al., 2011), a follow up study could not confirm MCT1 expression in the vasculature of several tumor samples (Pinheiro et al., 2012).

The proangiogenic signature of tumor ECs suggest a growth factor driven upregulation of key metabolic pathways and even dysregulation thereof, considering the genetic instability and harsh nutrient-deprived environment of tumor ECs. Indeed, GLUT1 expression, driving glucose uptake, is induced in normal ECs by VEGF secreted from cancer cells (Yeh et al., 2008). Furthermore, VEGF stimulates glycolysis via upregulation of PFKFB3 expression, whereas ECs show higher turnover of glycogen stores in low glucose (Vizan et al., 2009; De Bock et al., 2013). Use of glycolysis offers ECs an advantage compared with using oxidative metabolism, otherwise the sprouting into hypoxic regions would limit their energy production and growth. Other growth factors or cytokines stimulating glycolysis in ECs have not been reported so far but are of high interest given that glycolysis can be considered to be the engine that drives angiogenic growth in healthy and pathologic conditions (De Bock et al., 2013; Schoors et al., 2014).

As mentioned above, cells can use FAO for several purposes, i.e., dNTP synthesis, redox homeostasis, or energy production (Dagher et al., 2001; Pike et al., 2011; Jeon et al., 2012; Schoors et al., 2015). Healthy proliferating ECs use FAO for dNTP synthesis, but not only minimally for redox balance and ATP synthesis. Given that tumor ECs are even more proliferative, it will be interesting to explore whether they also upregulate
FAO to match the requirements for increased proliferation. Another outstanding question is whether tumor ECs actively engage in FAO for redox balance as well, because this might additionally equip them with higher reducing power and increase survival in the tumor microenvironment.

Last, it will be of interest to examine whether tumor acidosis affects tumor EC metabolism and signaling because of acetylation events, as recently shown in cancer cells (Corbet et al., 2014). In addition to global changes in the acetylation profile, a specific increase in Sirtuin 1-dependent deacetylation events in acidic regions could lead to changes in angiogenesis-limiting signals. For example, Notch and FOXO1 activity are reduced by Sirtuin 1-dependent deacetylation events (Potente et al., 2007; Guarani et al., 2011).

V. Pharmacological Targeting of Pathologic Angiogenesis

Because pathologic angiogenesis is relevant in cancer and ocular disease (Tah et al., 2015), antiangiogenic treatment approaches have entered routine clinical application more than a decade ago. On the basis of the belief that tumors could be starved to death (Folkman, 1971), multiple efforts were undertaken to develop antiangiogenic substances for the clinic. These finally led to the approval of the humanized monoclonal VEGF antibody bevacizumab for various types of cancer (Hurwitz et al., 2005; Miller et al., 2007) (Table 1). VEGF-targeted therapy is now also an accepted strategy or is being developed to treat ocular disease, characterized by neovascularization (Kim and D’Amore, 2012). For the treatment of different cancer types, various classes of agents targeting VEGF have been developed: monoclonal antibodies, VEGF decoy receptors, and small molecule tyrosine kinase inhibitors (Fig. 4). These agents are in clinical use or are being tested in clinical trials for further indications as monotherapy or in different combinations with standard chemotherapy regimens or chemoradiotherapy. The introduction of this armamentarium of drugs has changed current clinical oncology, showing effective initial responses in some patients and a prolongation of progression free survival for a relevant proportion of patients (Jayson et al., 2016).

However, treatment with agents targeting VEGF signaling often fails to yield sustained responses, because the majority of patients develop resistance (Schneider and Sledge, 2007; Bergers and Hanahan, 2008; Cortes-Funes, 2009; Grothey and Galanis, 2009; Mackey et al., 2012; He and Goldenberg, 2013; Vincent et al., 2015). This, together with the failure of these treatments to extend patients’ survival, highlights the need for alternative antiangiogenic therapies based on fundamentally different principles and mechanisms. We will briefly discuss some possible alternative routes.

First, targeting metabolic changes in cancer cells induced by antiangiogenic agents might increase the...
benefit of anti-VEGF therapy (Quintieri et al., 2014). Metabolic perturbations observed upon VEGF blockade are HIF-1α-driven, activated by hypoxia, which is induced by pruning of tumor vessels by the anti-angiogenic treatment (Keunen et al., 2011). For instance, glycolysis is increased while mitochondrial respiration is reduced, which could render tumors more susceptible to compounds targeting glycolysis (Keunen et al., 2011; Xu et al., 2013). Indeed, reactivation of mitochondrial respiration by dichloroacetate enhanced the effect of anti-VEGF therapy in a preclinical tumor model (Kumar et al., 2013). Also, upon arrest of anti-VEGF treatment, cancer cells can switch from a glycolytic phenotype to a more lipogenic phenotype, thereby fueling a more aggressive and metastatic tumor growth, which could be reverted by blocking FA synthase with the drug orlistat (Sounni et al., 2014). The metabolic evolution of tumors treated with anti-VEGF substances needs further investigation to better understand its etiology and might open future translational possibilities for tumor (angiogenesis) inhibition (McIntyre and Harris, 2015).

Second, targeting EC metabolism itself might be an attractive new therapeutic option. Indeed, pharmacological blockade of PFKFB3 or CPT1A reduced pathologic angiogenesis in ocular disease and inflammatory skin and bowel disease (Schoors et al., 2014, 2015). Antiglycolytic therapy with PFKFB3 blockers is safer than anticipated, because it only transiently and partially lowers glycolysis, without causing harmful systemic effects (Schoors et al., 2014). This new regimen of antiglycolytic therapy of pathologic angiogenesis provides a paradigm shift, because traditional strategies are aimed at blocking glycolysis permanently and maximally (for instance, by administering a high dose of 2-deoxyglucose). Although the latter strategy appears intuitively more efficient, it also causes undesired adverse effects, which limit its success. Hence, administration of a maximally tolerable dose of a glycolysis blocker may not always be the preferred strategy. Rather, the minimal dose of such compound to normalize the deregulated metabolism in activated ECs, sufficient to render activated ECs quiescent again, should be considered. An important difference with cancer cells is that ECs, in contrast to transformed malignant cells, are capable of reversibly switching back and forth their metabolism.

Third, tumor vessels are structurally and functionally highly abnormal, thereby impairing rather than improving tumor vessel perfusion and oxygenation. This creates a hostile microenvironment for cancer cells, deprived of oxygen, nutrients, and growth factors, from where they attempt to escape by metastasizing to distant tissues (Carmeliet and Jain, 2011; Siemann and Horsman, 2015). Recent studies show that strategies of healing and normalizing these tumor vessels improve tumor vessel perfusion, increase the delivery of oxygen and immuno/chemotherapeutics, and tighten the tumor EC barrier, all leading to reduced metastasis and improved chemotherapy delivery and response (Mazzone et al., 2009; Ebos and Kerbel, 2011; Maes et al., 2014). It will be interesting to explore whether targeting of EC metabolic pathways is able to induce tumor vessel normalization and to offer benefit in reducing tumor aggressiveness. Ongoing studies indeed suggest that blocking PFKFB3 might have such effects (unpublished findings).

VI. Conclusion and Outlook

EC metabolism is becoming increasingly recognized as a key determining mechanism of angiogenesis. However, of the >4,000 metabolic enzymes, only a few have been studied in ECs to date at both the metabolic, biologic, and translational level, leaving a very large residual number of unanswered questions on these other enzymes and transporters. Another untested approach is to study, via global untargeted metabolomics, the metabolic signature of ECs from mice and patients with disease, to explore whether deregulated EC metabolism could underlie the excessive vessel growth or, conversely, the EC dysfunction contributing to the particular disease. One more outstanding question is whether EC subtypes in different vascular beds have different metabolic profiles?

The ultimate goal is to translate insights from these preclinical studies into new therapeutic approaches, targeting EC metabolism. However, previous efforts have not always been successful, because clinical trials with aldose-reductase inhibitors, targeting the sorbitol pathway, did not show robust improvement of diabetic retinopathy (Sorbinil Retinopathy Trial Research Group, 1990; Giannoukakis, 2008; Hotta et al., 2012; Tang et al., 2012). Guiding translation of metabolic targets to the clinic will require not only an in-depth understanding of the role these metabolic targets have in EC biology but also careful pharmacokinetic profiling of efficacy versus toxicity and evaluating possible compensatory metabolic escape routes induced by blockade of a particular metabolic target. Finding an answer to these questions will be an interesting adventure for the future.

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