Role of glucose metabolism in ocular angiogenesis (Review)

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Abstract. Glucose metabolism, the major source of energy, plays a crucial role in physiological cell function and the maintenance of homeostasis. Glucose acts as the predominant source of metabolic fuel in the generation of ATP and is involved in biosynthesis and epigenetics. Thus, glucose metabolism maintains a key role in cell function, homeostasis, energy generation, biosynthesis and epigenetics. An increasing number of studies have revealed that glucose metabolism is intricately involved in angiogenesis, with the disruption of angiogenesis contributing to several vascular diseases. Ocular vascular diseases are common ophthalmological disorders, and the prevalence of these disorders is increasing annually. Ocular vascular diseases largely occur from abnormal congenital development or acquired disturbances to the vasculature. Thus, identifying the process of occurrence and development of physiological and pathological angiogenesis is of utmost importance, and this involves understanding the inseparable role of intercellular communications between vascular cells. Although vascular endothelial growth factor (VEGF) is a well-recognized therapeutic target for the management of ocular vascular diseases, VEGF-based therapy fails to achieve the desired therapeutic effects in several cases, partly due to drug resistance and non-compliance. In the present review, current knowledge on the processes and roles of glucose metabolism in governing both physiological and pathological ocular angiogenesis are summarized, highlighting vascular glucose metabolism as a promising strategy for maintaining or restoring the physiological functions of the vasculature, thus potentially ameliorating ocular vascular diseases.

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Contents

1. Introduction

Ocular angiogenesis is a complex pathophysiological process, which is coordinated by a series of intricate and precise molecular mechanisms. Pathological ocular angiogenesis, including retinal, choroidal and corneal neovascularization (CoNV), is a major cause of blindness globally (1). Amongst the most metabolically active tissues in the body, the retina and choroid consume high levels of oxygen and nutrients (2). The principal function of the retinal vasculature is to metabolically sustain the inner retina, while the outer retina is supplied by the choroidal vasculature (3). The innermost layer of all these ocular vessels is lined by endothelial cells (ECs), which are metabolically active and simultaneously maintain vascular homeostasis and systemic metabolism (4); they also play differential roles, depending on their location. ECs differentiate into three distinct subtypes, tip cells, stalk cells and phalanx cells, to permit their adaptation to changes in supply and demand (5). Following induction by a large variety of stimuli, including injury, infection and hypoxia, disruptions in the functions of vascular ECs may lead to a range of ocular vascular diseases due to abnormal angiogenesis, including diabetic retinopathy (DR), retinopathy of prematurity (ROP) and neovascular age-related macular degeneration (nAMD) (2). Anti-angiogenesis therapy, which targets vascular endothelial growth factor (VEGF), has become the primary therapy for the inhibition of pathological ocular neovascularization. This therapy is effective for the majority of patients. However, its use shows some limitations, which often become prominent gradually. These include drug resistance, partly due to the redundancy afforded by other angiogenic signals (6), and non-compliance due to the frequency of injections required. Thus, novel therapies are still needed.

Metabolism is a key feature required for ECs to survive, migrate, proliferate, and grow; thus, it is important for the ocular vasculature (7). Recently, metabolic pathways, including glucose metabolism, fatty acid oxidation and amino acid metabolism, have been identified to be crucial for

angiogenesis in health and disease (4,8). Glucose metabolism consists of two complementary pathways, glucose anabolism and glucose catabolism, which are kept in balance. Glucose catabolism is further divided into anaerobic glycolysis, aerobic oxidation, and the pentose phosphate pathway (PPP). There is increasing evidence to suggest that glucose metabolism controls EC proliferation, migration and neovascularization (9,10). EC activities primarily rely on glucose metabolism, particularly glycolysis, as the source of energy (11,12). It is estimated that only 0.04% of glucose is oxidized, while 98% of glucose is metabolized to lactate in in rat coronary microvascular ECs (13). In human umbilical vein ECs, compared with glutamine oxidation and fatty acid oxidation, glycolytic flux is ~200-fold higher (12). There are three types of ECs: tip, stalk, and phalanx cells. The phalanx cell, keeping a quiescent state, maintains vascular integrity and inhibits inflammation. The forkhead box O1 (FOXO1) protein induced-decrease in glycolysis can keep ECs in a quiescent state and limit the overgrowth of vessels (14). Concurrently, ECs store glucose as glycogen in their intracellular reserves (15). When angiogenesis occurs, phalanx cells activate and transform into tip/stalk cells, which migrate and proliferate to form new blood vessels. This process is highly dependent on glycolysis to provide energy. VEGF and FGF can enhance glycolysis by increasing 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) and hexokinase (HK)-2 to promote the activation of ECs (12,16). Hyperglycemia, a hallmark of diabetes, inhibits glucose phosphorylation and contributes to apoptosis of ocular microvascular ECs, causing diabetic retinopathy through the suppression of HK2 expression (17,18). Therefore, the perturbation of EC glycolytic metabolism results in EC dysfunction and vascular pathologies (10). As 85% of the energy required for ECs to function is obtained from glycolysis, glycolysis may be a potential target for the management of pathological retinal angiogenesis. Accordingly, the elucidation of the underlying metabolic perturbations that occur is crucial for the identification of EC metabolism-centric therapeutics (10).

Here, the processes involved in ocular angiogenesis and the role of cell glucose metabolism are summarized. A deeper understanding of the connection between glucose metabolism and retinal angiogenesis may assist in the development of future therapies and in prevention strategies.

2. Glucose metabolism

Glucose metabolism primarily consists of anaerobic glycolysis and aerobic oxidation, and serves three important aims: Energy generation, biosynthesis and metabolite production. Glucose is first metabolized to generate pyruvate; pyruvate has two different fates, depending on the availability of oxygen, namely anaerobic glycolysis and aerobic oxidation, the latter coinciding also with oxidative phosphorylation. The common factor between both is that they use glucose for the production and sustenance of sufficient supply of energy (19).

Glycolysis. Among the pathways of glucose metabolism present in a cell, the most prevalent and important one is glycolysis (Fig. 1). A glycolysis reaction, literally the lysis of glucose, requires the conversion of glucose into pyruvate and then into lactate as a waste product (20). The retina receives glucose and oxygen from the afferent blood (via hemoglobin), where glucose penetrates the cell membrane and enters cells by glucose transporters, especially glucose transporter 1 (GLUT1) (4). The moment glucose enters the cells, it is phosphorylated to glucose-6-phosphate (G6P) by HK, which is the first rate-limiting step. G6P then converts to fructose-6-phosphate (F6P). The conversion of F6P to fructose-1,6-bisphosphate (F1,6P2) by 6-phosphofructo-1-kinase is the second rate-limiting checkpoint of the glycolytic pathway. Prior to this step, G6P under specific conditions can enter the PPP or can be converted to glucose-1-phosphate, resulting in the initiation of gluconeogenesis (10). Subsequently, F1,6P2 undergoes a series of enzymatic reactions to produce phosphoenolpyruvate (PEP) (12). Pyruvate kinase (PK), the third rate-limiting enzyme, catalyzes the dephosphorylation of PEP to produce pyruvate. In the absence of oxygen, pyruvate is reduced to lactate, which is exported from the cell (20).

In cancer, the presence of a specific mode of glycolysis occurs, which is termed aerobic glycolysis and eponymously known as the 'Warburg effect'. Glycolysis can occur in the presence of oxygen to meet the increased energetic and biosynthetic demands (20). The Warburg effect is a hallmark of cancer and refers to a metabolic shift in cancer cells. In addition, it has been demonstrated that the mammalian retina also displays cancer-like metabolism (21).

Glucose oxidation. Under aerobic conditions, pyruvate can be delivered into a mitochondrion where it is converted to acetyl coenzyme A (acetyl-CoA), which enters the tricarboxylic acid (TCA) cycle, ultimately resulting in the production of ATP via oxidation reactions (Fig. 1). The TCA cycle is an ubiquitous metabolic chain in all aerobic organisms. In the first step, the pyruvate dehydrogenase complex catalyzes pyruvate to form acetyl-CoA. Subsequently, acetyl-CoA is further condensed with oxaloacetate to form citrate by citrate synthase. Citrate is converted to isocitrate by isocitrate dehydrogenase, which is further converted to α-ketoglutarate (α-KG) through oxidative decarboxylation. The α-ketoglutarate dehydrogenase complex catalyzes the conversion of succinyl-CoA from α-KG, and then succinyl-CoA is converted to succinate. Finally, succinate, through fumarate and malate, is reconverted into oxaloacetate. Additionally, during the biochemical reactions involved in the TCA cycle, NADH and FADH2 are generated as byproducts, which feed into the process of oxidative phosphorylation for ATP generation.

Energy. Glucose metabolism plays a critical role in energy generation, and it is the most efficient form of cellular energy generation. The entire glycolytic reaction produces 2 single molecules of ATP (22); however, the complete oxidation of glucose can produce approximately 30/32 total ATP molecules (23). Following the completion of the ocular vasculature development, the majority of vascular ECs are quiescent. Under pathological conditions, including hypoxia, infection and injury, the quiescent ECs are potently activated to produce new vessels (24), and this process requires an adequate supply of energy. Surprisingly, although glucose oxidation produces significantly more ATP than glycolysis, the energy consumed by ECs is primarily derived from glycolysis, accounting for 85% of the energy supply, even when the oxygen supply is

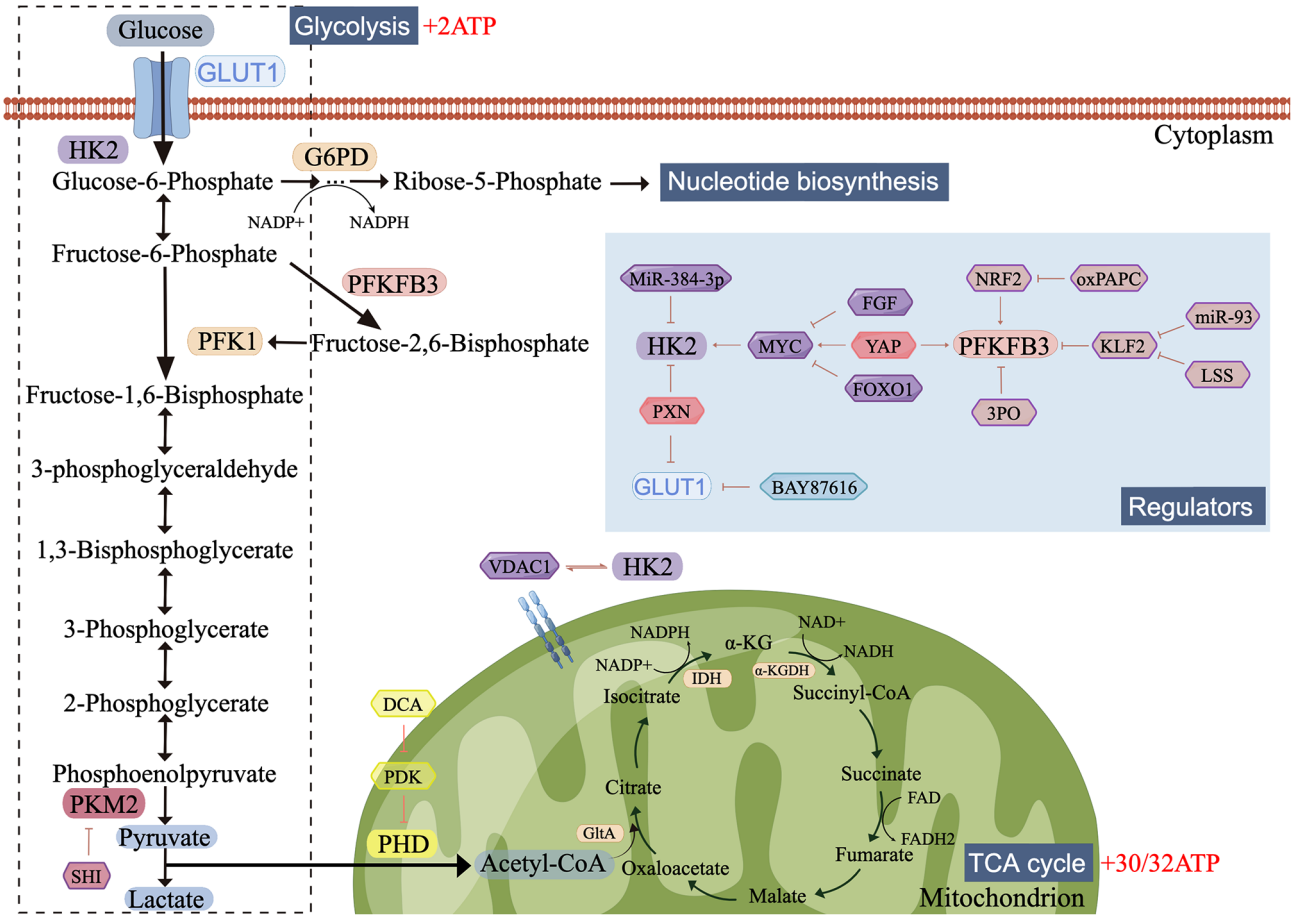

Figure 1. Glucose metabolism and the regulators involved. Glucose is first metabolized to generate pyruvate; pyruvate is then reduced to lactate or enters the TCA cycle, depending on the availability of oxygen. Several regulators, including growth factors, transcription factors and miRNAs, mediate glucose uptake and flux. The figure was created using Figdraw (www.figdraw.com). TCA, tricarboxylic acid; GLUT1, glucose transporter 1; HK2, hexokinase 2; G6PD, glucose 6-phosphate dehydrogenase; PFKFB3, phosphofructokinase-2/fructose-2,6-bisphosphatase-3; PFK1, phosphofructokinase-1; PKM2, Pyruvate kinase-2; PHD, prolyl hydroxylase domain; Acetyl-CoA, acetyl coenzyme A; VDAC1, Voltage Dependent Anion Channel 1; GltA, citrate synthase; IDH, isocitrate dehydrogenase; α-KGDH, α-ketoglutarate dehydrogenase; SHI, shikonin; PDK, pyruvate dehydrogenase kinase; DCA, Dichloroacetic acid; PXN, Paxillin; FGF, fibroblast growth factor; YAP, Yes-associated protein; FOXO1, Forkhead box O1; NRF2, Nuclear factor E2-related factor; 3PO, 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one; oxPAPC, 1-palmitoyl-2-arachidonoyl-sn-glycero-3phosphocholine; KLF2, Krüppel-like factor 2; LSS, laminar shear stress.

sufficient (12). This phenomenon may be explained by the observation that the mammalian retina exhibits a cancer-like metabolism known as aerobic glycolysis (25); thus, the dependence on glycolysis is not surprising. At first glance, glycolysis is a low-efficiency form of energy production; however, the rate of production is faster (12). Glycolysis is essential in allowing hypoxic tissues to restore blood supply in a timely manner. In addition, when the ECs migrate to avascular areas where the conditions are relatively hypoxic, a shortage of oxygen supply makes glycolysis the only route of energy production for ECs (26). Additionally, the mitochondrial content in ECs is relatively reduced (27) and glycolysis can result in the avoidance of oxidative damage in ECs, by reducing the production of reactive oxygen species (ROS) levels (28).

Carbon for nucleotide biosynthesis. In addition to energy generation, glucose metabolism has another significant function, being involved in biosynthesis directly. For example, intermediates of glucose metabolism can act as precursors for the *de novo* synthesis of nucleotides. Glycolytic intermediates can enter the PPP, also referred to as the phosphogluconate pathway and the hexose monophosphate shunt, to provide carbons for nucleotide biosynthesis (29). In the PPP, G6P is reduced to ribose-5-phosphate (R5P) by the rate-limiting enzymes glucose 6-phosphate dehydrogenase (G6PDH), 6-phosphogluconolactone (6PGL), and 6-phosphogluconate dehydrogenase (6PGDH), concurrently consuming NADP+ to generate NADPH, which is used for ROS scavenging (30). R5P then serves as the starting material in nucleotide biosynthesis. Thus, inhibition of G6PDH compromises nucleotide biosynthesis and vessel sprouting (31). In summary, glucose metabolism is essential for nucleotide biosynthesis. The key enzymes involved in these pathways also represent promising anti-neovascularization therapeutic targets.

Epigenetics and glucose metabolism. In addition to energy generation and biosynthesis, glucose metabolism is required for epigenetic modifications and gene regulation by providing metabolites that act as substrates or co-factors for several important enzymatic reactions (32). Epigenetics refers to the capability of the same genome to produce multiple distinct, yet stable, phenotypes through chemical modifications of chromatin, without alterations to the original DNA sequence (33,34). Epigenetics play crucial roles in regulating

gene expression and governing cellular phenotypes and consist of histone modifications, DNA methylation and RNA-mediated processes. In cancer, epigenetics and metabolism are highly interconnected in an interdependent manner. Variations of the expression of acetyl-CoA controlled by glycolysis greatly affect the histone acetyltransferase-mediated histone acetylation (35). Furthermore, high lactate levels caused by glycolysis result in the generation of a local acidic pH that promotes histone deacetylation (36). Promoter hypomethylation in turn upregulates HK2 and facilitates glycolytic flux in glioblastoma and hepatic carcinoma (37). Collectively, glucose metabolism and epigenetics affects each other to co-regulate a range of physiological and pathological activities including angiogenesis.

3. Glucose metabolism in physiological ocular angiogenesis

Physiological angiogenesis. The formation of the vasculature is primarily mediated by two mechanisms: Vasculogenesis and angiogenesis (3,38). Vasculogenesis refers to the process of *de novo* vessel formation from undifferentiated precursor cells during early embryonic development. Angiogenesis, a complex process of new vessel formation from existing vessels, is the predominant mode of retinal vessel growth (39). The mature retina consists of 10 layers from the inner limiting membrane to the retinal pigment epithelium (RPE) and is supplied with dual blood supplies by both the retinal and choroidal vasculatures that supply the inner and outer layers, respectively (40). During embryonic and early fetal development, oxygen and nutrients are delivered to the retina by hyaloid vessels (41). In humans, hyaloid vasculature formation, regression, and the majority of retinal vasculature development occur before birth. In mice, the retinal vasculature develops postnatally (3).

Hyaloid vasculature. The hyaloid vasculature arises from the central hyaloid artery (HA), which enters from the optic fissure and extends into the primitive vitreous at around 5 weeks gestation (WG) (42). Subsequently, the HA runs through the primitive vitreous to the posterior lens surface, forming a dense capillary network, known as the tunica vasculosa lentis (TVL), forming the vasculosa hyaloidea propria in a more proximal position of the vitreous. The TVL expands further around the anterior part of the lens forming the pupillary membrane and eventually drains into the choroidal veins (43). The hyaloid vasculature is characterized by the absence of veins; thus, all hyaloid vessels are arteries and the venous drain is accomplished by the choroidal vessels (43). The hyaloid vessels exhibit clear evidence of regression at ~13-15 WG and culminate in the involution of the entire hyaloid by 35-36 WG (44).

Retinal vasculature. The retinal vasculature is an ocular circulatory system that consists of the primary plexus, secondary plexus and deeper plexus. The primary plexus of the retinal vasculature emerges from an established capillary ring at the optic disc at ~15 WG and subsequently spreads across the inner surface of the retina to the ora serrata nasally at ~36 WG, finally reaching the ora serrata temporally at ~40 WG (38,39) (Fig. 2A). Consistent with the extension of the primary plexus, the deeper plexus originates from the primary plexus veins at the optic nerve head to the periphery in a manner of angiogenesis sprouting, at ~25 WG (3,38). The development of a deeper plexus continues after birth until the retinal vasculature is terminally matured. Notably, during the entire development process, there is a completely avascular region known as the fovea (38,45).

Retinal vasculature development is closely associated with a multitude of cells, including ECs, astrocytes, microglia and pericytes. Astrocytes develop from astrocyte progenitor cells (APCs) and are present only within the vascularized area; thus, the macular hole and the avascular region of the peripheral retina lack astrocytes (3,38). Under platelet-derived growth factor receptor (PDGF) stimulation, which is secreted by retinal ganglion cells (Fig. 2B), APCs invade the retina through the optic disc, then expand further across the nerve fiber layer toward the peripheral margins of the retina forming a meshwork. APCs invade prior to retinal vasculature development; however, APCs do not differentiate or mature at this point. Along with the transmigration of ECs into the retina, APCs are stimulated to differentiate into mature astrocytes. Differentiated astrocytes, located primarily at the leading edge of the invading ECs, and can sense hypoxia signals and secrete VEGF, which mediates the migration and proliferation of ECs. With the extension of vessels, a sufficient oxygen supply in turn also contributes to astrocyte maturation (38). In response, astrocytes decrease VEGF synthesis and limit further growth of retinal vessels by local feedback mechanisms (46).

ECs line the inner surface of vessels to support tissue growth and repair. The growth of the retinal vasculature depends on the sprout formation of ECs similar to plant germination, which requires ECs to display different phenotypes based on their location. ECs compete with each other for the leading tip position (47) (Fig. 2B). A specialized EC at the distal end of each sprout extend long filopodial protrusions, termed as the tip cell. Stalk cells form behind tip cells in endothelial sprouts (47,48). Tip cells are nonproliferative and can migrate, lead, and guide the vessel sprouts, while the extension and perfusion of vessels mainly rely on stalk cells and further vascular remodeling (48). The tip/stalk cell phenotype can be regulated by the balance between a variety of angiogenic factors and their downstream signaling pathways, such as VEGF/Notch signaling (49). VEGF expression is higher in the peripheral retina and this promotes the transcription of the notch ligand Delta like 4 (DLL4) in tip cells, concurrently inhibiting DLL4 transcription in adjacent cells (50,51). As a result, the adjacent cells finally differentiate into stalk cells. Tip cells tightly attach to astrocytes and radially extend to the avascular area via various long, dynamic actin-based filopodia along the astrocyte mesh template under the stimulation of VEGF, guiding the direction of growth (3). Following tip cells, stalk cells extend fewer filopodia but proliferate to support sprout elongation and form the vessel lumen. Adjacent sprouts fuse to establish vessel loops (52). The sprouting process iterates and creates a primitive plexus, which is later remodeled into structured, hierarchical vascular trees. There is a specialized population of ECs, the cobblestone-like appearance of quiescent ECs, termed phalanx cells (53). Under hypoxic conditions, oxygen sensors become inactive, then phalanx cells can express oxygen sensors to regulate vessel perfusion, mediated by prolyl hydroxylase domain proteins (52). At the initiation of angiogenesis, quiescent ECs are rapidly activated and switch to a proliferative state, ultimately enhancing glycolysis (52).

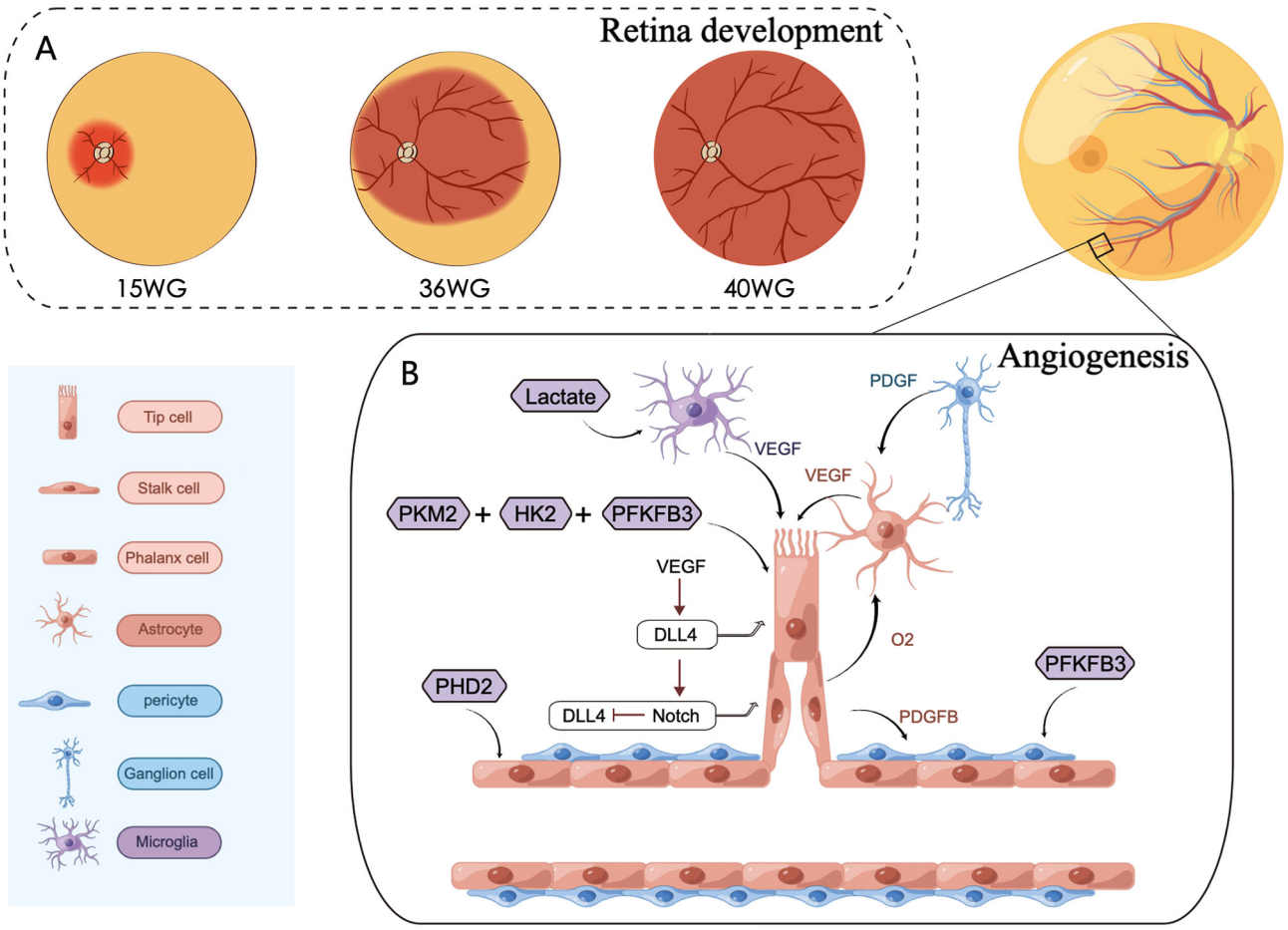

Figure 2. The process of retinal angiogenesis. (A) The primary plexus of the retinal vasculature emerges from an established capillary ring at the optic disc at ~15 WG and subsequently spreads across the inner surface of the retina to the ora serrata nasally, at ~36 WG, finally reaching the ora serrata temporally at ~40 WG. (B) Endothelial cells differentiate into three subtypes: Tip cells, stalk cells and phalanx cells, depending on their location, and interact with angiogenesis-related cells to promote retinal vasculature development. This process is regulated by metabolic enzymes. The figure was created using Figdraw (www.figdraw.com). WG, week gestation; PKM2, pyruvate kinase-2; HK2, hexokinase 2; PFKFB3, phosphofructokinase-2/fructose-2,6-bisphosphatase-3; PHD, prolyl hydroxylase domain; VEGF, vascular endothelial growth factor; PDGF, platelet derived growth factor receptor; DLL4, Delta like 4.

During sprouting, tip cells migrate to avascular areas, where oxygen is absent and VEGF mRNA expression is higher. In this condition, glycolysis provides adequate energy for rapid vessel growth and mature. In turn, the new vessels can provide adequate oxygen (12). In response to adequate oxygen levels, the expression of VEGF decreases, ECs stops migrating and switches from an activation state (tip cells) into a quiescent state (phalanx cells). Thus, the migration and proliferation of activated ECs relies on glycolysis. However, with the establishment of vessels, ECs will again transform into a quiescent state and reduce the glycolytic rate (26).

Pericytes are specialized mural cells located at the abluminal surface of capillary blood vessels (54) and together with ECs play a major role in angiogenesis, participating in vessel formation, remodeling, and stabilization (55). During angiogenesis, the EC-specific ligand PDGFB can bind with high affinity to PDGF receptor B (PDGFRb) secreted by pericytes, inducing the recruitment and attachment of pericytes (Fig. 2B) (54). Studies have revealed that the inactivation of PDGF-B/PDGFRb signaling results in reduced retinal pericyte coverage, leading to endothelial hyperplasia, abnormal vascular morphogenesis, and the formation of microaneurysms (52,56,57). Compared to glucose oxidation, glutamine oxidation and fatty acid oxidation, glycolysis also provides up to 85% of the ATP required by pericyte (58). Under high glucose levels, Notch3 gene downregulation in pericytes reduces PDGFR levels, resulting in disorders of endothelial-pericyte interactions, finally leading to pericyte apoptosis (59). In cancer, the treatment of pericytes with 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO), an inhibitor of PFKFB3, has been shown to reduce glycolysis, whereas adherence to ECs is enhanced, which may be explained by the fact that 3PO upregulates N-cadherin levels in pericytes (58). Overall, appropriate glycolysis may facilitate the maintenance of pericytes in a quiescent state and their adherence to ECs. However, whether this phenomenon exists in the retina remains to be further confirmed.

Macrophage/microglia originate from retinal myeloid cells, and possess high glycolytic characteristics similar to ECs (11). Microglia are resident macrophages in the central nervous system (60). Several unique stimulatory factors, such as lactate, have been demonstrated to alter macrophage metabolism (61) and induce macrophage differentiation into an M2 phenotype, which in-turn enhances retinal angiogenesis (11) (Fig. 2B). For example, proangiogenic cytokines, including VEGF, released from macrophages, also facilitate EC glycolysis, resulting

in retinal neovascularization (RNV) (11). It also has been reported that the deletion of macrophages by mannosylated clodronate liposomes suppressed pathological angiogenesis and facilitated physiological angiogenesis (62).

Choroidal vasculature. The development of the choroidal vasculature precedes that of the retinal vasculature, and occurs via a mechanisms termed hemo-vasculogenesis, which is distinguished from vasculogenesis and angiogenesis (63). Hemo-vasculogenesis refers to the blood vessels, blood cells having a common precursor, the hemangioblast. The choroidal vasculature is divided into three layers, namely the anterior choriocapillaris (CC), Sattler's layer of intermediate vessels, and the outermost Haller's layer (63). The choroidal vasculature is accountable for nourishing the outer retinal layers, particularly photoreceptors and RPE. The CC initiates the formation of a single layer, originating from islands of progenitors by hemo-vasculogenesis during 6-8 WG (64). At 6-7 WG, erythroblasts can be observed in the CC layer and distributed in the forming choroidal stroma (63). By 8.5 WG, the vascular lumens become apparent (64). At 11-12 WG, the deeper choroidal vasculature can be observed in the posterior pole and the equatorial choroid follows closely. Simultaneously, certain ECs proliferate and sprout from the scleral side of CC, indicating that the formation of intermediate vessels is mediated by angiogenesis, apparently promoting the development of intermediate vessels and the confluence of capillaries and large vessels (64). These developing vessels in the central choroid are more mature, as pericyte-like cells are also present in that position (65). At 14-16 WG, cells surrounded by pericytes contact with ECs lining the lumen via peg-in-socket-like contacts, a feature of the normal adult microvasculature (65). By 21 WG in the posterior pole region, three layers of blood vessels of CC have manifested and further expand and remodel after 22 WG (63).

Key regulators of glucose metabolism in physiological angiogenesis. In recent years, given the highly plastic phenotype of ECs in retinal angiogenesis, the understanding of the regulators controlling the glucose metabolism of ECs has substantially increased, including the involvement of metabolic enzymes and transcription factors. Targeting these regulators may hold significant potential in maintaining vascular homeostasis. Crucial elements regulating glucose metabolism are outlined below (Fig. 1):

HK2. HK2 is an isoform of the HK modulating the first rate-limiting step in glycolysis, as described above. As previously demonstrated, HK2 knockdown significantly reduced, while HK2 overexpression increased, glycolysis and retinal angiogenesis (16). High glucose reduced HK2 levels and interactions with voltage-dependent anion channel, a protein enriched in the outer mitochondrial membrane, resulting in apoptosis of human umbilical vein ECs (HUVECs) by impairing mitochondrial permeability (18).

Fibroblast growth factor (FGF) is an essential growth factor that binds to the FGF receptor (FGFR) to activate various signaling pathways (66). FGFR1 and FGFR3 belong to the FGFR family and are expressed in mice and humans. FGFR3 levels in human dermal lymphatic ECs were previously demonstrated to be upregulated by FGFR1 knockdown; however, FGFR3 knockdown did not affect FGFR1 levels (16). FGFR1 knockdown resulted in the inhibition of HUVEC proliferation, decreased tip cell numbers, and impaired retinal vascular branching and growth, whereas FGFR3 knockdown had no effect (16). Further investigations indicated that FGF or FGFR1 knockdown decreased HK2 expression (16). Mechanistically, the link between FGF and HK2 involves MYC regulation, which can coordinate cellular proliferation and metabolism (16,67). FGF thus controls glycolysis-mediated retinal angiogenesis through MYC-dependent regulation of HK2 expression.

PFKFB3. PFKFB, a member of the bifunctional enzyme family, can facilitate the synthesis of fructose-2,6-bisphosphate (F2,6BP2), which is an allosteric activator of phosphofructokinase-1 (68). Among all the PFKFB isozymes, PFKFB3 has the highest kinase activity (69). In a previous study, the knockdown of PFKFB3 *in vitro* or the genetic silencing of PFKFB3 in developing retina decreased F2,6BP2 levels, reduced glycolytic flux, and decreased vessel sprouting and vascular plexus expansion under physiological conditions in mice. Conversely, PFKFB3 overexpression exerted the opposite effect (12). Mechanistically, PFKFB3 inhibition induced these defects partly due to impaired EC proliferation, but more prominently through the reduced competitiveness of tip cells, and abrogated tge interactions between PFKFB3 and actin (12).

1-Palmitoyl-2-arachidonoyl-sn-glycero-3phosphocholine (PAPC) is a complex phospholipid enriched in cell membranes and is easily susceptible to oxidation to oxPAPC, which can stimulate EC sprouting and proliferation (70). Nuclear factor E2-related factor (NRF2), a transcription factor, has been reported to be a mediator of the oxPAPC response (71). miR-93 is the most abundant miRNA in ECs; EC proliferation and glycolysis were previously observed to be significantly induced following miR-93 overexpression (72). The overexpression of oxPAPC led to similar results as those for miR-93. Additionally, the effects of miR-93 and oxPAPC overexpression on glycolysis and proliferation could be reversed by the silencing of NRF2. Together, NRF2 regulates endothelial glycolysis and proliferation via miR-93 and oxPAPC (71).

Shear stress, a force that blood flow exerts on the ECs, is broadly categorized into disturbed or laminar shear stress (LSS) (73,74). LSS plays a crucial role in the activation of ECs from a quiescent state (73). In a previous study, when HUVECs were exposed to LSS for 72 h, the glycolysis and mitochondrial activity of the ECs was observed to be reduced. The silencing of Krüppel-like factor 2 (KLF2), a transcription factor that stabilizes the quiescent EC phenotype, reversed this alteration (9). Subsequently, RNA sequencing revealed that PFKFB3 was downregulated, following the overexpression of KLF2. Thus, the simultaneous overexpression of KLF2 and PFKFB3 revealed that the reduction of PFKFB3 was the cause of KLF2-mediated inhibition of glycolysis (9). Overall, LSS inhibited EC glycolysis contributing to the repression of PFKFB3 mediated by KLF2; however, whether this occurs *in vivo* also remains to be established (9). Taken together, multiple means of inhibition of PFKFB3 reduce vessel formation under physiological conditions.

PKM2. PKM2 is an isoform of PK, which is the final catalytic step involved in glycolysis. As previously demonstrated, PKM2 silencing inhibited glycolysis, generated fewer and shorter sprouts with few filopodia in ECs *in vitro*, and diminished radial vascular growth, although it had

no effect on vascular density *in vivo* (75). High-resolution confocal microscopy revealed that PKM2 was enriched at the VE-cadherin-mediated endothelial junctions and accumulated at F-actin-rich filopodia and lamellipodia of tip cells (75). The lower number of junctions and impaired endothelial barrier were detected in PKM2-deficient ECs. In another study, ECs treated with shikonin (SHI), a pharmacological inhibitor of PKM2, also exhibited a reduced migratory ability; however, their proliferation levels remained unaltered (75). Taken together, whereas PKM2 is pivotal in retinal angiogenesis BY regulating EC migration, the number of filopodia and VE-cadherin-mediated EC junctions, it is not required for EC proliferation. However, another study procured the opposite results, demonstrating that PKM2 knockout suppressed EC proliferation via the upregulation of p53, a transcription factor blocking cell cycle progression, a process that is independent of the activity of PK (76). In cancer, PKM2 has been demonstrated to interfere both with NF-κB/p65 and HIF-1α activation, which ultimately triggers VEGF-A secretion and subsequent blood vessel formation (77).

GLUT1. GLUT1 is one of the primary transporters of glucose and is required for endothelial glycolysis. It has been revealed that GLUT1 inhibition by BAY87616, a highly selective GLUT1 inhibitor, reduces glucose transport and glycolysis in human retinal microvascular ECs and HUVEC. Of note, GLUT1 inhibition attenuates EC proliferation and sprouting angiogenesis, but not migration or viability (78). Surprisingly, the number of tip cells was not significantly altered under GLUT1 starvation, despite the fact that tip cells are highly dependent on glycolysis. Overall, GLUT1 can regulate retinal angiogenesis via glycolysis-mediated EC proliferation, but not via migration (78). The Notch and VEGF signaling pathways have been revealed to modulate the expression of GLUT1 in ECs (12,78,79).

Other mediators. FOXO, a transcription factor, is a downstream effector of the PIK3/AKT pathway that connects vascular growth with metabolism (74,80). FOXO1 is a member of the FOXO family and is highly enriched in ECs, according to the immunofluorescence analysis data of a previous study (14). In that study, the loss of FOXO1 in ECs resulted in abnormal retinal angiogenesis, and the ECs became dense and hyperplastic, resulting in the inability of tip cells to correctly sprout (14). The overactivation of FOXO1 in ECs led to a hyper-pruned vasculature network and a thinner lumen in retinal vessels (14). Thus, FOXO1 is a suppressor of EC proliferation and retinal angiogenesis. As evidenced by a reduction in glucose uptake, glycolytic flux and lactate production, FOXO1 activation was shown to lead to a robust reduction in glycolysis. Moreover, transcriptional analysis revealed that MYC, a potent driver of glycolysis, was downregulated under the same conditions. Taken together, FOXO1 inhibited retinal angiogenesis via the decreased glycolysis capacity mediated by MYC (14).

4. Glucose metabolism in pathological ocular angiogenesis

Abnormal vessel growth, insufficient vessel growth, or uncontrolled vessel growth, promotes ocular disease and poses a threat to normal vision. As a consequence of dysregulated angiogenesis, oxygen and nutrients are not correctly delivered, leading to an imbalance in metabolic demand and supply and disturbed neural retinal function (2). The pathological process of angiogenesis is associated with several diseases, including ROP, DR and age-related macular degeneration (AMD), amongst others.

Retinal neovascularization

ROP and DR. In humans, the majority of retinal vessels complete development before birth, whereas normal retinal angiogenesis is arrested in the preterm infant. Consequently, pathological compensatory mechanisms are excessively triggered, and this is hypothesized to finally result in the aberrant vascularization of the retina, known as RNV (81). This process is known as ROP. ROP has two postnatal phases: An initial phase of vessel loss, followed by a second phase of vessel over-proliferation (81,82). In the first stage, the relative hyperoxic environment post-birth compared with that *in utero* suppresses the expression of oxygen-regulated angiogenic growth factors in astrocytes, Müller cells, pericytes and the RPE through HIF-1α, leading to EC apoptosis, the cessation of retinal vessel growth and the regression of existing vessels (2,83). At this stage, only partial vascularization has a chance to be observed in the developing retina. In the second stage, given the increase in metabolic activity, dysplastic retinal vessels fail to provide an adequate amount of oxygen and nutrients, resulting in the pathological proliferation of vessels in response to VEGF upregulation (81). HIF-1α is also upregulated to initiate transcription of the genes as a response to hypoxia, including VEGF (84). Ultimately, the retinal leakage and detachment contribute to impaired vision.

DR, one of the most common complications of diabetes, remains a leading cause of visual impairment and blindness (85). Hyperglycemia and other metabolic dysregulations lead to microvascular damage and retinal function disorder (86). At the onset of DR, the vessel wall is compromised due to the loss of supporting pericytes and/or glial attachment leading to capillary wall dilatation (microaneurysms), leakage (edema and hard exudates), and rupture (hemorrhages) (86). As the severity of DR progresses, capillary occlusion leads to retinal ischemia, which, in turn, induces the upregulation of VEGF, driving pathological neovascularization. Severe retinopathy may end with macular edema and retinal detachment.

As described above, the neovascularization of ROP and DR are both attributed to the severe imbalance in blood and oxygen between demand and supply. The disruption of angiogenesis reduces the oxygen supply, thus resulting in the upregulation of pro-angiogenic factors, which can act directly on ECs to stimulate excessive retinal angiogenesis. The mouse model of oxygen-induced retinopathy (OIR) is a well-recognized model for RNV (87). As previously demonstrated, mice, from postnatal day 7 (P7), were exposed to a hyperoxic environment (where the concentration of oxygen was maintained at 75%) for 5 days (until P12), after which mice were subsequently maintained under normal conditions (21% oxygen). RNV peaked at P17 (87). As described above, this process involves initial vessel loss (P7-P12), neovascularization (P12-P17), and neovascular regression (P17-P25) (87).

Key regulators of glucose metabolism in RNV. miRNAs are small non-coding RNAs involved in almost all biological

processes, playing critical roles in cell proliferation, growth, apoptosis and vascular neovascularization (88). miR-384-3p has been confirmed to inhibit the proliferation of human retinal microvascular ECs (HRMECs) via the downregulation of HK2 and the inhibition of RNV in DR (89). Moreover, the genetic loss of GLUT1 results in a significant reduction in glycolysis, EC proliferation and the branch point density of retinal vessels in developing postnatal mice (78).

ECs treated with 3PO or 7,8-dihydroxy-3-(4-hydroxyphenyl)-chromen-4-one, small molecule inhibitors of PFKFB3, present with dose-dependently reduced glycolysis in ECs and the formation of RNV in mice with OIR (69). However, 2-deoxyglucose (2-DG), another glycolytic blocker, induced EC disintegration and eventual death, and this may be attributed to the near-complete inhibition of glycolysis achieved by 2-DG (69). In tumors, 3PO treatment had no effect on tumor growth and cancer cell proliferation. However, decreased glycolysis in pericytes, resulting in the higher coverage of pericytes, which promoted tumor vessel normalization (58). It remains to be determined whether 3PO normalizes the previously formed pathological vessels in the retina; however, it was previously demonstrated that in adult healthy mice treated with 3PO for 15 days, there was no effect on the healthy retinal vasculature system, and the perfusion of retinal vessels (90).

Yes-associated protein (YAP) is a critical downstream effector of the Hippo signaling pathway and functions as a transcription cofactor that binds the TEA domain transcription factor (TEAD) to initiate the expression of target genes (91,92). There is increasing evidence to indicate that YAP is associated with both physiological and pathological angiogenesis (93). The YAP-TEAD1 complex has been demonstrated to activate the transcription of PFKFB3 via binding to the PFKFB3 promoter, and it has also been shown that blocking the YAP/PFKFB3 axis significantly inhibits hypoxia-induced glycolysis by decreasing the secretion of VEGFA and VEGFR1 (94). Based on this observation, the role of the YAP/PFKFB3 axis in retinal angiogenesis was further explored and it was found that the inhibition of either YAP or PFKFB3 could restrict the biological function of ECs and suppress RNV (94). Conversely, as demonstrated in another study, the suppression of YAP transcription also reduced glycolysis, damaged filopodia protrusion and contributed to impaired retinal angiogenesis, and these effects were dependent on the downregulation of MYC, another potent driver of glycolysis (93).

Adenosine/adenosine receptor-mediated signaling has been implicated in ischemic diseases and is regulated by hypoxia. Ischemic proliferative retinopathy-induced hypoxia involves an increase in adenosine and adenosine receptor levels (95). Peak adenosine levels are temporally related to active vasculogenesis in the retina in the model of OIR (96). The expression of adenosine A2a receptor (Adora2a), an adenosine receptor, was shown to increase markedly via a HIF-2α-dependent mechanism in HRMECs and in mice with OIR. Activated Adora2a, subsequently promoted glycolysis and retinal angiogenesis through HIF-1α accumulation. The deletion of Adora2a reversed the increase in the number and length of sprouts induced by the pharmacological inhibition of Notch signaling (97). Collectively, as critical metabolic regulators in ECs, miR-384-3p, HK2, PFKFB3, GLUT1, YAP and Adora2a may be potential targets in the management of RNV.

Choroidal neovascularization (CNV)

AMD. AMD is also one of the leading causes of vision loss. AMD can be classified into early and late stages. nAMD is the advanced stage of AMD and is accompanied by pathological angiogenesis; new blood vessels from pre-existing choroidal vessels intrude through Brunch's membrane (BM) into the RPE or sub-retinal space (98). This destructive process is termed CNV and is referred to as subretinal neovascularization (98). The junction of the choroid and the retina is comprised of the RPE, BM, and choroidal capillaries (63). When certain triggers upregulate VEGF expression in RPE cells and the BM is disrupted by proteases, choroidal vessel growth becomes disorderly and extrudes into sub-retinal space, resulting in CNV (98). Given that the choroidal vasculature has not YET been adequately studied, the pathogenesis of nAMD is not well defined and it is currently hypothesized to be multifactorial (99). Laser-induced CNV is the most efficient *in vivo* model available to study the mechanisms of CNV. In mice with experimental CNV, laser injury caused by the rupture of the BM has been shown to result in cell damage and hypoxia, culminating in neovascular lesions occurring on day 5 and reaching their peak on day 7 (100).

Key regulators of glucose metabolism in CNV. The levels of lactic acid, the end-product of glycolysis, can induce reprogramming in several cells and are considered a marker of an underlying pathologies, including types of cancer (101). Lactic acid levels have been shown to be notably increased in the serum of mice with laser-induced CNV on day 5 (102). Dichloroacetic acid (DCA) can suppress pyruvate dehydrogenase kinase (PDK), which is a modulator of lactate levels, by inactivating the pyruvate dehydrogenase complex involved in the pyruvate conversion into acetyl-CoA. As previously demonstrated, treatment with DCA significantly inhibited CNV, with the optimal inhibition observed during the late-angiogenic period (days 4-7) (103). Moreover, lactate has extra functions in maintaining the M1/M2 macrophage balance in favor of M2 macrophages by promoting the transformation M1 macrophages to M2 macrophages (103). Another study further demonstrated that VEGFA mRNA and VEGFA protein expression levels were only elevated in lactic acid-treated macrophages. Thus, high VEGF levels enhanced neovascularization. Finally, inhibiting lactate acid transport by the intravitreal injection of α-cyanohydroxycinnamic acid, a monocarboxylate transport blocker, downregulated VEGF levels and the subsequent CNV (101). Vallée *et al* (104) revealed that enhanced WNT/β-catenin pathway activity stimulated the PI3K/Akt pathway and HIF-α, which activated glycolytic enzymes (Glut, HK, PDK and LDH-A). This process resulted in aerobic glycolysis, representing the accumulation of lactate that initiates the expression of VEGF to promote angiogenesis in nAMD. A previous study also demonstrated that pyruvate, lactate levels and the lactate/pyruvate ratio were increased in urine samples collected from patients with typical AMD, indicating that glycolysis may be involved in the aggravation of AMD (105).

In clinical samples, higher levels of the intermediates of the TCA cycle were similarly detected in patients with active nAMD. In a previous study, using ultrahigh-performance liquid chromatography-tandem mass spectrometry, the detection of energy metabolites in the aqueous humor of with AMD revealed that citrate and isocitrate levels were significantly increased in the AMD group, whereas succinate and α-ketoglutarate levels were significantly decreased compared with the control group (106). Low α-ketoglutarate levels contribute to the stabilization of HIF-1α and the secretion of VEGF-A, which further promotes progression to CNV (107). Thus, the dysregulation of the TCA cycle may also be a driving force in nAMD. However, the underlying mechanisms require further investigation (106).

Another study also revealed that the deregulation of glucose metabolism was associated with CNV (108). Following feeding with a high-fructose/high-fat (HFHF) diet, rats began to exhibit fasting hyperglycemia, glucose intolerance and insulin resistance, indicative of an impaired glucose metabolism, which promoted CNV compared to rats fed a standard diet. Simultaneously, using laser photocoagulation to trigger CNV and feeding with an HFHF diet induced the exacerbation of CNV. This result indicated that an HFHF diet promotes a favorable environment for neovascular events. Moreover, an HFHF diet increased the expression of glial fibrillary acidic protein, an activator of glial cells. Sustained injury induced by lasers in the BM also lead to the activation of glial cells and an HFHF diet further promoted this activation to propagate to the rest site of the retina (108).

3PO has been demonstrated to reduce the CNV lesion volume in laser-injured mice by inhibiting PFKFB3 (69). SHI oral gavage has been confirmed to alleviate the leakage, area and volume of mouse laser-induced CNV lesions, and inhibit macrophage infiltration without any evidence of ocular cytotoxicity through inhibiting PKM2 (109). Taken together, these findings highlight a potential strategy for the management of CNV.

CoNV. The cornea is optically clear with the absence of blood vessels, which is required for corneal transparency and the maintenance of vision. The corneal epithelium combined with protective factors forms a barrier that protects the cornea from injuries and corneal infiltration by blood vessels. By contrast, CoNV results from the invasion of blood vessels from the limbal vascular plexus into the cornea in multiple pathological states, including hypoxia, inflammation, infection, injury and degeneration (110).

For example, inflammation-related injury disrupts the homeostasis between proangiogenic cytokines and anti-angiogenic factors (111). Along with the accumulation of proangiogenic stimuli, including VEGF, the vascular ECs (VECs) surrounding the limbal vasculature begin to activate and release proteolytic enzymes that degrade the BM of vessels and the corneal extracellular matrix, ultimately permitting VECs to invade the corneal stroma. The invaded VECs further proliferate, migrate, sprout and grow, resulting in neovascularization in the cornea (110). Metabolism plays a critical role in the transportation of oxygen through the cornea. High lactate levels were observed in the hypoxic cornea, resulting in an increase in stromal lactate concentration (110).

Paxillin (PXN) is a vital component of focal adhesion. The deletion of PXN reduces the levels of HK2 and GLUT1, resulting in a decrease in lactate and ATP levels in HUVECs or the VEGF-treated cornea, protecting against VEGF-induced EC invasion and angiogenesis (112). In addition, as previously demonstrated, rats with streptozotocin-induced diabetes presented with a reduced degree of alkali injury-induced CoNV compared with the control rats, although the difference was not significant. This may be related to the effect of high glucose on wound healing and the dissimilar to circulatory high-glucose stimulation responses in CoNV development, amongst different species (113).

To date, the relevance between CoNV and glucose metabolism has not been intensively investigated. Considering the strong link between glucose metabolism and ECs, however, the regulators of glucose metabolism mentioned above may have potential roles on CoNV, and further research is required in order to fully elucidate this.

5. Conclusions and future perspectives

Over the past several decades, ocular vascular development has been thoroughly studied at the molecular level; however, studies at the metabolic level are lacking to a certain extent. Glucose metabolism, particularly glycolysis, is a key factor involved in ocular angiogenesis, both under physiological and pathophysiological conditions. There are several factors, including metabolic enzymes, transcription factors and epigenetics involved in the regulation of glycolysis on angiogenesis. Pre-pathological ocular angiogenesis can be suppressed from the source, if the safe inhibition of EC glycolysis can be achieved clinically. To date, the treatment of RNV by targeting VEGF provides an effective therapy in clinical practice. However, anti-VEGF therapy is only suitable for advanced ocular vascular diseases. Therefore, the control of disease progression by directly regulating endothelial metabolism is one of the most efficient approaches during the early stages of ocular vascular diseases. Thus, the process of ocular angiogenesis and the central involvement of glucose metabolism were summarized in the present review. Although an increasing number of studies have illustrated the therapeutic potential of targeting glucose metabolism, additional research is required to fully utilize this therapeutic approach clinically.

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Authors' contributions

HS, WS and QL reviewed and edited the manuscript. XG, HZ, WZ and RZ wrote part of the manuscript and prepared the figures. All authors have read and approved the final manuscript. Data authentication is not applicable.

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Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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