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Review

Cross-talk between signaling and metabolism in the vasculature

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ABSTRACT

The link between signaling and metabolism was first recognized with insulin signal transduction. Efficient glucose uptake by the endothelium requires insulin receptor activation to deliver GLUT receptors to the cell surface. More recently however, additional evidence has emerged for a broader crosstalk as signaling events have been shown to regulate a large number of metabolic enzymes. Changes in the metabolic status of endothelial and smooth muscle cells are observed at times of increased proliferative activity and these coincide with activation of cell surface receptors. Intriguingly, a rise in glycolysis appears to be associated with remodeling of the actin cytoskeleton during migration and angiogenesis. Overall, understanding how do signaling and metabolic pathways intersect and cross-regulate each other has become an important question and an emerging cornerstone in vascular biology.

Contents

1. Introduction

To meet their bioenergetic needs, cells rely on glucose as one of the primary sources of energy. Through glycolysis, glucose is metabolized into pyruvate in the cytosol. Pyruvate can then either enter the tricarboxylic acid (TCA) cycle in the mitochondria, where it undergoes oxidative phosphorylation (OXPHOS) in a process that requires oxygen, or it can be further converted into lactate (anaerobic glycolysis). OXPHOS is a very efficient process, generating a total of 32–36 ATPs per glucose molecule and therefore it is not surprising that the path is used by most mammalian cells [1]. In contrast, glycolysis only generates a total of 2 ATPs per glucose molecule, and thus, it is much less efficient than oxidative phosphorylation. The relative use of OXPHOS versus glycolysis varies in cells, and while it was originally thought that only cancer cells switch their metabolism to glycolysis, several examples in the recent literature highlight instances in which normal cells opt for glycolysis even in the presence of high levels of oxygen, a process called

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aerobic glycolysis, and in response to cell surface signaling. Why normal cells, in oxygenated tissues, would select a less efficient mode for ATP generation is an interesting puzzle and it has revealed that aerobic glycolysis is intertwined with signaling pathways that regulate anabolic metabolism, proliferation, and cytoskeletal regulation.

2. The Warburg effect is not exclusive to cancer cells

The switch from OXPHOS to aerobic glycolysis was first described by Otto Warburg. He discovered that cancer cells mainly use aerobic glycolysis even in the presence of oxygen to sustain their rapid and unlimited growth [2]. This phenomenon became quickly recognized as the "Warburg effect" and it has been prevalently studied in cancer cells [3,4]. However, the Warburg effect is not exclusive of transformed cells; normal cells also upregulate aerobic glycolysis, especially in situations of rapid growth (Table 1). In fact, increased glycolytic metabolism was recently described in proliferating lymphocytes [5–7], activated macrophages [8], proliferating thymocytes [9–11], proliferating fibroblasts [12,13] and regenerating skeletal muscle cells [14]. In the absence of proliferation most of these cell types have a low baseline level of glycolysis that increases by 20- to 30-fold (in the case of lymphocytes) upon activation [15,16].

Table 1
Aerobic glycolysis in non-transformed cells.

Cell type	Experimental setup	Reference
Hematopoietic cells, lymphocytes	Cytokine stimulation (IL-3) of immortalized murine hematopoietic cells (FL5.12); IL-2 stimulation of primary human T-cells; IL-7 stimulation of immortalized murine B cell progenitors	Bauer et al. [6]
Lymphocytes	Rat thymus lymphocytes stimulated with concanavalinA	Hume et al. [5]
Lymphocytes	Primary human T cells co-stimulated with CD28 and insulin	Frauwirth et al. [15]
Lymphocytes	Primary murine T cells activated with CD3 and CD28	Wang et al. [16]
Macrophages	Bone marrow derived macrophages activated with LPS	Palsson-McDermott et al. [8]
Thymocytes	Primary rat thymocytes stimulated with concanavalinA and IL-2	Brand and Hermfisse [11]
Thymocytes	Primary rat thymocytes stimulated with concanavalinA and IL-3	Greiner et al. [10]
Thymocytes	Primary rat thymocytes stimulated with concanavalinA and IL-4	Guppy et al. [9]
Adipocytes	Differentiation of human bone marrow mesenchymal stem cells (MSCs)	Meleshina et al. [42]
Stem cells	Human embryonic stem cells (hESCs) and induced pluripotent stem cells (IPSCs)	Varum et al. [43]
Stem cells	Reprogramming of derived induced pluripotent stem cells	Folmes et al. [44]
Stem cells	Human bone marrow mesenchymal stem cells	Fillmore et al. [45]
Stem cells	Hematopoietic stem cells	Simsek et al. [46]
Stem cells	Hematopoietic stem cells	Takubo et al. [47]
Stem cells	Osteogenic differentiation of human mesenchymal stem cells (hMSCs)	Chen et al. [48]
Stem cells	Human embryonic stem cells (hESCs)	Turner et al. [49]
Stem cells	Mouse embryonic stem cells	Schieke et al. [50]
Stem cells	Human embryonic stem cells and hematopoietic stem cells	Ochocki et al. [51]
Fibroblasts	Primary human dermal fibroblasts, proliferating vs. quiescent (contact-inhibited)	Lemons et al. [12]
Smooth muscle cells	Human carotid artery vascular smooth muscle cells stimulated with PDGF	Lambert et al. [33]
Smooth muscle cells	Primary rat aortic vascular smooth muscle cells stimulated with PDGF	Perez et al. [34]
Myoblasts	Differentiation of immortalized mouse skeletal muscle cell line C2C12	Leary et al. [52]
Endothelial cells	EC spheroids, mouse retina, zebrafish	De Bock et al. [21]
Endothelial cells	Primary rat pulmonary microvascular endothelial cells (PMVECs) and primary rat pulmonary artery endothelial cells (PAECs)	Parra-Bonilla et al. [18]
Endothelial cells	Primary pig aortic endothelial cells	Culic et al. [19]

A common link between the examples cited above is proliferation. Interestingly, endothelial cells (EC) even in a quiescent state are highly glycolytic despite their close proximity to oxygen. Importantly, while all ECs are glycolytic [17], there seem to be differences in the extent of glycolysis used by ECs from different vascular beds. Parra-Bonilla et al. described that rapidly proliferating pulmonary microvascular ECs are highly glycolytic, while the slower growing arterial ECs are less glycolytic and consume more oxygen [18]. Changes in rates of glycolysis were also described at times of vascular expansion. The endothelium has been shown to double glycolytic rates when switching from quiescence to an angiogenic phenotype [17]. Angiogenic ECs generate more than 80% of ATP through glycolysis, converting glucose into lactate, but less than 1% using OXPHOS in the TCA cycle [19,17]. Oxidative pathways only account for 15% of the total amount of ATP generated in ECs [17]. It is thus not surprising that, compared to other cell types, ECs have a rather small mitochondrial volume fraction (5% vs. 30% in oxidative hepatocytes) that mainly serves as signaling hubs rather than for generating energy/ATP [20].

The preference of ECs for aerobic glycolysis might seem surprising considering the abundance of oxygen in the blood and the direct and unlimited access of blood vessel-lining ECs to oxygen. Using glycolysis as the major metabolic pathway to generate energy might be beneficial when ECs sprout into avascular tissue. Furthermore, a low-oxidative metabolism limits the formation of reactive oxygen species (ROS) and potential ROS-mediated damage. Another advantage of glycolysis over OXPHOS is the fact that it can generate ATP much more rapidly, facilitating adaptation to quickly changing energy-demands of proliferating/migrating cells. Finally, by using less oxygen, more oxygen is available for perivascular cells [21]. But are these the only reasons for the preferential use of glycolysis by endothelial cells and what are the underlying signaling pathways?

3. Crosstalk between signaling and metabolism

It seems obvious that cell signaling and metabolism are linked events, but their cross-talk and regulatory pathways are poorly understood. Insulin signaling was the first of such links, as this pathway is required for the uptake of glucose by glucose transporters (GLUT). In the absence of insulin, GLUT is efficiently sequestered intracellularly in vesicles that are only recruited to the plasma membrane upon phosphorylation of the insulin receptor [22]. In turn, the insulin receptor activates phosphatidylinositol 3-phosphate kinase (PI3K)/AKT protein kinase [23], which subsequently activates AS160, a GTPase-activating protein (GAP) specific for RAB that facilitates anchorage of GLUT vesicles to the plasma membrane (Fig. 1). In fact, inactivating mutations of AS160 result in attenuation of GLUT translocation [23]. Importantly, PI3K and AKT also contribute to regulation of glycolysis through alternative mechanisms described below, further intertwining signaling and metabolism.

A recent publication has demonstrated that PI3K activation increases glycolysis in epithelial cells by enhancing cytoplasmic availability of aldolase A (Fig. 2) [24]. This activity is independent of AKT because specific inhibition of AKT through MK2206 had no effect on NADH/NAD+ ratio and extracellular acidification rate (ECAR), which are both proxies for glycolysis [24]. In contrast, the pan inhibitor BKM120 or the specific PI3K-alpha inhibitor BYL719 resulted in significant attenuation of glycolysis. Importantly, blockade of PI3K was found to be the direct result of a decreased release of aldolase A from the actin cytoskeleton [24]. Therefore, it appears that signaling that activates PI3K unifies several important activities in the cell notably glucose uptake, actin cytoskeletal dynamics, and glycolysis (Fig. 2). While these effects downstream of PI3K are yet to be demonstrated in other cell types, it is likely that the same outcome would be observed in vascular cells. It should be stressed that PI3K is one of the main pathways activated downstream of multiple

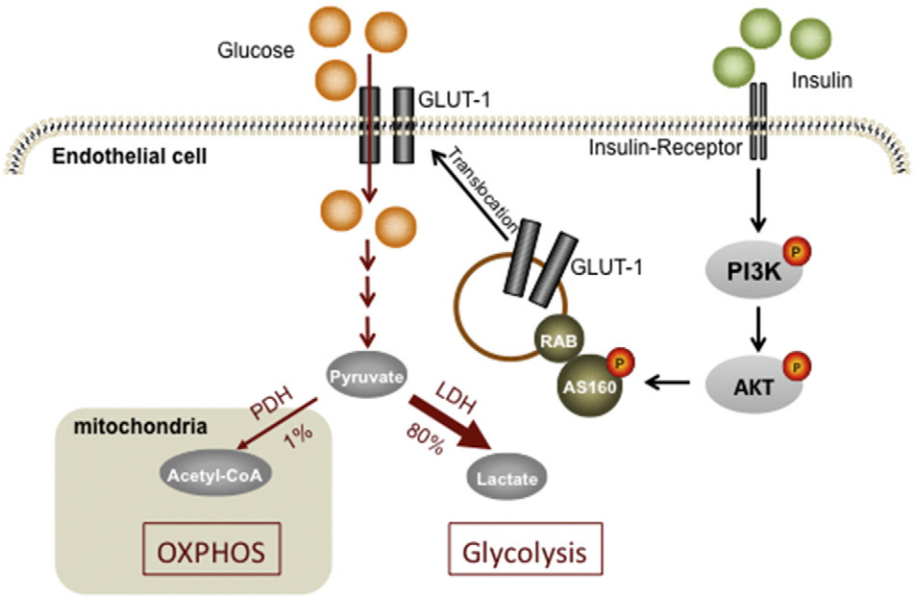

Fig. 1. Insulin signaling and glucose metabolism in ECs. Insulin signaling induces GLUT translocation to the cell membrane, enabling glucose uptake and metabolism. ECs gain 80% of their energy through aerobic glycolysis, but only 1% through oxidative phosphorylation (OXPHOS). Black arrows represent signaling pathways, red arrows represent metabolic pathways.

tyrosine kinase signaling events and therefore it appears to be a hub for the coordination between signaling and metabolism.

The AKT pathway, downstream of PI3K has been well recognized to promote aerobic glycolysis in cancer cells [25]. More recently, studies performed in cancer cell lines showed that inhibition of Fms-related tyrosine kinase 3 (FLT3) reduces glycolysis and promotes macroautophagy through direct targeting of hexokinase II (*HK2*), a key enzyme in glucose metabolism [26]. Importantly, the study uncovered information that directly connects metabolic collapse to cell death. Since HK2 is regulated by signaling pathways in several non-transformed cells, these findings are likely to extend to non-transformed cells as well.

4. Signaling and metabolism in endothelial and smooth muscle cells

In ECs, lactate, the end product of glycolysis in the cytoplasm, increases migration and tube formation in vitro by activating the nuclear factor kappa-light-chain-enhancer of activated B cells/interleukin 8 (NFkB/IL8) pathway [27]. It also activates hypoxia-inducible factor-1 alpha (HIF1a), leading to an increased expression of vascular endothelial

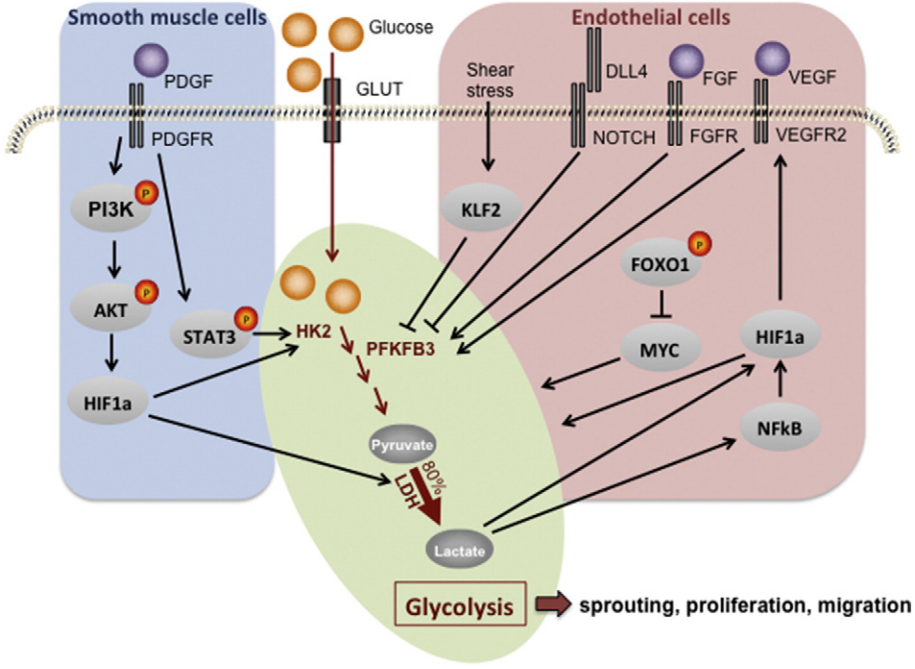

Fig. 2. Metabolism and actin cytoskeleton. PI3K activation increases glycolysis by inducing the release of F-actin bound aldolase after remodeling of the actin cytoskeleton. PFKFB3 interacts with F-actin in migrating ECs. Black arrows represent signaling pathways, red arrows represent metabolic pathways.

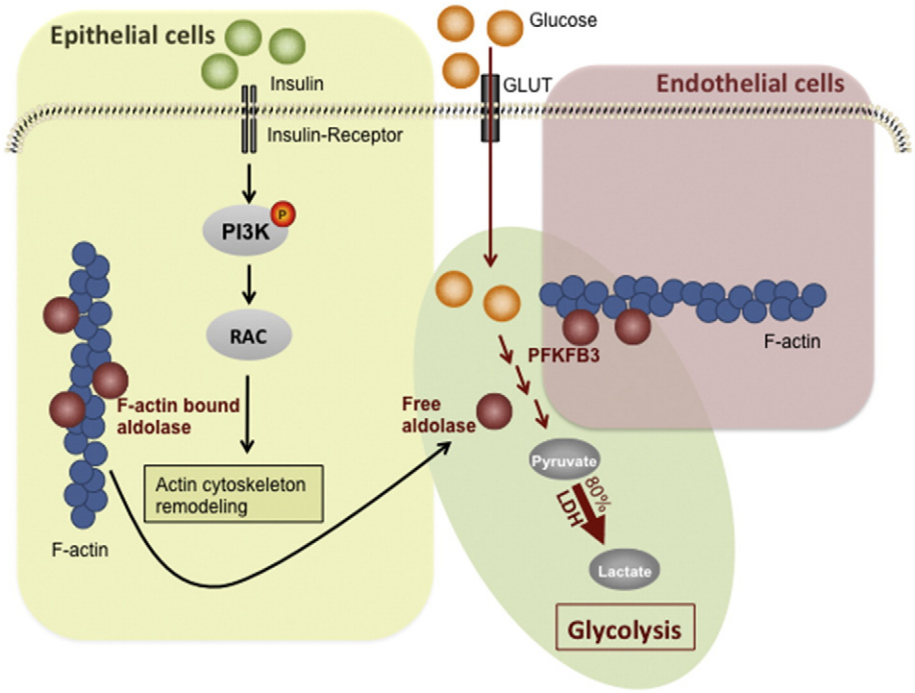

Fig. 3. Signaling and metabolism in ECs and SMCs. Angiogenic signaling pathways through receptor tyrosine kinases in ECs and SMCs induce glycolysis and thus proliferation, migration and sprouting by stimulating rate limiting glycolytic enzymes. *Anti*-angiogenic signals such as FOXO1, NOTCH and KLF2 inhibit rate limiting glycolytic enzymes and thus glycolysis. Glycolytic metabolites such as lactate, in turn, induce HIF1a and NFkB-mediated expression of pro-angiogenic VEGFR2. Black arrows represent signaling pathways, red arrows represent metabolic pathways.

growth factor 2 (VEGFR2) and Fibroblast growth factor (FGF) [28] (Fig. 3). This activation of HIF1a can occur when lactate is metabolized into pyruvate leading to the inhibition of prolyl-hydroxylases (PHDs) [27,29,28]. Furthermore, the enzyme necessary for metabolizing pyruvate into lactate, lactate dehydrogenase (LDH), has been shown to be necessary for angiogenesis in pulmonary microvascular ECs. In fact, shRNA-mediated reduction of LDH-A expression resulted in decreased cell growth and reduced vascular network formation in matrigel assays [30].

Another rate-limiting enzyme in the glycolytic pathway, phosphofructokinase-2/fructose-2,6-bisphosphatase (PFKFB), regulates EC sprouting. PFKFBs synthesize fructose-2,6-bisphosphate ($F2,6P_2$), an activator of 6-phosphofructo-1-kinase (PFK-1), which converts fructose-6-phosphate (F6P) to fructose-1,6-biphosphate ($F1,6P_2$). Even though PFKFB3 deficiency only reduces glycolysis by ~40%, it leads to a significant decrease of proliferation and sprout formation in EC spheroids and in the retina of neonate mice. In addition, these cells form fewer and smaller lamellipodia and show reduced motility [17]. These same effects were observed when blocking PFKFB3 with the small molecule inhibitor 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO) [31].

Interestingly, PFKFB3 expression and thus glycolysis are upregulated by angiogenic stimuli (VEGF and FGF signaling) and downregulated by differentiation/stabilization stimuli (NOTCH signaling) [17]. This hints towards a possible crosstalk between (angiogenic) signaling and metabolism in ECs (Fig. 3). Furthermore, PFKFB3 deficiency reduces the tip cell phenotype induced by lack of NOTCH in EC spheroids, while PFKFB3 overexpression in EC spheroids as well as in a zebrafish model restores vascular sprouting in NOTCH activated cells and therefore counteracts NOTCH-induced stalk cell phenotype [17]. In contrast, NOTCH has been reported to increase glycolysis by transcriptionally regulating metabolic genes such as GLUT, HEXA and MYC in Drosophila [32]. To what extent this is applicable to mammalian cells is at this point unclear.

Curiously, in migrating ECs, PFKFB3 and F-actin interact at the leading edge in lamellipodia. This is the second example where enzymes involved in metabolism bind to the cytoskeleton (Fig. 2). The effect of PFKFB3 on endothelial directional migration could potentially be the consequence of its compartmentalization in actin filaments [17].

Similar to angiogenic stimuli in ECs, PDGF-stimulation of smooth muscle cells (SMCs) promotes glycolytic activity. Mechanistically, PDGF-mediated activation of the PI3K/AKT pathway leads to increased expression of the glycolytic enzymes LDH and HK2, accompanied by reduced apoptosis or increased proliferation (Fig. 3) [33,34]. Adding support to this notion, Heiss et al. in the present issue of *Vascular Pharmacology* showed that PDGF-induced HK2 expression, and thus increased glycolytic activity in SMCs, is also regulated by STAT3 activation [35]. Furthermore, they show that inhibition of glycolysis using pharmacological glycolytic inhibitors or si-mediated knock-down of HK2 reduces PDGF-induced migration in SMCs, while cell viability and cytoskeleton remain unchanged [35]. This indicates that SMCs depend on glycolysis and activity of glycolytic enzymes for proliferation, similar to what has been described for HK2 in cancer cells [36], and PFKFB3 in ECs [17].

Since angiogenesis is associated with increased glycolysis, one might anticipate reduced glycolytic activity in a quiescent state of the endothelium. Indeed, shear stress-induced upregulation of KLF2 in ECs directly represses transcription of glycolytic enzymes including PFKFB3, HK2 and PFK-1 (Fig. 3). This reduces the glycolytic activity of endothelial cells subjected to higher shear stress [37].

Another important regulator of quiescence in the endothelium is FOXO1. Its endothelial-specific overexpression leads to reduced EC proliferation and growth, while endothelial-specific depletion induces increased EC proliferation in postnatal mouse retinas [38]. Interestingly, HUVECs overexpressing a constitutively active form of FOXO1 show reduced extracellular acidification rate, glucose uptake, glycolytic flux and lactate production, indicating reduced glycolysis in these cells [38]. Mechanistically, FOXO1 suppresses expression of MYC, a known activator of glycolysis and mitochondrial metabolism [39], leading to the downregulation of genes involved in metabolism [38]. FOXO1 therefore not only mediates a quiescent phenotype by inhibiting proliferation, but also by shutting down glycolysis in ECs (Fig. 3).

5. Conclusions

Signaling and metabolic pathways are clearly linked in vascular cells within an intricate and reactive web of interactions. How these interactions are selected, maintained, or terminated remain important questions that will likely be the focus of much attention in the near future. It is extremely likely that transcription factors play an important role in this process. Much of this has been already realized for FOXO, MYC and HIF1a, but other players are likely to emerge. In fact, the ZBTB family of transcription factors has been shown to repress several glycolytic enzymes in fibroblasts and in lymphocytes [40,41]. It is anticipated that these master regulators will also mediate similar responses in multiple cell types to quickly promote alterations in metabolic pathways that can be solidified by external signaling.

Taken together, a large volume of new information has been accumulated in the last five years and has uncovered how signaling can be translated into particular metabolic programs to enable important cellular functions like proliferation and differentiation.

Authorship contributions

MU generated the figures. MU and MLIA wrote the manuscript.

Conflict of interest disclosures

The authors declare no competing financial interests.

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