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Minireview

Endothelial cell metabolism and implications for cancer therapy

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Tumour tissue is characterised by fluctuating oxygen concentrations, decreased nutrient supply, and acidic pH. The primarily glycolytic metabolism of tumour cells contributes to this, with increased glucose consumption and increased lactate secretion. Endothelial cells are particularly challenged when recruited towards the tumour metabolic environment. They are required to proliferate and form functional networks in order to establish continuous blood flow. Considering that deregulated metabolism is an emerging hallmark of cancer and target of tumour therapy, it is of importance to incorporate the current knowledge about how the tumour metabolic environment, as a therapy target, can affect endothelial cell metabolism and the angiogenic response. Recent studies have shown differences in metabolic pathways in endothelial cells compared with other normal or tumour tissues. Therefore, we have reviewed relevant literature on endothelial metabolism and the response to angiogenic activation in conditions of metabolic stress.
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Tumour cell metabolism and angiogenic stimulation

Tumour cells are exposed to fluctuating concentrations of oxygen and nutrients during growth and metastasis. Although oxidative phosphorylation (OxPhos) represents an efficient way to produce energy, proliferating tumour cells rely on increased aerobic glycolysis to produce energy, and a constant supply of metabolic intermediates to synthesise nucleotides and lipids. Genetic changes such as mutation of the tumour suppressor *p53*, the oncogene *c-myc,* and hypoxic/oncogenic activation of hypoxia-inducible factor (HIF)-1α, enhance the glycolytic flux, even in the presence of oxygen (Warburg effect). This is based on induction of glucose transporter 1 (Glut1), hexokinase 2 (HK2), pyruvate kinase M2 (PKM2), lactate dehydrogenase A (LDHA), or pyruvate dehydrogenase kinase 1 (PDK1) (Semenza, 2010). Tumour cells secrete high amounts of lactate as a glycolytic end product, which contributes to the acidic pH in the tumour tissue (acidosis). Finally, oncogene activation and hypoxia lead to increased secretion of vascular endothelial growth factor (VEGF) A by tumour cells, leading to the stimulation of angiogenic sprouting and blood vessel development (Bridges *et al*, 2011; Vander Heiden, 2011).

Angiogenic sprouting: the VEGFR2/Dll4 balance

An endothelial cell that expresses high levels of VEGF receptor (VEGFR) 2 is likely to respond to the tumoural VEGFA cue and become the leading cell of an angiogenic sprout (tip cell). The VEGFA/VEGFR2 signalling in the migratory tip cell induces expression of Delta-like ligand 4 (Dll4), which is then exposed on the cell surface. Delta-like ligand 4 binding to the Notch receptor on neighbouring cells leads to the paracrine induction of Notch signalling. Notch signalling stimulates transcription of VEGFR1 and inhibits transcription of VEGFR2, thereby limiting the response to VEGFA. These cells, being adjacent to the tip cell, take on the more proliferative stalk cell phenotype. As Notch signalling also induces Dll4 expression, the signal for stalk cell determination is further being transduced to adjacent cells, that become part of the growing sprout (Bridges *et al*, 2011; Potente *et al*, 2011).

Tumour vasculature and metabolic conditions

Intratumoural endothelium is exposed to imbalanced growth factor signalling, favouring proangiogenic factors such as VEGFA and angiopoietin 2 and leading to excessive angiogenesis and abnormal vasculature. Areas of hyper- and hypovascularisation are in close proximity inside the tumour mass, leading to spatial and temporal heterogeneity of blood flow and frequently hypoxic areas (Goel *et al*, 2011).

Metabolome analyses of tumour tissue show significantly lower glucose concentrations compared with normal tissue, for example in colorectal and stomach tumours (Hirayama *et al*, 2009). Although increased glucose consumption in tumour cells correlates with hypoxia, decreased blood flow and accumulation of HIF-1α are not a prerequisite for glucose deprivation and increased glucose consumption in tumours since mutations of cancer-related genes such as *p53* and *c-myc* also increase glycolytic activity (Vander Heiden, 2011). Enhanced tumour cell proliferation results in decreased blood perfusion because the development of supporting vasculature lacks behind. New forming vessels inside the tumour are therefore frequently exposed to nutrient scarcity, acidosis, and hypoxia. In turn, the vascular survival ability inside the tumour mass also determines the level of nutrient supply and oxygen perfusion of the tumour. Accordingly, high vascular survival ability in the tumour correlates with tumour aggressiveness (Giatromanolaki *et al*, 2002).

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Angiogenic network formation in the tumour environment is therefore dependent on the ability of endothelial cells to (a) survive and migrate in conditions of low nutrient availability and hypoxia, (b) maintain sufficient energy supplies for growth and proliferation, and (c) utilise alternative energy substrates to compete with the metabolic requirements of tumour cells. In the following sections, we review how endothelial cells adapt their metabolism to nutrient starvation and hypoxia, and how signalling by angiogenic factors is able to modulate their metabolic pathways. These pathways potentially provide new targets for inhibitors of metabolism in endothelial cells and tumour cells. Mechanisms of angiogenesis were recently reviewed by Potente, Gerhardt, and Carmeliet, who described the emergence of endothelial metabolism as an important component of angiogenesis (Potente *et al*, 2011).

METABOLISM AND ANGIOGENIC SIGNALLING IN ENDOTHELIAL CELLS

Exogenous glucose as a fuel for glycolytic energy production

Endothelial cells show a preference for glycolytic energy production even in the presence of oxygen, which is uncommon for non-malignant cells. For example, human umbilical vein endothelial cells grown *in vitro* show high glycolytic enzyme activities and capacity for lactate production, independently from their proliferative state (Peters *et al*, 2009). This has been confirmed numerous times in macro- and microvascular endothelial cells (Krutzfeldt *et al*, 1990; Tretyakov and Farber, 1995; Parra-Bonilla *et al*, 2010). It seems a paradox that cells most closely apposed to oxygenated blood are not utilising oxygen. However, this may reduce oxidative damage and allow oxygen to diffuse into deeper proliferating tissues. Furthermore, constitutively using glycolysis may prepare endothelial cells for rapid sprouting and migration.

Endothelial cells are regulated by HIF-1α and HIF-2α in response to hypoxia. Hypoxia signalling mediates a glycolytic shift (Tretyakov and Farber, 1995), but as endothelial cells are already glycolytic, effects on other pathways may be more critical, for example basement membrane breakdown, invasion of vessels, and upregulation of Dll4 signalling. It is well established that HIF-1α can directly activate the Notch signalling pathway (Qiang *et al*, 2011). In addition, HIF-2α has an endothelial cell-autonomous role, as it has been shown to regulate angiogenic factors, such as fibronectin, integrins, endothelin B receptor, and Dll4, and is required for vessel integrity and tumour neovascularisation (Skuli *et al*, 2009).

Lactate as a regulator of metabolism and HIF signalling

Exogenous lactate, once taken up by neighbouring cells, can be channelled into the tricarboxylic acid (TCA) cycle and fuel OxPhos, if the cells have access to oxygen. A subpopulation of tumour cells readily takes up and metabolises lactate. In these cells, the membrane expression of the monocarboxylate transporter (MCT)1 enables lactate influx in a concentration-dependent manner (Sonveaux *et al*, 2008). Tumour-associated fibroblasts and normal endothelial cells may also exploit this process, as they have been described to show high MCT1 expression, and it has been shown that coronary and pulmonary endothelial cells readily oxidise lactate (Krutzfeldt *et al*, 1990; Koukourakis *et al*, 2006; Parra-Bonilla *et al*, 2010; Vegran *et al*, 2011).

Increased concentrations of exogenous lactate and increased endothelial import of lactate influences endothelial cell signalling, by stimulating autocrine IL8/NFκB signalling (Vegran *et al*, 2011), as well as increasing VEGFA levels through stabilisation of HIF-1α (Hunt *et al*, 2007) (Figure 1). These studies suggest a proangiogenic role of lactate. Although MCT1 expression has been described to be low in tumour-associated vasculature in some cases (Koukourakis *et al*, 2006), it does not exclude that increased lactate in the tumour tissue can act proangiogenic and serve as a metabolic substrate for tumour endothelial cells. This is supported by the following: (a) inhibition of endothelial MCT1 reduces lactate-induced angiogenesis in tumours (Sonveaux *et al*, 2012) and (b) tumour endothelium gene expression studies show an upregulation of LDHB, which facilitates integration of exogenous

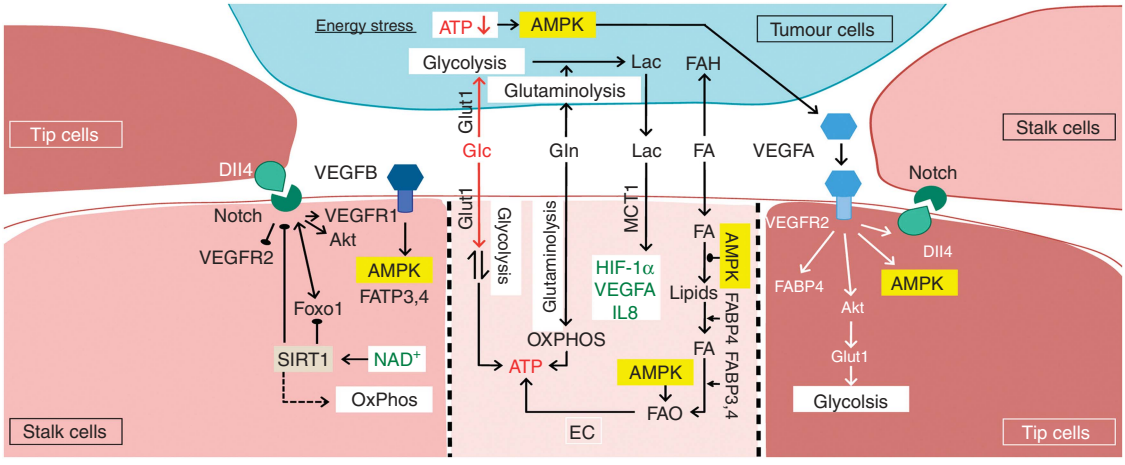

Figure 1 Metabolic signalling in tumour angiogenesis. Tumour cells (TC) depend on glucose (Glc) and glutamine (Gln) for glycolysis and glutaminolysis, and secrete high amounts of lactate. Once entered into endothelial cells (EC) through MCT1, lactate can increase VEGFA, HIF-1α and interleukin 8 (IL8) signalling. Fatty acids (FA) are utilised by TC (FA handling, FAH). Tumour cells undergoing energy stress (Glc in red) have depleted ATP levels, leading to activation of Adenosine monophosphate kinase (AMPK). Energy metabolism in EC depends on glucose (uptake through Glut1) and glycolysis. In glucose starvation (Glc in red), EC utilise Gln for energy production. Fatty acids taken up from outside or produced from lipolysis can be used for lipid synthesis (inhibited by AMPK) or FA oxidation (activated by AMPK). Fatty acid- binding protein 4 (FABP4) and FATP3,4 are involved in lipolysis and FA transport, respectively. Adenosine monophosphate kinase activation in TC leads to VEGFA secretion and stimulation of endothelial tip cell formation through VEGF receptor (VEGFR) 2 signalling. VEGFA/VEGFR2 signalling induces Dll4, FABP4, Akt, and Glut1, potentially promoting glycolysis in the tip cell. Dll4-induced Notch activation regulates expression of VEGFR2 (inhibition), VEGFR1 (activation), and Akt (activation) in the trailing stalk cells. Sirtuin 1 (induced by NAD+) inhibits Notch activity in stalk cells, thereby potentially counteracting a glycolytic switch induced by Notch signalling and shifting the balance towards OxPhos. VEGFB/VEGFR1 signalling activates AMPK and FATP3, 4, potentially promoting FAH in stalk cells.

lactate in metabolism, once it has entered the cells (van Beijnum *et al*, 2006).

A shift from the consumption of glucose to lactate represents a metabolic niche for cells growing closely to highly glycolytic, lactate-secreting tumour cells (Figure 1). Thus, increased exogenous lactate and its import by endothelial cells may contribute to survival in a glucose-deprived environment, and promote tumour angiogenesis.

Glutamine and glycogen utilisation to replenish intracellular substrates

Glutamine is an essential amino acid and functions in protein synthesis, pH regulation, energy production and protection against reactive oxygen species (ROS). Tumour cells show increased glutamine metabolism and cannot survive in the absence of glutamine (Vander Heiden, 2011).

Endothelial cells have a high capacity for glutamine transport and utilisation. A prosurvival effect of glutamine has been shown in conditions of oxidant injury. Increased ROS inhibit glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a key glycolytic enzyme. In conditions of this glycolytic inhibition, glutamine contributes to endothelial adenosine tri-phosphate (ATP) synthesis and improves cell viability (Hinshaw and Burger, 1990). This indicates that glutamine is a substrate for endothelial cells with impaired glycolytic capacity, and can be used in conditions of decreased glucose supply.

Furthermore, decreased supplies of glucose can be potentially compensated by the glycogenolytic production of glucose-1-phosphate, which is converted into glucose-6-phosphate (Glc-6-P, an intermediate of the glycolytic pathway). Inhibition of glycogenolysis in endothelial cells leads to a reduction in cell survival (Vizan *et al*, 2009). This observation may be due to indirect inhibition of the two major pathways that utilise Glc-6-P, which are the glycolytic pathway and the pentose phosphate pathway (PPP).

The PPP generates nicotinamide adenine dinucleotide phosphate hydrate (NADPH) and ribose-5-phosphate (Rb-5-P), respectively, required for lipid and nucleotide synthesis. NADPH is also required for the protection against ROS. Direct inhibition of the NADPH and CO_2 producing oxidative PPP branch (oxPPP) by targeting Glc-6-P dehydrogenase results in decreased endothelial cell survival (Vizan *et al*, 2009). When coronary endothelial cells are incubated in medium containing glucose as the sole exogenous substrate, more than 90% of CO2 production is derived from the PPP, as exogenous glucose is mainly converted into lactate during glycolytic energy production (Krutzfeldt *et al*, 1990). Because glycogenolysis-derived Glc-6-P only minimally contributes to endothelial energy production in normal glucose and glucose-starved conditions (Krutzfeldt *et al*, 1990), its catabolism in the oxPPP might be more important. Also, further metabolism of Rb-5-P in the reversible non-oxidative PPP branch can feed back into glycolysis through the production of fructose-6-phosphate. Inhibition of this by targeting transketolase reduces endothelial cell survival (Vizan *et al*, 2009), underlining the significance of the PPP in endothelial cell metabolism.

The role of adenosine monophosphate (AMP) kinase and fatty acid oxidation to fuel energy production

Lipid metabolism enables cell growth and proliferation, because it provides biosynthetic modules for membrane renewal, regulates cell signalling, and produces energy through the oxidation of fatty acids (FA). Fatty acid oxidation (FAO) requires import of fatty acids into the mitochondria. This can be stimulated *in vitro* by incubation in carnitine, which binds free FAs in the cytoplasm. The complexes are then transported into the mitochondria by carnitine-palmitoyl transferase 1 (CPT1). Interestingly, endothelial cells stimulated with carnitine are able to increase FAO to such a level, that the rate of consequent ATP production exceeds the rate of glycolytic ATP production by 50% (Hulsmann and Dubelaar, 1988).

Adenosine monophosphate kinase (AMPK) is a key regulator of FAO in cells that undergo energy stress, and counteracts ATP depletion by inhibiting anabolic processes including protein, FA, and glycogen synthesis, and by activating ATP-producing catabolic processes such as FAO. Stimulation of endothelial AMPK by drug treatment decreases FA synthesis by inhibiting acetyl-CoA carboxylase (ACC) activity, and promotes FAO and ATP production by increasing CPT1 activity. When starved of glucose, endothelial cells have steady ATP levels, increased AMPK activity, and decreased ACC activity, suggesting that AMPK-mediated regulation of ACC and CPT1, and increased FAO as a consequence is an important process for endothelial cells to survive energy stress (Dagher *et al*, 2001).

Adenosine monophosphate kinase is an interesting target in diabetes and cancer therapy. Its activation improves insulin sensitivity, hyperglycaemia, and hyperlipidemia. Also, it is described as an enforcer of metabolic checkpoints in cancer, as AMPK phosphorylates such tumour suppressors as tuberous sclerosis complex-2 and p53, and controls cell growth, autophagy, and apoptosis. Metformin, an antidiabetic drug that acts partially by activation of AMPK, improves endothelial function and reduces cancer-related mortality in diabetic patients (Mather *et al*, 2001; Zou *et al*, 2004; Landman *et al*, 2010). Regarding the above-described effects of AMPK activation on FA metabolism in addition to this, it is interesting to investigate in how far Metformin affects endothelial metabolism and function in a tumour setting. This may be important for future therapeutic strategies that aim at improvement and normalisation of the tumour vasculature.

Effects of VEGFA and VEGFB on metabolism

Endothelial cells form networks in response to VEGFA, even in conditions of oxygen, glucose, and serum deprivation (Helmlinger *et al*, 2000). This indicates that VEGFA is an important regulator of the endothelial response to changes in metabolic substrate availability. Vascular endothelial growth factorA is known to activate kinases such as Akt and AMPK, which have been described for their effects on energy metabolism in endothelial cells (Yeh *et al*, 2008; Reihill *et al*, 2011).

For example, in brain endothelial cells, VEGFA enhances Glut1 expression via Akt, and may promote glycolytic energy production for angiogenic growth (Yeh *et al*, 2008). Also, VEGFA/VEGFR2 signalling increases the turnover of glycogen during proliferation (Vizan *et al*, 2009), potentially to replenish metabolic intermediates such as Glc-6-P. Furthermore, VEGFA can increase endothelial FA uptake via AMPK, which may be important for endothelial cell survival and FA transport to adjacent tumour tissue (Reihill *et al*, 2011). This is supported by recent research, showing that VEGFA/VEGFR2 signalling may regulate intracellular FA handling in endothelial cells by inducing FA binding protein 4 (FABP4, transports FA). Here, loss of FABP4 reduces endothelial cell proliferation, indicating its significance in endothelial cell homoeostasis (Elmasri *et al*, 2009). Vascular endothelial growth factorB, considered to promote endothelial cell survival and to be less angiogenic than VEGFA, induces FA uptake via AMPK (Reihill *et al*, 2011), and induces FA transport proteins (FATP) 3 and 4 via signalling through VEGFR1 (Hagberg *et al*, 2010). Thus, also VEGFB may act as a regulator of the transfer and delivery of FAs to adjacent tissues (Hagberg *et al*, 2010).

Tumour cells as potential FA users increase the secretion of lipoprotein lipase for extracellular lipolysis and expression of FA transporter FAT/CD36 for FA uptake, which promotes tumour cell

proliferation *in vitro* (Kuemmerle *et al*, 2011). These mechanisms may also help to support the vasculature *in vivo*.

Notch signalling and metabolic regulation

Dll4/Notch signalling contributes to anti-VEGFA therapy resistance in Dll4-overexpressing glioblastoma xenografts. These tumours are less hypoxic, owing to a more functional vasculature, and blockade of Dll4 signalling in these tumours leads to reduced therapy resistance (Li *et al*, 2011).

In breast cancer cells, up- or downregulation of Notch activity can induce a glycolytic switch. This glycolytic switch is mediated by distinct signalling processes, which are a decrease of p53 signalling after Notch inhibition, and an increase of Akt/phosphatidylinositol 3-kinase signalling after Notch activation. The glycolytic switch is reversible only in cells with activated Notch signalling, which can switch back to OxPhos in conditions of glucose starvation. Cells with inhibited Notch signalling have an impaired mitochondrial function and cannot use OxPhos as a back-up function. This indicates that the modulation of Notch signalling may have an important function in retaining metabolic flexibility in conditions of metabolic stress, to promote a highly proliferative state in areas of fluctuating nutrient supply (Landor *et al*, 2011). This is of interest in angiogenic sprouting, in which the regulation of Notch signalling is crucial for the maintenance of tip and stalk cells in the sprout, and potentially also for the modulation of glycolysis and OxPhos. Notch signalling might therefore be essential for the endothelial adaptation to hypoxia and nutrient deprivation that might occur during anti-angiogenic treatment.

Tricarboxylic acid cycle intermediates such as acetyl-CoA are required for epigenetic regulation of signalling pathways, and imbalances in the metabolic flux can have a direct influence on signalling. Deacetylating enzymes such as sirtuins are important regulators in the response to metabolic stress. In times of nutrient scarcity, elevated levels of nicotinamide adenine dinucleotide (NAD^+) result in increased enzymatic activity of Sirtuin 1 (SIRT1). Its activation helps to redirect cellular metabolism from glycolysis to OxPhos by deacetylating and activating transcription factors and cofactors, such as Foxo1 (Chalkiadaki and Guarente, 2012). Sirtuin 1 loss in endothelial cells reduces endothelial sprouting and branching (Potente *et al*, 2007), and Notch intracellular domain is a direct target of SIRT1 (Guarani *et al*, 2011). Foxo1, a transcription factor involved in glucose homeostasis, is also a target of SIRT1 and a negative modulator of angiogenesis (Potente *et al*, 2007). Foxo1 is required for Notch-mediated gene transcription in hepatic cells (Pajvani *et al*, 2011), and may potentially have a role in the endothelial regulation of Notch signalling. As SIRT1 is mainly expressed in stalk cells (Guarani *et al*, 2011), which have activated Notch signalling, SIRT1- and Foxo1-dependent modulation of Notch signalling might be relevant for angiogenic sprouting in conditions of metabolic stress.

Metabolic communication between tumour and endothelial cells, and metabolic adaptation in endothelial cell phenotypes

In energy-depleted tumour cells, activation of AMPK increases tumoural VEGFA secretion (through stabilisation of VEGFA mRNA), thereby promoting angiogenesis (Yun *et al*, 2005). Exogenous lactate, secreted as a glycolytic end product by tumour cells, is taken up by stromal and endothelial cells and can increase mitochondrial biogenesis and angiogenesis (Hunt *et al*, 2007; Sonveaux *et al*, 2008; Vegran *et al*, 2011; Sonveaux *et al*, 2012). The activation of endothelial AMPK and Akt (downstream of VEGFA), and the activation of endothelial metabolic sensors such as SIRT1 and Foxo1 may modulate the utilisation of alternative energy substrates and the activity of Notch signalling, which is supported by several studies (Potente *et al*, 2007; Yeh *et al*, 2008; Guarani *et al*, 2011; Reihill *et al*, 2011). Therefore, the glycolytic switch of tumour cells is coupled to angiogenic activation, producing signals that prime and control endothelial metabolism and function (Figure 1).

The modulation of Notch activity has a role in fine-tuning the balance of glycolysis and OxPhos in breast cancer cells (Landor *et al*, 2011). Transferring of these findings to Notch signalling in endothelial cells may give further insight into the mechanism of angiogenic sprout formation. Here, Notch signalling is known to have a crucial role in tip and stalk cell determination. Tip cells have very low Notch activity and high VEGFR2 expression in relation to stalk cells, and may be dependent on glycolytic energy metabolism. This is for two main reasons: (a) low Notch activity may induce a glycolytic shift and a decrease in OxPhos, as shown in breast cancer cells (Landor *et al*, 2011) and (b) high VEGFA/VEGFR2 signalling may promote glucose metabolism by Akt/Glut1 signalling (Yeh *et al*, 2008). Also, VEGFA/VEGFR2 signalling to FABP4 (Elmasri *et al*, 2009) and potentially increased fatty acid uptake (Reihill *et al*, 2011) may be crucial for membrane remodelling during the migration of tip cells towards the angiogenic cue (Figure 1).

In contrast, stalk cells have high Notch activity, and increased VEGFR1 expression. Vascular endothelial growth factor B/VEGFR1 signalling induces FATP3 and 4 (Hagberg *et al*, 2010). Thus, the increase of VEGFR1 may promote VEGFB signalling and FA handling in stalk cells. Also, high Notch activity in breast cancer cells promotes glycolysis, and renders OxPhos intact, whereas low Notch activity promotes glycolysis, but inhibits OxPhos (Landor *et al*, 2011). If stalk cells show a similar regulation, then high Notch activity may promote glycolytic energy production for stalk cell proliferation. However, Notch activity can be limited by SIRT1 signalling (Guarani *et al*, 2011). Stalk cells show high expression of SIRT1 (Potente *et al*, 2007), which can promote mitochondrial respiration, as shown in different cell types (Chalkiadaki and Guarente, 2012). Thus, SIRT1 may restrain Notch-induced glycolysis and redirect stalk cell metabolism to OxPhos, by limiting Notch activity and activating mitochondrial respiration (Figure 1).

This means that tip and stalk cells may use different energy production pathways. Fatty acids handling induced by VEGFB, and balancing between glycolytic and mitochondrial energy production, regulated by Notch and SIRT1, might be critical in the proliferating stalk cells, whereas glycolytic energy production might be predominant in the migrating tip cells. It seems a paradox that the proliferative stalk cells, that mainly compose the sprout, may have a higher capacity for OxPhos than the migratory tip cells. However, this instance may help to retain the growth of the angiogenic sprout in fluctuating nutrient supply, and before blood flow is completely established in the developing network.

Tumour stem-like cells and bone marrow-derived cells have been shown to contribute to tumour vasculature. Both these populations are maintained in hypoxic areas (hypoxic tumour stem cell niche, and hypoxic bone marrow) (Wang *et al*, 2010; Qiang *et al*, 2011), which means that they are more likely to survive hypoxic stress in the tumour. Notch signalling is required for stem cell maintenance (Wang *et al*, 2010), and may induce pathways that help the cells to survive in hypoxic stress. Furthermore, increased Notch signalling in Dll4-overexpressing tumours makes endothelial cells resistant to VEGFA-stimulated excessive angiogenesis and anti-VEGFA therapy (Li *et al*, 2011). Owing to abnormal tumour vasculature, and during the course of anti-VEGFA therapy, the tumour environment becomes oxygen- and nutrient-deprived, and increased Notch signalling may help the vasculature to survive in these conditions. In order to understand the adaptive mechanisms that enable endothelial cells to build functional vasculature in oxygen- and nutrient-deprived

conditions, it will be important to further investigate the regulation of changes in metabolic pathways in endothelial cells.

CONCLUSION

In depth exploration of endothelial metabolic changes in response to angiogenic stimulation and stress conditions, may give more insight into survival strategies of endothelial cells and their sensitivity to therapeutic strategies that target tumour metabolism. Drugs targeting metabolic enzymes are under preclinical and clinical investigation; potential targets include enzymes of glycolysis (e.g., HK2, PKM2, LDHA), enzymes of the TCA cycle (e.g., PDK, glutamate dehydrogenase) or enzymes of the PPP (Glc-6-P dehydrogenase, transketolase) (Vander Heiden, 2011). In order to understand the effects of metabolic drugs in anti-tumour therapy, the tumour vasculature needs to be considered as a responder to metabolic drug treatment, especially for the ability of endothelial cells to preferably employ aerobic glycolysis, similar to tumour cells.

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