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Review

Targeting fatty acid metabolism in cancer and endothelial cells

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ABSTRACT

Tumour angiogenesis has long been recognised as a target for anti-cancer therapy. The current approach of inhibiting the VEGF pathway has shown benefit in the clinic, though less than anticipated. We recently documented that glycolytic metabolism in endothelial cells (ECs) fuels angiogenesis, rendering it a possible target for inhibiting vascular growth in pathological conditions. More recently, we reported that the oxidation of fatty acids (FA) is irreplaceable for EC proliferation by providing carbons for *de novo* nucleotide synthesis. Furthermore, ECs are rather unique in this respect, creating novel therapeutic opportunities. Here, we review and compare the current understanding of FA utilisation in ECs and tumour cells (TCs).

1. Introduction

Tumour progression is stimulated by the growth of new blood vessels (angiogenesis), which is activated when pro-angiogenic factors become prevalent (angiogenic switch). The result of the overstimulation of angiogenic growth is an abnormal tumour vasculature, with hyperpermeable vessels, abnormal arteriolar-venous shunts, blind ends, and unperfused channels (Dewhirst et al., 1996; Konerding et al., 2001). This in turn leads to areas of insufficient oxygen (hypoxia) and nutrient supply within the tumour, reduced drug delivery, and more aggressive tumour growth when adaptive pathways such as the hypoxia-inducible factor (HIF) signalling pathways come into play (McIntyre and Harris, 2015). The dysfunctional tumour vasculature impairs tumour perfusion and restricts the supply of oxygen and nutrients, thus creating a hostile microenvironment, from where cancer cells attempt to escape through intravasation and metastasis.

Anti-angiogenic therapy in tumours mainly targets growth factor signalling. The most prominent target is the VEGF signalling pathway, for which several drugs have been developed, but their success in patients is less prominent than expected. This is partly due to intrinsic refractoriness or acquired resistance, relying for example on the upregulation of alternative angiogenic pathways, the switch to VEGF-independent modes of vessel formation, and

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the recruitment of pro-angiogenic cells from the bone marrow, amongst other mechanisms (McIntyre and Harris, 2015). It is therefore important to develop alternatives to the current approach of targeting growth factor signalling to inhibit tumour angiogenesis.

Several tumour cell (TCs) types have high glycolytic rates, even though sufficient oxygen is available for oxidative metabolism; a phenomenon called the Warburg effect. The potential of targeting the glycolysis addiction of TCs has been under investigation, and several compounds have been clinically tested, but none have been approved (Granchi and Minutolo, 2012). In general, anti-glycolytic strategies aim to inhibit glycolysis completely and permanently, but their efficacy and safety is abated by the fact that they also block other (vital) glucose metabolism pathways, and that high, toxic levels of these compounds are needed, as their mechanism, for example in case of 2-deoxyglucose (2DG), is often based on competing with mM levels of glucose.

Endothelial cells (ECs) are very plastic cell types. They can rapidly switch from protracted periods of quiescence to intense proliferation during angiogenesis (Potente et al., 2011). We recently demonstrated that despite their close proximity to oxygen in the blood, ECs mainly rely on glycolysis to produce ATP (De Bock et al., 2013). Moreover, glycolysis rates of *in vitro* cultured ECs were comparable to or even higher than glycolysis rates in TCs and exceeded the levels of glucose oxidation and fatty acid oxidation (FAO) flux by more than 200-fold (Eelen et al., 2015). Both genetic silencing and pharmacologic inhibition of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) reduced vessel branching and outgrowth *in vivo* (De Bock et al., 2013; Schoors et al., 2014a). Strikingly, glycolysis even overruled the genetic signals that regulate EC specification during vessel branching, and favoured the more migrative tip cell phenotype. Indeed, tip ECs lead the forefront of the vessel sprout, while stalk cells elongate the sprout through proliferation. Notch is the most potent pro-stalk cell signal known to date. Notably, overexpression of PFKFB3 converted genetically instructed (by Notch overexpression) stalk cells into tip cells, while silencing of PFKFB3 caused opposite effects (De Bock et al., 2013). In addition, silencing of PFKFB3 decreased the proliferation rate of ECs (Xu et al., 2014).

Activated ECs are hyperglycolytic and depend on glycolysis for angiogenic growth. Hence, anti-glycolytic strategies impair pathological angiogenesis (De Bock et al., 2013; Schoors et al., 2014a). Permanent and complete glycolytic inhibition by 2DG, previously evaluated in the clinic for anti-tumour therapy, is toxic for ECs *in vitro*. In contrast, the glycolytic inhibitor 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO), targeting PFKFB3, only leads to a partial and transient decrease of glycolysis *in vivo*, which is sufficient to reduce pathological neovascularization, while not affecting blood vessels in healthy tissue. Thus, the finding that reducing only the hyper-glycolysis (*i.e.* the extra amount of glycolysis, induced to increase vessel sprouting), rather than completely blocking glycolysis, suffices to impair pathological angiogenesis, provides a paradigm shift in the design of anti-glycolytic therapy (Schoors et al., 2014a,b).

In addition to glycolysis, other metabolic pathways also contribute to the altered behaviour of cancer cells and tumour ECs. One of them is fatty acid (FA) metabolism. In most TC types, *de novo* lipid synthesis is activated to generate lipids for cell signaling, membrane expansion and other cellular functions, required for rapid TC growth (Carracedo et al., 2013; Currie et al., 2013). In contrast, we recently reported that ECs consume FAs as a carbon source for DNA synthesis, pushing FA metabolism pathways into the spot light as a potential basis for anti-angiogenic strategies (Schoors et al., 2015). In the following sections, we will discuss FA metabolism in ECs and TCs, and overview the potential of targeting FA metabolism for inhibiting tumour angiogenesis (Fig. 1).

2. FA metabolism in ECs

2.1. FA synthesis in ECs

ECs express the enzymes required for FA synthesis: (i) ATP citrate lyase (ACLY), producing acetyl-coenzyme A (CoA) from citrate, (ii) acetyl-CoA carboxylase (ACC), converting acetyl-CoA into malonyl-CoA, and (iii) fatty acid synthase (FASN), a multi-enzyme complex that catalyses the synthesis of long-chain fatty acids such as palmitate from malonyl-CoA and acetyl-CoA. One might perhaps argue that *de novo* lipogenesis would be irrelevant for ECs, since they are continually exposed to FAs in the blood stream. However, endothelial FA synthesis is dynamically regulated in different EC-related disease e.g. in diabetes or arteriosclerosis, and regulates enzymes crucial for vascular function, for example through means of lipid modification (Semenkovich, 2004; Wei et al., 2011). For instance, FASN knock down leads to decreased bioavailability of endothelial nitric oxide synthase (eNOS) through decreased palmitoylation of the enzyme. This leads to translocation of eNOS away from the membrane and to decreased EC migration, EC sprouting in the aortic ring model and EC permeability. Since eNOS regulates vasodilation, the membranal translocation in response to FASN-dependent palmitoylation could facilitate increased perfusion in hypoxic areas. This would increase FA availability from the blood stream and enable FA uptake rather than energy-heavy FA synthesis (Wei et al., 2011). Orlistat, a drug used in the treatment of obesity, targets lipases and has been used to inhibit FASN. It has been shown that in ECs, orlistat targets the metabolic function of FASN, thereby reducing EC FA synthesis, EC proliferation and exposure of the VEGF receptor 2 (VEGFR2) on the cell surface (Browne et al., 2006), though the mechanism has not been investigated. Other reports have confirmed the anti-angiogenic effects of FASN knock down and orlistat treatment (Seguin et al., 2012).

2.2. FA uptake and transport in ECs

Uptake of FAs is the first step in cellular FA utilisation and a key node of metabolic regulation. Inside the cell, FAs are free or bound to FA binding proteins (FABPs), which transport FAs to sites of destination. These can be the endoplasmic reticulum (ER) for elongation and desaturation processes, the nucleus for regulation of gene expression, or lipid droplets upon formation of triglycerides (Currie et al., 2013; Furuhashi and Hotamisligil, 2008). The FA translocase FAT/CD36 and passive diffusion (flip-flop) are responsible for the transfer of FAs across the cell membrane (Glatz et al., 2010). Passive diffusion is dependent on a gradient of free FAs, determined by their levels in- and outside the cell. The pool of free (unbound) FAs inside the cell is influenced by the activity of various proteins, including (i) FA transport proteins (FATP1-6, also called very long-chain fatty acid-CoA ligase (ACSVL), which have been implicated in transport and also in activation of FAs as an initial step of their metabolism (Mashek and Coleman, 2006), (ii) acyl-CoA synthetase long-chain family members (ACSL 1–5), which have been implicated in the activation of FAs, and (iii) the above mentioned intracellular FABPs (FABP1–9). Hence, an increase in the activity of these enzymes would lower the intracellular pool of free FAs and therefore increase the FA gradient and hence passive diffusion (influx) of FAs (Mashek and Coleman, 2006).

The vascular endothelium expresses FAT/CD36, FABP4, FATP3, FATP4, FABP3, and FABP5 (Antohe et al., 1998; Elmasri et al., 2009; Greenwalt et al., 1990; Masouye et al., 1997; Watanabe et al., 1993). Compared to other FA transporter genes, FAT/CD36 expression levels in cardiac ECs are high, suggesting an important role for FA uptake in the heart (Coppiello et al., 2015). However, since FAT/CD36 also binds other ligands including thrombospondin-1, oxidized phospholipids, oxidized low-density lipoprotein and long-