Targeting Metabolism to Counteract Tumor Angiogenesis: A Review of Patent Literature

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Abstract: Background: Massive vessel recruitment is required to sustain rapid tumor growth by delivering oxygen and nutrients. Current strategies to counteract angiogenesis are mostly aimed at reducing tumor vessel density. However, many of these drugs have been shown to trigger hypoxia, thus exacerbating tumor aggressiveness. Promising results come from a completely different approach based on the “normalization” of the endothelial layer and the consequent improvement of the vascular function. This new strategy would ameliorate drug delivery to the tumor meanwhile reducing invasiveness and metastatization.

Objective: Since endothelial metabolism has proved essential in the regulation of the angiogenic switch, many recent patents focus on agents able to inhibit specific metabolic pathways in Tumor-Associated Endothelial Cells (TECs) in order to provide vessel normalization. Here, we provide a review of the recent advances in the development of patents on agents targeting endothelial metabolism that have proved effective in several vascular disorders.

Methods: Results of genetic and pharmacologic studies that brought to the development of patents for methods to counteract aberrant angiogenesis were analysed and sub-divided according to the specific metabolic pathway targeted.

Results: Growing evidences indicate that targeting specific molecular players involved in the endothelial metabolic remodelling required to sustain aberrant angiogenesis, is a valuable therapeutic strategy that can be exploited in vascular disorders as well as in tumor angiogenesis.

Conclusion: These findings might have important implications in clinics and could be particularly relevant to patients developing resistance to traditional anti-angiogenic drugs.

Keywords: Angiogenesis inhibition, endothelial cell metabolism, tumor angiogenesis, vessel normalization.

1. INTRODUCTION

1.1. The Angiogenic Process

New blood vessels are formed from pre-existing ones through the angiogenic process which involves migration, proliferation and differentiation of Endothelial Cells (ECs) lining the blood vessels wall [1]. ECs in the adult organism are mostly quiescent but retain the ability to rapidly activate in response to growth factors such as the Vascular Endothelial Growth Factor (VEGF) or under the hypoxic condition and switch towards an angiogenic state [2, 3]. In accordance with the model of vascular sprouting, in the presence of pro-angiogenic stimuli, ECs differentiate into specialized subtypes. In particular, “tip” cells feel the pro-angiogenic cues and guide the new sprout thanks to the presence of several motile structures as filopodia and lamellipodia, which allow directional locomotion. Meanwhile, “stalk” cells proliferate to elongate the sprout and to form a vascular lumen. When the new branch is established and properly perfused, ECs acquire again the quiescent “phalanx” phenotype.

Importantly, ECs need to adapt their metabolic profile when switching from quiescence to vascular branching [2, 4]. In the recent years, growing evidence highlighted the pivotal role of endothelial metabolism in controlling angiogenesis [5-8]. Despite their direct access to blood oxygen, ECs preferentially rely on glycolysis for energy supply, with most glucose entering the cell converted in lactate [2]. This metabolic feature allows ECs to face the high-to-low oxygen availability cycles deriving from the continuous vessel remodelling. Notably, the glycolytic flux is further enhanced
upon induction of the angiogenic switch in order to increase the production of Adenosine Triphosphate (ATP) and macromolecules for cell division [9]. Interestingly, glycolytic ATP production has been shown to compartmentalize within the motile cytoplasmic projections of “tip” cells where it has to rapidly provide energy for cytoskeletal remodelling during migration [9].

Several papers have recently pointed out the critical and direct involvement of glyclosis and Fatty Acid Oxidation (FAO) in the regulation of most endothelial functions [10-12]. For instance, genetic or pharmacological inactivation of the glycolytic activator Phosphofructokinase-2/Fructose-2,6-Bisphosphatase-3 (PFKFB3) leads to impaired endothelial proliferation and migration as well as reduced vascular branching both in vitro and in vivo [9]. Conversely, inhibition of mitochondrial respiration has been shown not to affect angiogenesis [9]. More than 85% of glucose entering ECs is then converted into lactate [9]. Importantly, lactate has a pro-angiogenic role by increasing VEGF production in ECs through stabilization of HIF-1α-Inducible Transcription Factor-1a (HIF-1α) and promoting cell migration [13, 14]. The FAO pathway has proved essential for angiogenesis too. Indeed, endothelial loss of the mitochondrial importer of Fatty Acids (FAs), i.e. CPT1a, reduces cell proliferation in vitro and affects retinal vascular development in mice [15]. This phenotype is likely caused by depletion of the precursors for nucleotide biosynthesis, which is in turn required for DNA replication and cell proliferation. Glutamine is a key amino acid involved in ECs metabolism [16]. Firstly, glutaminolysis is an important anaplerotic pathway of the Krebs cycle by replenishing the cellular pool of α-ketoglutarate. Furthermore, glutamine is an important source of carbons for protein synthesis as well as a precursor of Glutathione (GSH), a key molecule involved in cell redox homeostasis. Notably, glutamine deprivation in ECs results in impaired proliferation and reduced vessel sprouting in vitro [17]. Moreover, genetic ablation of Glutaminase-1 (GLS1), the enzyme responsible for glutamine conversion into glutamate, causes vascular defects in the retina of 5-days pups [17]. These indications, taken together, widely demonstrate that specific metabolic pathways regulate the angiogenic response in ECs.

1.2. Tumor Angiogenesis

Blood vessels deliver oxygen and nutrients to all tissues, including tumors. Excessive and deregulated angiogenesis is an important hallmark of cancer progression [18-20]. To sustain rapid growth, cancer cells secrete in the tumor microenvironment high levels of pro-angiogenic factors as Vascular Endothelial Growth Factor-A (VEGF-A), Placental Growth Factor (PIGF) and Basic Fibroblast Growth Factor (bFGF). The exposure of tumor ECs to imbalanced growth factor signals promotes vessel overgrowth [21]. However, in a counterintuitive manner, this aberrant angiogenesis leads to the formation of a non-functional vasculature. Indeed, tumor vessels appear highly disorganized, tortuous and dilated [22-25]. Moreover, the loose association with mural cells results in excessive permeability, poor perfusion and hypoxia [23]. These structural and functional alterations eventually promote cancer cell metastatisation and limit drugs delivery to the tumor. For these reasons and since blood vessels are essential to sustain tumor growth, strategies to inhibit tumor angiogenesis have been developed to treat cancer [26].

1.3. Therapeutic Strategies - Canonical Approaches

As a master regulator of angiogenesis, the VEGF pathway is the main target of anti-angiogenic therapies. In particular, among the molecules that are approved and/or in clinical development, there are Monoclonal Antibodies (mAb) against VEGF-A (i.e. Bevacizumab) or its receptor (VEGFR2) (i.e. Ramucirumab) and small molecules able to block Receptor Tyrosine Kinases (RTKs) as VEGF and PDGF receptors (e.g. Sunitinib, Sorafenib) [27-35]. Another strategy to inhibit tumor angiogenesis focuses on the block of Angiopoietin-2 (ANG2), one of the ligands of the Tie2 receptor (i.e. AGM386) [36, 37].

Anti-angiogenic therapies are currently providing survival benefits to many cancer patients, in particular in combination with chemotherapy [38, 39]. However, these therapies have been shown to only slightly improve the overall survival [40-44]. The most important reason why most anti-angiogenic drugs failed to pass the clinical trial phase relies on the radical difference between normal and tumor ECs [45, 46]. For instance, it has been recently demonstrated that, unlike normal ECs (NECs), endothelial cells isolated from prostate tumors show a reduced sensitivity in term of proliferation, survival and motility to the oral non-selective tyrosine kinase inhibitor Sunitinib [47]. Moreover, ECs behaviour is widely influenced by the specific stimuli associated with the tumour microenvironment and some important mechanisms of cell migration observed in NECs are not active in TECs [48-54]. In conclusion, the important functional differences between NECs and TECs together with the onset of resistance mechanisms and the side effects associated to canonical anti-angiogenic treatments highlighted the need for new molecular targets as well as novel approaches [55, 66].

1.4. Therapeutic Strategies - Tumor Vessel Normalization

A different strategy, based on the concept of tumor vascular normalization, is aimed at improving the vascular function instead of destroying vessels [67-72]. This effect involves the attenuation of hyper-permeability, the increase in pericytes coverage and the restoration of perfusion [73, 74]. In combination with chemotherapy, endothelial normalization, therefore, enhances drug delivery and prevents the induction of hypoxia linked to the drastic block of tumor’s blood supply, likely limiting the risk of metastasis formation [21, 68, 75-77]. Importantly, some anti-angiogenic agents as Bevacizumab, have been shown to give just a partial reduction of vessel density when administrated at low doses, therefore promoting an overall normalization of the vasculature with numerous advantages [70, 78, 79].

In addition to addressing the VEGF / VEGFR and ANG / TIE-2 axes, vascular normalization can be achieved also by targeting ECs metabolism [7, 80, 81]. Indeed, the metabolic switch associated with the angiogenic response of tumor ECs has recently revealed to be a new promising therapeutic target [82]. For instance, a partial inhibition of glycolysis has already proved effective in counteracting tumor angiogenesis.
in vivo by promoting vessel normalization. In particular, PFKFB3 haplo-deficiency in ECs results in better-functioning tumor vasculature, thus leading to reduced metastatization and improved delivery of chemotherapeutics to the tumor [83-85]. In this study, the authors induced ectopic tumor growth by injecting syngeneic cells subcutaneously to generate a model of tumor angiogenesis. Moreover, B16-F10 cancer cells were injected intravenously via the portal vein or tail vein in syngeneic wild-type or transgenic mice to obtain liver or lung metastasis, respectively. Similar results can be obtained also through a pharmacological approach, by injecting the PFKFB3-blocker 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propan-1-one (3PO) at low doses in mice [83]. Consistently, a recent patent provides siRNAs directed against PFKFB3 for the treatment of pathological angiogenesis. This patent concerns not only the treatment of cancer but involves also applications for the treatment of pathological angiogenesis such as for example macular degeneration [86]. Other patents (not specifically directed against tumor vessels) are based on a PFKFB3 inhibitor in combination with an immune checkpoint inhibitor to treat cancer and to stimulate anti-tumor immunity [87].

An alternative way to counteract pathological angiogenesis by targeting ECs metabolism is based on the inhibition of FAO. Indeed, interfering with the FAO rate-limiting enzyme CPT1a impairs endothelial sprouting without affecting cell migration [15]. Consistently, pharmacological blockade of FAO reduces angiogenesis in vivo, in a mouse model of retinopathy of prematurity [15]. However, despite targeting CPT1a was shown effective in treating pathological angiogenesis conditions such as age-related macular degeneration, diabetic retinopathy, diabetic maculopathy and proliferative retinopathies, this strategy may not be suitable to the tumor context. Indeed, CPT1a silencing has been shown to induce hyper-permeability of ECs monolayers in vitro and leakage of blood vessels in vivo [88]. A patent from Carmeliet and Shoors indeed provides both siRNAs and inhibitors directed against CPT1a, which have proven effective for the treatment of pathological angiogenesis different from the tumor context [89].

Cancer cells need to reprogram their metabolism to sustain high division rates and to survive under hypoxic conditions. In particular, they switch their metabolism towards aerobic glycolysis, the so-called Warburg effect, to fulfill the growing tumor energy demands [90]. Because of this substantial increase in the glycolytic flux, high levels of lactate are found in the tumor microenvironment, which contributes to acidosis. As stated above, the glycolytic product lactate drives angiogenesis in ECs through HIF-1α stabilization. Hence, the lactate secreted by cancer cells has a paracrine effect on ECs and works as a signalling molecule aimed at increasing angiogenesis. Consistently, high levels of lactate in human cancer correlates with the invasiveness and poor prognosis of the disease [91]. Targeting the endothelial lactate importer, i.e. Monocarboxylate Transporter 1 (MCT1), actually results in reduced tumor angiogenesis in mice upon injection of syngeneic tumor cells [92]. To date, patents aimed at inhibiting MCT1 are only related to the treatment or diagnosis of some type of cancer [93]. Anyway, in the future, a dual effect targeting both cancer cells and tumor-associated ECs could bring important advantages.

Glutaminolysis is enhanced in both tumor-associated ECs and cancer cells. Interestingly, the inhibition of Glutamine Synthetase (GS) strongly affects vascular branching, in both developmental and pathological angiogenesis. Agents targeting GS can be used for the treatment of diseases characterized by pathological angiogenesis such as macular degeneration. In particular, a specific patent concerns both siRNAs and specific inhibitors aimed at reducing the activity of the GS enzyme [94].

Another in vivo study showed that a decrease in the activity of the oxygen-sensor protein PHD2 induces endothelial normalization thus suppressing tumor invasiveness and metastasis [73, 95]. In this syngeneic tumor model, vessel normalization is due to a shift from “tip” towards “phalanx” morphology of tumor-associated ECs, which helps to restore a normal endothelial layer. The endothelial re-adaptation is therefore responsible for improving perfusion and oxygenation so that the resulting tumor appears less glycolytic and aggressive [95, 96]. A patent of 2014 refers these beneficial outcomes due to the administration a PHD2 inhibitor wherein the inhibitory effect is achieved at the DNA or RNA level [97].

Other promising results come from studies on the anti-malarial drug chloroquine. This agent acts as an autophagy inhibitor and shows anti-cancer activity at high doses. Conversely, a low dose of chloroquine is able to induce a more quiescent phenotype in tumor ECs, therefore, promoting vessel normalization via autophagy-independent mechanisms [98, 99]. A related patent shows a method to treat cancer by using a pharmaceutical composition of an Anti-Angiogenic Drug (i.e. Avastin) together with the autophagy inhibitor chloroquine [100].

Taken together, these data highlighted the pivotal role of endothelial metabolism in driving angiogenesis and investigated different strategies to target specific metabolic pathways to treat cancer

CONCLUSION

The present work reviews the recent patents on strategies to counteract tumor vascularization. In particular, great efforts have been made to elucidate the role of endothelial metabolism in the control of angiogenesis in order to open new windows of therapeutic intervention. Promising results come from studies on strategies aimed at inhibiting the metabolic pathways involved in the endothelial angiogenic response such as glycolysis, FAO and glutaminolysis, as resumed in Fig. (1). The difficulty of translating these findings from basic science to the clinical use still places a great challenge and requires further investigation.

CURRENT & FUTURE DEVELOPMENTS

Abnormal and sustained angiogenesis has a pivotal role in tumor growth. Many in vitro studies, as well as studies on murine models, demonstrated that pharmacological inhibition of new vessel formation could be a valuable way to counteract tumor growth. However, most anti-angiogenic drugs were actually found to be unsatisfactory when transferred to human patients due to the onset of resistance mechanisms or to the severe side effects. Nowadays, it is clear that tumor vascula-
tissue has unique features and TECs strongly differ from NECs in morphology, metabolism and angiogenic potential. For this reason, great efforts have been carried out in the recent years to discover new therapeutic approaches able to exploit these intrinsic differences between TECs and NECs. In particular, there are some evidence showing that inhibition of the metabolic switch that fuel TECs angiogenic potential has actually proved effective in mouse models of tumor angiogenesis. Despite a more complete understating of the metabolic pathways specifically and differentially active in TECs is required, these data open new windows of therapeutic intervention for the treatment of cancer patients.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

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CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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REFERENCES


