# Angiogenesis and Metabolism: Entwined for Therapy Resistance

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## Keywords

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## Abstract

Angiogenesis and metabolism are entwined processes that permit tumor growth and progression. Blood vessel supply is necessary for tumor survival by providing oxygen and nutrients for anabolism, but also by removing waste products from cellular metabolism. On the other hand, blocking angiogenesis with antiangiogenic therapies shows clinical benefits in several tumor types. Nevertheless, resistance to therapy emerges over time. In this review, we will discuss a novel mechanism of adaptive resistance involving metabolic adaptation of tumor cells, as well as provide examples of tumor adaptation to therapy, which may represent a new mechanism of resistance in several types of cancer. Thus, targeting this metabolic tumor adaptation could be a way to avoid resistance in cancer patients.


## Angiogenesis and Metabolism as Therapeutic Target

In 1971, Judah Folkman proposed that tumor growth is dependent on angiogenesis and its inhibition could be used for cancer treatment. Since then, several antiangiogenic drugs have been developed and are currently in clinical use[1]. More recently, the “Hallmarks of Cancer” highlighted the role of angiogenesis and metabolism in tumor progression [2]. And in the update published in 2011 ("Hallmarks of cancer: the next generation"), Weinberg and Hanahan proposed the deregulation of cellular energy as a new hallmark[3]. Thus, angiogenesis and metabolism are key for tumor progression. But more importantly, they are highly entwined processes that share common molecules and signaling pathways[4].Therefore, these common molecular hubs are not only logical targets for therapy but also are critical regulators of tumor adaptation to anti-vascular or anti-metabolic therapies. During the last years, the important role that hypoxia and metabolism play in tumor adaptation to antiangiogenic treatment has been described. Metabolic reprogramming in tumors contributes to their growth either by directly supporting cancer cell proliferation or by shaping the microenvironment potentially favoring tumor cell survival. Pre-clinical studies combining antiangiogenic therapies with anti-metabolic therapies have shown great promise, and clinical trials are being performed. Here, we

review the link between these two important processes with a particular focus on new therapeutic opportunities to prevent tumor metabolic adaptation.

## Angiogenesis, Hypoxia, and Metabolism in Tumor Tissues

Angiogenesis is stimulated when tumor tissues require nutrients and oxygen and is necessary for tumor growth and progression[5]. The growth of new blood vessels is regulated by a balance of pro- and antiangiogenic signals, including the increase in secretion of various proangiogenic growth factors such as vascular endothelial growth factor (VEGF), fibroblast growth factors (FGFs), angiopoietins (Ang), placental growth factor (PlGF) and some integrins, and concomitantly decrease of several anti-angiogenic factors, such as: angiostatin, endostatin, interferons, platelet factor 4, thrombospondin, and tissue inhibitor of metalloproteinase-1, -2, and -3 [6]. When this balance is lost, an abnormal vascular network that is characterized by dilated, tortuous, and hyperpermeable vessels is created [7]. Therefore, tumor vasculature is typically chaotic with dead-end vascular branches and areas of inverted and intermittent blow flow. Some of these areas have impaired vascular function and lead to regions of lowered perfusion and hypoxia[8]. Indeed, hypoxia promotes vessel growth by up regulating multiple pro-angiogenic pathways that mediate key aspects of endothelial, stromal, and vascular support cell functions[9].

Clinically, tumor hypoxia is associated with poor patient prognosis and resistance to chemotherapy[10]. Hypoxia regulates the expression of many genes under the transcriptional control of hypoxia-inducible factors (HIF1α and HIF2α), which heterodimerize with HIF1β and bind to the hypoxia response element (HRE) [11].HIF1α and HIF2α phosphorylation and activation can be modulated by growth factors' signaling cascades such as PI3K/AKT/mTOR and MAPK [12].Many of the genes regulated by HIF lead to more aggressive growth and survival of tumor cells that contribute to cancer development and progression, as HIF is a key regulator of tumor growth, particularly of angiogenesis and metabolism.

The metabolic characteristics of normal and tumor cells are different. Tumor metabolic needs are higher based on cancer phenotypic changes including increased proliferation and survival in a tumor microenvironment with low levels of oxygen, nutrients, and acidic

extracellular pH[13]. Indeed, cancer cells have an altered metabolism that induces metabolic reprogramming producing the activation of target genes by HIF, which decreases cellular dependency on oxygen. Oncogenic transformation itself with genes such as RAS, MYC, and AKT can also upregulate glucose consumption, glycolysis and the loss of phosphorylation of TP53 (best known as p53). This transformation may also recapitulate the features of the Warburg effect, that is, the uncoupling of glycolysis from oxygen levels [14].Thus, tumor metabolism is highly related with tumor initiation and progression, and may also play a role in tumor response to anti-cancer treatments.

On the other hand, hypoxia and HIF signaling regulate many metabolic processes and metabolic intermediates. One of these processes is the increment of glutamine uptake. Glutamine is used in the tricarboxylic acid (TCA) cycle as an alternative to pyruvate, and also reduces oxidative phosphorylation by preventing pyruvate from entering the TCA [15, 16].The pentose phosphate pathway (PPP) is up-regulated in cancer, and stabilization of HIF1α increases expression of genes involved in the PPP [17]. Furthermore, previous studies indicated a link between glutamine metabolism and mTOR signaling, which led investigators to consider the possible involvement of glutamine and the metabolism of lactate in the induction of mTOR signaling [18, 19]. Overall, it is well established that there is a bidirectional relationship between HIF/hypoxia and metabolism, both at the glycolysis/lactate and TCA/glutamine levels.

Tumor hypoxia also triggers the production of metabolic acids, such as lactic acid, as products of anaerobic glycolysis [20]. Therefore, fine regulation of pH is a critical aspect for maintaining the optimum conditions of cell functions[21]. Thus, under hypoxia many pH regulatory proteins are upregulated or show increased activity, e.g. monocarboxylate transporters 1 and 4 (MCT1 and MCT4) that export lactate, which are important for pH regulation in the tumor extracellular microenvironment [20].The resulting acidosis from upregulated glycolysis is considered to be a key factor in the invasiveness and metastatic activity of cancer cells as they try to escape the toxic microenvironment [22]

Furthermore, cancer cells may also have altered metabolic interactions within subpopulations of cancer cells or with the microenvironment, both of which may alter overall tumor metabolite levels [23]. Moverover, metabolic requirements of in tumors are define by tissue of origin, epigenetic drivers, aberrant signaling and tumor

microenvironment [24]. Furthermore, is important to mention that endothelial cells' metabolism can also considered as a possible novel therapeutic target [25]. Concomitantly understanding the dynamics of endothelial and cancer cell metabolism will provide new avenues for clinical strategies. On the other hand, it has been described that for each cancer types, its different metabolism supports the oncogenic phenotype. It is therefore important to evaluate the therapeutic potential of metabolism targeting, based on the concepts of metabolic normalization and metabolic depletion (See Box 1).

Overall, angiogenesis and metabolism are entwined in tumor growth: Hypoxia leads to angiogenic growth factors production that initiates angiogenesis; angiogenesis provides oxygen to the tumor; this angiogenesis also provides nutrients for cell metabolism, which produces energy for angiogenesis and cell proliferation. As oxygen is scarce, tumor metabolism is predominantly glycolysis which acidifies the environment; this acidity can impede metabolic enzymes. Cancer cells can also avoid apoptosis by ignoring apoptotic signals, which can ultimately alter the outcome of anti-cancer therapies. Indeed, hypoxia decreases the efficacy of chemotherapy and radiotherapy.

Therefore, these entwined tumor processes could be exploited therapeutically: angiogenesis is targeted by antiangiogenic agents, metabolic inhibitors could halt ATP production, buffer therapies could normalize acidity, and molecular inhibitors could overcome therapy resistance.

## Tumor Responses to Antiangiogenic Therapy

Many antiangiogenic drugs are clinically used in several types of cancer to block angiogenesis, impair tumor growth, progression and dissemination[26]. Most antiangiogenic therapies target VEGF and its receptors (VEGFRs) [27]. The initial hypothesis was that antiangiogenesis therapy would not induce resistance ("resistant to resistance") because it targeted the genetically more stable endothelial cells instead of the more unstable tumor cells [28].

Nevertheless, as in most systemic therapies, resistance to antiangiogenic treatments occurs, involving both upfront refractoriness (intrinsic resistance), and acquired resistance that is gained over the duration of the treatment. **Intrinsic resistance** is characterized by tumor

indifference to antiangiogenic therapy, and in patients receiving antiangiogenics such as bevacizumab, sorafenib or sunitinib, tumors continue to grow in spite of treatment [29]. On the other hand, **acquired resistance** to antiangiogenics seems to stem from tumor adaptations to therapy instead of mutations or gene amplifications that typically characterize acquired resistance to other therapeutic strategies. In this form of resistance, alternative mechanisms are created that lead to activation of additional proangiogenic signaling even when the target of the drug remains inhibited [30-33]. In fact, clinical evidence of this plasticity has been described in metastatic renal cell carcinoma (RCC) treated repeatedly with VEGFR inhibitors[34]. Indeed, several clinical trials report an upfront failure of these therapies in some patients, and also a lack of long-lasting effects of antiangiogenic agents as consequence of tumor adaptation to the therapy.

## Antiangiogenic Resistance via Metabolic Symbiosis

Recently, a new mechanism of resistance to antiangiogenic therapies was described that involves an induction of metabolic symbiosis between subpopulations of tumor cells [35-37]. Surprisingly, in this case, emergence of resistance is not associated with tumor revascularization but rather with metabolic changes occurring in tumor cells (Figure 1, Key Figure).

Some years ago, Sonveaux et al described a coordinated mechanism in the metabolism of cancer cells allowing the establishment of **metabolic symbiosis**: tumor cells in hypoxic areas up-regulate glycolysis, increase lactate production, and export lactate through *MCT4*. On the other hand, this excess of lactate is taken up by tumor cells in more oxygenated areas of the tumor via *SLC16A1* (best known as *MCT1*), and aerobically metabolize it via the mitochondria [38].

Recently, three independent laboratories have reported that this mechanism is used by tumors to evade antiangiogenic treatment [35-37].

Allen et al. observed in pancreatic neuroendocrine tumor (PanNET) mouse models that antiangiogenic inhibitors, sunitinib and axitinib, elicit compartmentalization of cancer cells into symbiotic clusters, which are the spatial relationship between the cell populations in the metabolic symbiosis: when glucose and oxygen concentrations are high (near blood

vessels), the cells preferentially utilize glucose-fuelled respiration; but when the oxygen supply is depleted (far from vessels), the cells rely on anaerobic glycolysis. The glycolytic cells produce large quantities of lactate which are consumed by cells close to vessels. In the study, the authors also present data to support a mechanism whereby cancer cells take up and metabolize lactate in the context of bioavailable glutamine in normoxic, but not hypoxic conditions, thereby up regulating mTOR signaling. Moreover, they described that co-inhibition of *mTOR* with rapamycin disrupts the symbiosis by up regulating glucose transport in normoxic cells[35].

On the other hand, Pisarsky et al. described in preclinical mouse model of breast cancer the role of metabolic symbiosis as a mechanism underlying evasive resistance to antiangiogenic therapy with the multikinase inhibitors nintedanib and sunitinib. Inhibition of glycolysis or genetic ablation of *SLC16A4*caused disruption of metabolic symbiosis, suppression of tumor growth and prevented the emergence of resistance [37]. In yet another study, we described the induction of MCT1/MCT4 lactate transporters in a pattern of metabolic symbiosis in RCC patient-derived orthoxenograft mouse models treated with sunitinib. This symbiosis was blocked using *mTOR* inhibitors, affecting cells close to vessels and eliminating the hypoxic regions and impairing tumor growth [36]. The concept of metabolic symbiosis is not new; some years ago it was described by Dewhirst, Sonveaux, Feron and colleagues [38]. However, there is clear evidence that this concept can now be extended to the metabolic symbiosis that occurs in response to treatment with antiangiogenic drugs as a new mechanism of resistance to the therapy. This is caused by stress in the tumor microenvironment due to decreased tumor vasculature and exacerbated intratumor hypoxia.

Thus, here again, the close implications between the process of angiogenesis inhibition and changes in metabolism is well established, in this case as an adaptive mechanism in response to treatment. Furthermore, from a therapeutic perspective, blocking this metabolic adaptation could have a significant value, as we envision the use of anti-metabolic drugs in combination with antiangiogenics upfront or when resistance emerges.

## Clinical trials

Antiangiogenic drugs used in the clinic extend survival in the order of months in some cancer settings while failing to induce survival benefit in others, in part because of intrinsic refractoriness or evasive escape[39]. Very recently, exciting novel concepts involving blocking angiogenesis and metabolic adaptation have emerged from preclinical research, which could prevent the emergence of resistance in the clinics.

Clinical trials using mTOR inhibitors as a second line treatment in combination or not with other therapies including antiangiogenics have been initiated. In particular, there are 17 open clinical trials and 19 clinical studies already completed with or without results, based on CinicalTrials.gov (Table 1).For years it has been thought that double inhibition of two important pathways such as VEGF and mTOR was unfavorable in terms of efficacy in particular due to increased toxicity [40]. Nowadays, this trend is changing because there are new preclinical and clinical data providing evidence of effectiveness and moderate toxicity of this combination. In particular, Motzer and colleagues tested a new VEGF receptor inhibitor, levantinib, alone or in combination with everolimus for a second line therapy in patients who had progressed to a first line antiangiogenic. They observed promising efficacy results with the dual combination not only in progression free survival but also in overall survival [41]. They also observed tolerable side effects in 20%of patients in the combinatory group. Recently, in the RECORD-4 clinical trial everolimus demonstrated a favorable benefit-risk profile used as a second-line in mRCC (metastatic renal cell carcinoma) patients who progressed after a first-line anti-VEGF therapy [42]. In addition, final overall survival (OS) analysis supports everolimus as a second-line option in mRCC patients who were previously treated with sunitinib, other anti-VEGF therapy, or cytokines. Based on these results, the efficacy of everolimus as a second-line treatment in mRCC patients has been demonstrated [42]. Nevertheless, it is necessary to keep adequate patient follow-up and control the dose to avoid the occurrence of side effects.

## Concluding Remarks

The process of angiogenesis and the metabolic pathways in a tumor cell are intimately entwined during cancer growth and disease development. Hypoxia is a key element in the

induction of neovessel formation, but is also critical in the regulation of metabolism. Indeed, hypoxia response programs typically include many metabolic genes, but also many angiogenesis-regulatory molecules [43]. Therefore, it is not surprising to see the close implication of these processes also in therapeutic resistance. Indeed, a recently described form of tumor resistance to antiangiogenic therapies involves a metabolic rewiring of the carbohydrate energy pathways in the form of metabolic symbiosis. But even more importantly, it also opens new avenues for treatment strategies aimed at inhibiting both metabolism and angiogenesis.

The current challenge is to overcome the idea that cancer metabolism is a unique and consistent entity, and analyze tumor metabolism in the context of tissue origin, genetics and epigenetics change of individual tumors, signaling aberrations, heterogeneity of cancer cells and the associated tumor microenvironment. Nowadays, tumor metabolism offers a wide range of targeted drugs that can be exploited for cancer therapy.Therefore, taking into account tissue variability and the specific metabolism of each tumor could give us the possibility to select specific drugs and use therapeutic strategies based on metabolism. Therefore, for the combination of anti-metabolic drugs together with antiangiogenics, a logical therapeutic strategy could be the use of an antiangiogenic drug as first-line treatment and, at the moment of resistance, follow on by second-line treatment aimed at blocking metabolic adaptation. Nevertheless, it is not yet clear whether suppression of VEGF pathway should be maintained in the second-line treatment in order to sustain the tumor metabolic adaptation. In this case, addition of anti-metabolic drugs on top of antiangiogenics (added combination) could demonstrate extended benefits. While these particular combinations have not been fully tested yet in patients, the current clinical approaches tend to use combination strategies rather than sequential monotherapies [44]. Overall, targeting angiogenesis and tumor metabolic reprogramming could be a new opportunity for cancer treatment (See Outstanding Questions). Furthermore, identifying new predictors of response or biomarkers of resistance to antiangiogenic therapies would facilitate the applicability of these new combinations in cancer patients.

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# Legend to Key Figure

## Figure 1: Response to antiangiogenic therapies and resistance by metabolic symbiosis.

Vascularized angiogenic tumors are treated with antiangiogenic therapy, which elicits regression of tumor vasculature causing an increase in hypoxia and tumor shrinkage. Hypoxia modulates different growth factors and signaling cascades, such as the mTOR pathway, which can trigger tumor adaptation to therapy. Resistance to antiangiogenic therapies involves tumor plasticity mechanisms, such as the establishment of metabolic symbiosis between cancer cells. Tumor cells in hypoxic regions (blue) up-regulate glycolysis, increase lactate production and export lactate through the transporterMCT4. On the other hand, lactate is taken up by normoxic cancer cells via MCT1 and is aerobically metabolized in the mitochondria. This symbiotic mechanism is used to evade antiangiogenic therapies and allows resistance and tumor progression.

# Glossary

**Anaerobic glycolysis:** transformation of glucose to lactate when limited amounts of oxygen ($O_2$) are available.

**Angiogenesis:** formation of new blood vessels from pre-existing vessels.

**Antiangiogenics:** class of anti-cancer therapies that target the tumor vasculature.

**Antiangiogenic resistance:** process that involves different mechanisms in the tumor that allow evasion of therapy.

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**Endothelial cells:** form a single *cell* layer that lines all blood vessels and regulates molecule exchanges between the bloodstream and the surrounding tissues.

**Hallmarks of cancer:** constitute an organizing principle for rationalizing the complexities of neoplastic disease.

**Hypoxia:** condition where the tissues are not oxygenated adequately, usually due to an insufficient concentration of oxygen in the blood.

**Hypoxia-inducible factor:** key regulator that plays an integral role in the body's response to low oxygen concentrations

**Hypoxic and metabolic adaptation to antiangiogenic therapy:** changes that occur in tumor cells in response to low levels of oxygen and nutrients that allow tumor cells to survive in these conditions.

**Glycolysis:** metabolic pathway that converts glucose into pyruvate.

**Metabolic symbiosis:** mechanism of resistance where hypoxic cancer cells import glucose and export lactate, while normoxic cells import and catabolize lactate.

**Warburg effect:** describes the increased utilization of glycolysis rather than oxidative phosphorylation by tumor cells for their energy requirements under physiological oxygen conditions. This effect has been the basis for much speculation on the survival advantage of tumor cells, tumorigenesis and the microenvironment of tumors.

Key Figure

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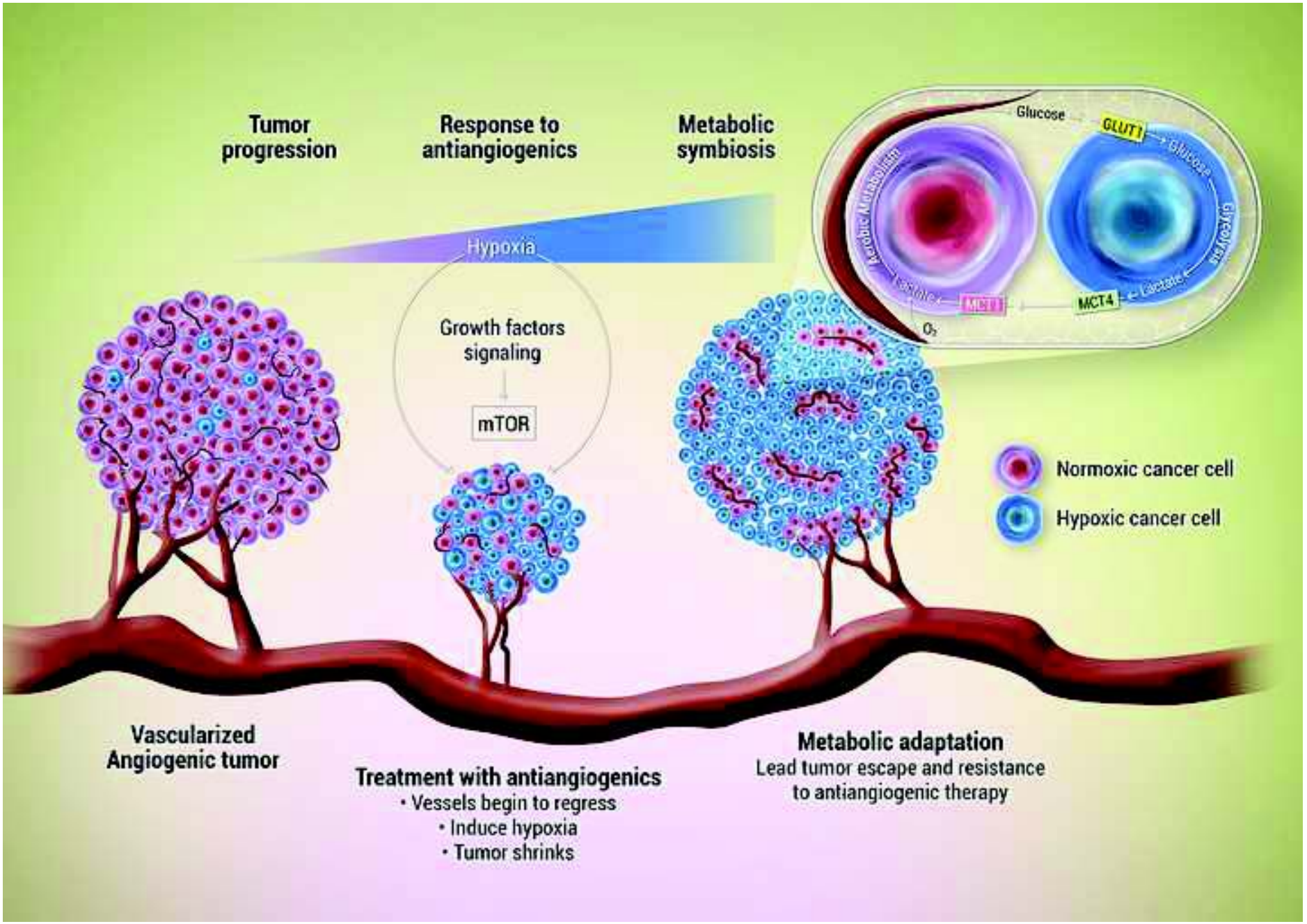

## Trends Box

- Angiogenesis and metabolism are entwined processes during tumor development and their interface offers unprecedented opportunities for therapeutic intervention.
- Antiangiogenic drugs are currently used in the clinic but therapy resistance emerges over time with disease progression. Recently, metabolic symbiosis has emerged as a new mechanism of resistance to these therapies.
- Metabolic symbiosis is a tumor compartmentalization where hypoxic regions (far from blood vessels) are highly glycolytic and they generate high amounts of lactate. In contrast normoxic regions (close to blood vessels) uptake the excess of lactate and metabolize it by aerobic mitochondrial respiration. With this mutualistic balance tumors evade antiangiogenic therapies and continue to grow.
- Targeting angiogenesis and metabolic adaptation could substantially extend the benefits of antiangiogenic therapies.

# Outstanding Questions Box

- How could the Warburg effect influence drug efficacy?
- Can we exploit tumor energetics knowledge to improve drug development?
- Could there be synergy in combining antiangiogenic therapies and metabolic inhibition?
- Would they interact with standard chemotherapy? What about the scheduling of these combinations of therapies?
- Could they be used in different disease stages (metastatic, adjuvant, neoadjuvant)?
- Are there potential predictive biomarkers of response to these combination therapies?
- Should we develop metabolic drugs into personalized cancer medicines?
- Which are the biological consequences of sustained suppression of angiogenesis on tumor biology and normal tissue homeostasis?
- Should we combine antiangiogenic drugs with anti-resistance targeting agents at the time of resistance or earlier?
- Why are surrogate markers or biomarkers of angiogenesis and antiangiogenesis still so elusive and not yet clinically applied?