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# GLIOBLASTOMA: EVOLVING NICHES AND CHALLENGES

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**Abstract ▶** Glioblastoma multiforme (GBM) are one of the most refractory brain tumors characterized by aggressive invasive growth and resistance to therapy. These tumors are highly heterogeneous at the molecular and histological levels. Specific hallmarks like necrosis and microvascular proliferation distinguish GBM from lower-grade gliomas. GBMs are one of the most hypoxic as well as angiogenic tumors. GBMs consist of specific niches within the tumor microenvironment that regulates metabolic needs, tumor survival and invasion, as well as cancer stem cell (CSC) maintenance. This review features the distinct GBM niches, the functional status of the vasculature, and discusses the prospects of therapeutically targeting GBM niche constituents.

**Key Words:** Glioblastoma multiforme, neovascularization, hypoxia, necrosis, niche.

## Introduction

Glioblastoma multiforme (GBM) develop with a well formed yet inefficient neo-vascular system compared to the normal brain tissue[1]. Intra-tumoral necrosis due to insufficient vascular supply is a hallmark feature of GBM and sometimes it may involve molecular or genetic changes intrinsic to the tumor[2]. The overall prognosis for GB has changed little since the 1980s, despite major improvements in neuroimaging, neurosurgery, radiotherapy, and chemotherapy techniques (Figure 1). Evidence suggests that hypoxia is a well-recognized component of the tumor microenvironment and plays a role in the malignant transformation of cells and subsequent tumor growth. It has been linked to poor patient outcome and resistance to therapies in different cancer types. In a number of human cancers, hypoxia predicts the chances of metastases[3], tumor relapse, and resistance to chemo- as well as radiation therapy[5], and poor prognosis. In fact, the degree of necrosis within a GBM correlates inversely with the survival of the patient. Hypoxic stress has been linked to phenotypic changes in tumors wielded through genomic instability, *anoikis*, altered gene expression, and neo-angiogenesis.

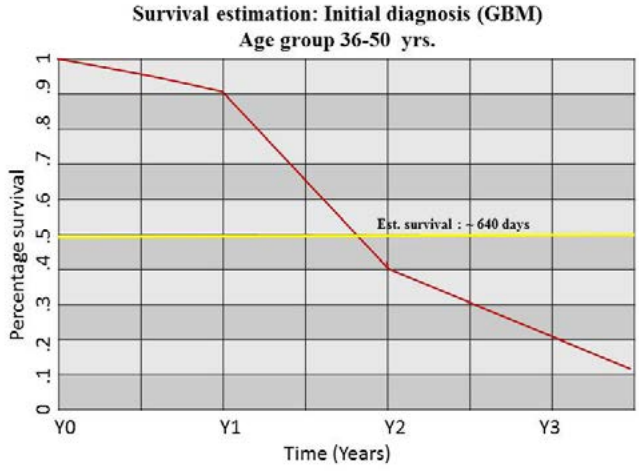



Figure1. Diagrammatic representation of the survival estimation of GBM

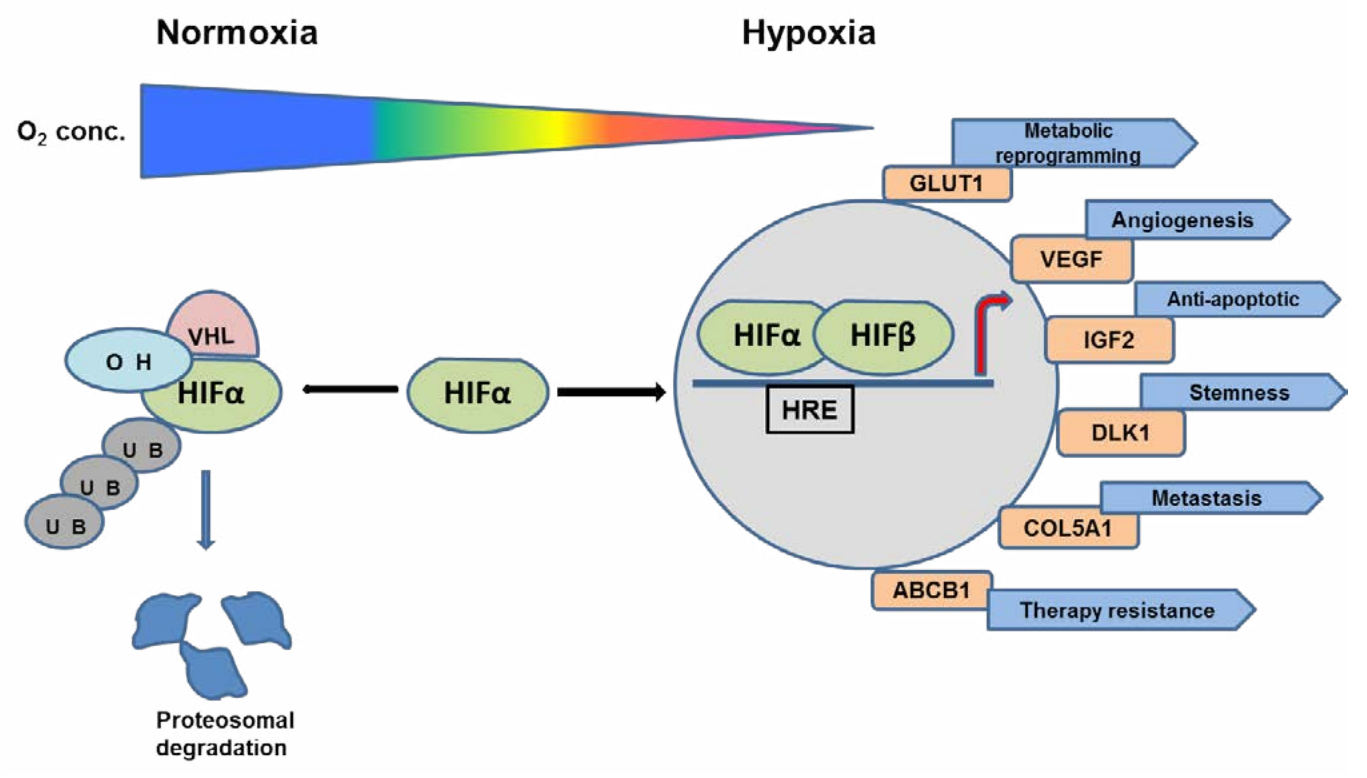
## Hypoxia in Glioblastoma

Recent advancements in genomic sequencing and analysis have stratified GBM into different molecular subtypes, of which the mesenchymal (MES) and proneural (PN) subtypes appear to be the most pronounced[6]). Interestingly, high levels of tumor necrosis were observed in tumors of patients having a mesenchymal subtype. The MES phenotype in GBMs has been associated with refractory tumors with elevated metastatic potential[7]. GBM cells surrounding necrotic zones with hypoxic core express high levels of the mesenchymal transcription factors like C/EBP-β and C/EBP-δ. Incidentally, the expression of these transcription factors is associated with a poor disease prognosis[8].

GBMs invariably display swift cell proliferation coupled with insufficient vascularization leading to generation of niches with scarce oxygen supply. This chronic exposure to extremely low levels of oxygen produces necrotic zones surrounded by densely packed hypoxic tumor cells called "pseudopalisading" GBM cells. These "pseudopalisading" GBM cells are shown to express hypoxia-regulated genes that control angiogenesis, extracellular matrix degradation, and invasive behavior[9]. The cellular responses to hypoxia are generally mediated by the hypoxia-inducible factor (HIF) family of transcription factors. HIFs function as heterodimers composed of an oxygen-sensitive HIFα subunit and a constitutively expressed HIFβ subunit. Under normoxia HIFα undergoes proteasomal degradation by ubiquitination mediated by the von Hippel–Lindau (vHL) tumor suppressor gene product. However, under hypoxic conditions, the interaction between HIFα and vHL is abolished leading to the stabilization of the HIFα subunit. This allows its dimerization with HIFβ and subsequent binding to hypoxia responsive elements (HREs) in the promoters of hypoxia-regulated genes[10]. Hence, the transcription of hundreds of downstream genes that can modulate cell survival, mobility, metabolism and angiogenesis in order to restore oxygen homeostasis are well regulated (Figure 2)

Two HIFα subunits, HIF1α and HIF2α, though structurally similar in their DNA binding and dimerization domains, are known to have non-overlapping biological roles and require different levels of oxygen for activation. HIF-1α and HIF-2α regulate different subsets of genes, although they do share common targets such as *VEGF* and *GLU*. In arginine homeostasis, HIF-1α induces *iNOS* expression

Figure 2. Regulation of HIF-1α under normoxic and hypoxic conditions



and increases nitric oxide production from arginine, whereas HIF-2α stimulates *arginase* expression, and suppresses NO production. Therefore, identification of differential roles of adipocyte HIF-1α and HIF-2α is essential to understand the molecular mechanisms of the metabolic consequences of adipose tissue hypoxia in obesity.

Another feature of GBM is extensive and abnormal angiogenesis that leads to disorganized and leaky blood vessels. This is predominantly induced by the remarkable elevation of vascular endothelial growth factor (VEGF) activity. It has been demonstrated that hypoxia induces both increased transcription and decreased degradation of VEGF mRNA in solid tumors[11]. The vascular abnormalities in GBM have can cause the disruption of the blood–brain barrier (BBB) as a result of which the circulating immune cells and derived chemokines and cytokines enter the brain. Monocytes, neutrophils, and myeloid-derived suppressor cells (MDSC) [12, 13] are all commonly found in the perivascular tumor niche. These cells convey immune-suppressive functions, and interact with tumor cells and cancer stem cells (CSCs), thereby promoting tumor propagation and progression.

Several studies from GBM and other tumor types have provided evidence that hypoxic tumor niches including peri-necrotic regions of human glioblastoma biopsies are rich in CSCs[14]. Hypoxia promotes stem-ness through the activation of genes implicated in self-renewal and dedifferentiation, and shields tumor cells and CSCs from chemo- and radiotherapy. The increased stem-ness of the tumor cells can be measured by an increase in neurosphere forming ability of CSCs, with increased expression of several cancer stem cell markers, including CD133, SOX2, OCT4, and nestin[15, 16]. The necrotic cell death in the center of the hypoxic niche releases pro-inflammatory signals into the surrounding tissue microenvironment that tunes inflammatory cells such as tumor associated macrophages (TAMs) and neutrophils to lose their original function of removing necrotic debris and transformed them into immune-suppressive and angiogenesis-promoting cells. In addition, hypoxia appears to enhance trans-differentiation of CSCs into endothelial-like cells, and promotes their incorporation into tumor vessels[17]. Micro vascular hyperplasia often observed in close proximity to pseudopalisading necrosis enables tumor cells and CSC to grow towards newly formed vasculature and thus contributes to a vicious cycle.

## The Invasive GBM Niche

GBMs not only migrate away from hypoxic regions within the tumor mass, but have the propensity to invade normal tissue as well. GBM infiltrate as single cells or move along white-matter tracts and basement membranes, including those of blood vessels to invade normal brain parenchyma[18, 19]. It is notable that several membrane metalloproteinases (MMPs) have been associated with GBM invasion mode of GBM[20-22]. A search for MMP-inducing factors in tumour cells led to the identification of CD147, also known as EMMPRIN (extracellular matrix metalloproteinase inducer), a highly glycosylated cell-surface trans-membrane protein which stimulates MMP synthesis in neighboring fibroblasts and tumor cells[23] (Figure 3).

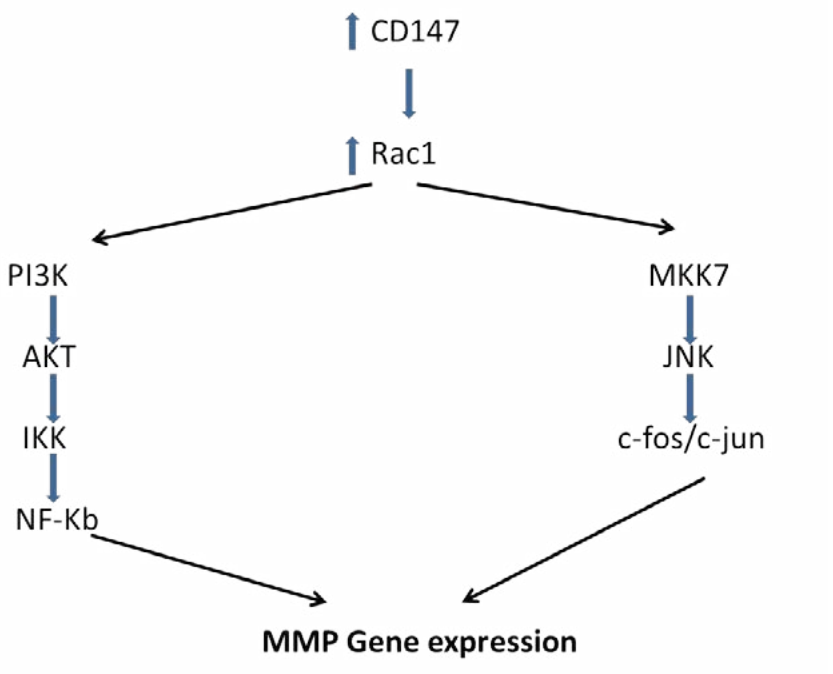



**Figure 3. Transcriptional control of membrane metalloproteinases (MMPs) by CD147.**

CD147 is highly expressed in various human carcinoma tissues and cell lines, correlating with tumour progression under experimental and clinical conditions. Glioma cells have been shown to over- express CD147 and it is associated with more aggressive tumor type and poor prognosis. The best characterized function of CD147 is its

ability to induce the expression of MMPs, including MMP-1, MMP-2, MMP-3, MMP- 9 and MMP-11 in stromal cells. In view of the high expression of CD147 in malignant tissues and its potential as a target for cancer therapy, many studies have investigated how CD147 modulates MMP in cancer. As a trans membrane glycoprotein, CD147 forms homo-oligomers in both heterotypic and homotypic cell–cell interactions to induce production of MMPs [24]. Moreover, full-length EMMPRIN is released by tumor cells via vesicle shedding[25]. Secreted soluble CD147 in conditioned medium is equally capable of inducing MMP production, either from surrounding fibroblasts or tumor cells themselves.

Our laboratory at Central Inter-Disciplinary Research Facility is actively engaged in elucidating the role played by CD147 in glioblastomas pathology. Up-regulation of CD147 in solid tumors imparts higher viability to them not only by facilitating the metastasis, but also by enabling the glycolytic switch that allows cancer cells to survive under low oxygen tension. We are working with human GBM cell lines under normoxic and hypoxic conditions to elucidate the role played by CD147 in their metabolic reprogramming as well as enhanced metastatic capacity.

## Conclusion

There is a growing support for the concept of presence of structurally and functionally distinct tumor niches in GBMs and other invasive tumor types with hypoxic features. The high proliferative rate of GBMs and subsequent neovascularization converts these niches into hypoxic and subsequent perivascular tumor niches. These distinct tumor niches undergo dynamic flux in a temporal and spatial manner creating tumor microenvironments to accommodate the aggressive growth of GBM into normal tissue. The prevailing view so far has focused on the signaling events in a tumor as a whole. However given the cellular and functional diversity of the vasculature, the cross-talk between the tumor and non-tumor niche constituents may vary widely. This opens up various avenues for further exploration into the crucial dialogues between these "in-flux" tumor and non-tumor compartments that generate tumor niches, about the type of tumor niche that will be generated out of this cross-talk, and how these distinct niches might react to cancer therapies. The forthcoming knowledge will provide key information from such studies may identify new opportunities that can be exploited for blanket therapeutic strategies aimed at all tumor niches in GBMs to enhance survival of cancer patients.

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