

Genomic profiling of glioblastoma: convergence of fundamental biologic tenets and novel insights

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Received: 8 May 2011 / Accepted: 13 September 2011 / Published online: 16 October 2011
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Abstract With advances in genomic profiling and sequencing technology, we are beginning to understand the landscape of the genetic events that accumulated during the neoplastic process. The insights gleaned from these genomic profiling studies with regards to glioblastoma etiology has been particularly satisfying because it cemented the clinical pertinence of major concepts in cancer biology—concepts developed over the past three decades. This article will review how the glioblastoma genomic data set serves as an illustrative platform for the concepts put forward by Hanahan and Weinberg on the cancer phenotype. The picture emerging suggests that most glioblastomas evolve along a multitude of pathways rather than a single defined pathway. In this context, the article will further provide a discussion of the subtypes of glioblastoma as they relate to key principles of developmental neurobiology.

Keywords Glioblastoma · TCGA · Genomics · RTK · RB · p53 · IDH1 · Telomerase · Angiogenesis · Invasion · Subtype · Cancer

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Introduction

In the landmark review by Hanahan and Weinberg [1], the authors distilled the essence of cancer into six distinct phenotypes, including evasion of apoptosis, self-sufficiency in growth signals, insensitivity to anti-growth signals, tissue invasion and metastasis, limitless replicative potentials, and sustained angiogenesis. The widely accepted paradigm suggests that cancer arises as a result of mutations or epigenetic events, which alter the functions of genes critical for attaining these phenotypes. These gene functions are intimately linked to the regulation of developmental processes [2]. As such, their aberrant functions inevitably lead to cell states that resemble stages during normal development. These cell states can be captured using genomic technologies to define distinct transcriptomal subtypes. With the advent of large scale genomic profiling, [3, 4], we now have a glimpse of the genetic events underlying glioblastoma pathogenesis as well as distinct transcriptomal signatures. In this review, the genomic profiles of glioblastoma will be reviewed in the context of the properties described by Hanahan and Weinberg. Transcriptomal subtypes of glioblastoma will be discussed in the context of developmental biology and the cell of origin.

Glioblastoma

Glioblastoma is the most common form of primary brain tumor, with dismal prognosis. The incidence of this tumor is fairly low, with 2–3 cases per 100,000 people in Europe and North America. Despite its rarity, overall mortality related to glioblastoma is comparable to the more prevalent tumors [5]. This is, in large part, due to the near uniform

fatality of the afflicted patients. Indeed, glioblastoma is one of the most aggressive of the malignant tumors. Without treatment, the median survival is approximately 3 months [6]. The current standard of treatment involves maximal surgical resection followed by concurrent radiation therapy and chemotherapy with the DNA alkylating agent, temozolomide [7]. With this regimen, the median survival is approximately 14 months. For nearly all affected, this treatment combination remains palliative.

Studies carried out over the past three decades suggest that glioblastomas, like other cancers, arise secondary to the accumulation of genetic alterations. These alterations can take the form of epigenetic modifications, point mutations, translocations, amplifications, or deletions, and modify gene function in ways that deregulate cellular signaling pathways leading to the cancer phenotype [1]. The exact number and nature of genetic alterations and deregulated signaling pathways required for tumorigenesis remains an issue of debate [8], although it is now clear that CNS carcinogenesis requires multiple disruptions to the normal cellular circuitry [3, 4].

Cancer genomics

In the history of cancer research, the current decade will likely be known as the decade of cancer genomics. It is during our time that the marriage of technology and annotated specimen collection led to fruition to provide us a glimpse of the complex genomic landscape that underlies cancer pathogenesis. Impressively, these efforts demonstrated true collaborative spirits between clinicians and laboratory scientists with common goals of furthering translational science.

The Cancer Genome Atlas (TCGA) constitutes the largest of the genomic efforts. It is a comprehensive and coordinated effort to catalogue the genetic and epigenetic changes in the cancer genome, with goals of identifying those responsible for carcinogenesis. The project constitutes a joint effort of the National Human Genome Research Institute (NHGRI), National Cancer Institute (NCI), and the U.S. Department of Health and Human Services, and collects tumor specimen from major cancer centers spanning across the continental USA. The project aims to provide the genomic profile of 500 specimens of various cancer types using state-of-the-art platforms for sequencing, microRNA, mRNA, single-nucleotide polymorphisms, and methylation profiling. TCGA started as a pilot project in 2006 with focus on glioblastoma as the first cancer type for study. With the success of the pilot project, TCGA plans to expand its efforts to aggressively pursue 20 or more additional cancers.

While acknowledging the importance of the TCGA in cancer research, one cannot neglect the value of the

pioneering genomic efforts that, in many ways, laid the groundwork for the TCGA [9]. This article will review the paradigms established by the various major genomic efforts, including the TCGA, in the context of the cancer phenotypes proposed by Hanahan and Weinberg [1] and fundamental tenets of developmental biology.

The cancer phenotype

The aggregate of cancer research investigation spanning the past three decades suggest that cancer is a genetic disease characterized by mutations or epigenetic events that abrogate or compromise regulatory circuitry governing cell proliferation and homeostasis [8]. In the landmark review by Hanahan and Weinberg [1], the authors distilled the essence of these regulatory circuits into six distinct phenotypes, including evading apoptosis, self-sufficiency in growth signals, insensitivity to anti-growth signals, tissue invasion and metastasis, limitless replicative potentials, and sustained angiogenesis (Fig. 1). The following section will review the TCGA findings pertinent to these phenotypes.

Self-sufficiency in growth signals—the receptor tyrosine kinase (RTK)/phosphoinositidyl 3 kinase (PI3K) signaling cascade

Active cellular proliferation in normal cells requires signals from its environment. These signals typically involve the binding of a transmembrane receptor to growth factors, extracellular matrix components, or cell surface components. This mitogenic signaling process is under stringent regulation in normal cells. Typically, multiple ligand-receptor interactions in a permissive cellular state are required before cellular proliferation can take place. This regulation minimizes the probability of dysregulated, autonomous cell growth [1, 10].

To abridge this stringent growth regulation, tumors often mutate the transmembrane receptors or their downstream effectors in ways that constitutively activate the pathway. The pathway most commonly mutated to achieve this end in glioblastoma involves the RTK–PI3K pathway [10, 11]. RTKs are cell surface receptors that are normally activated only in response to growth factor binding [10]. Results from the TCGA revealed that nearly all glioblastomas harbor activating mutations or amplifications in genes required for this signaling cascade [3, 4, 12, 13]. Epidermal growth factor receptor (EGFR) and platelet derived growth factor receptor (PDGFR) are two prototypical members of RTKs [3, 4, 13].

For EGFR and PDGFR, binding of the growth factor receptor to the ligand leads to homo- or hetero-

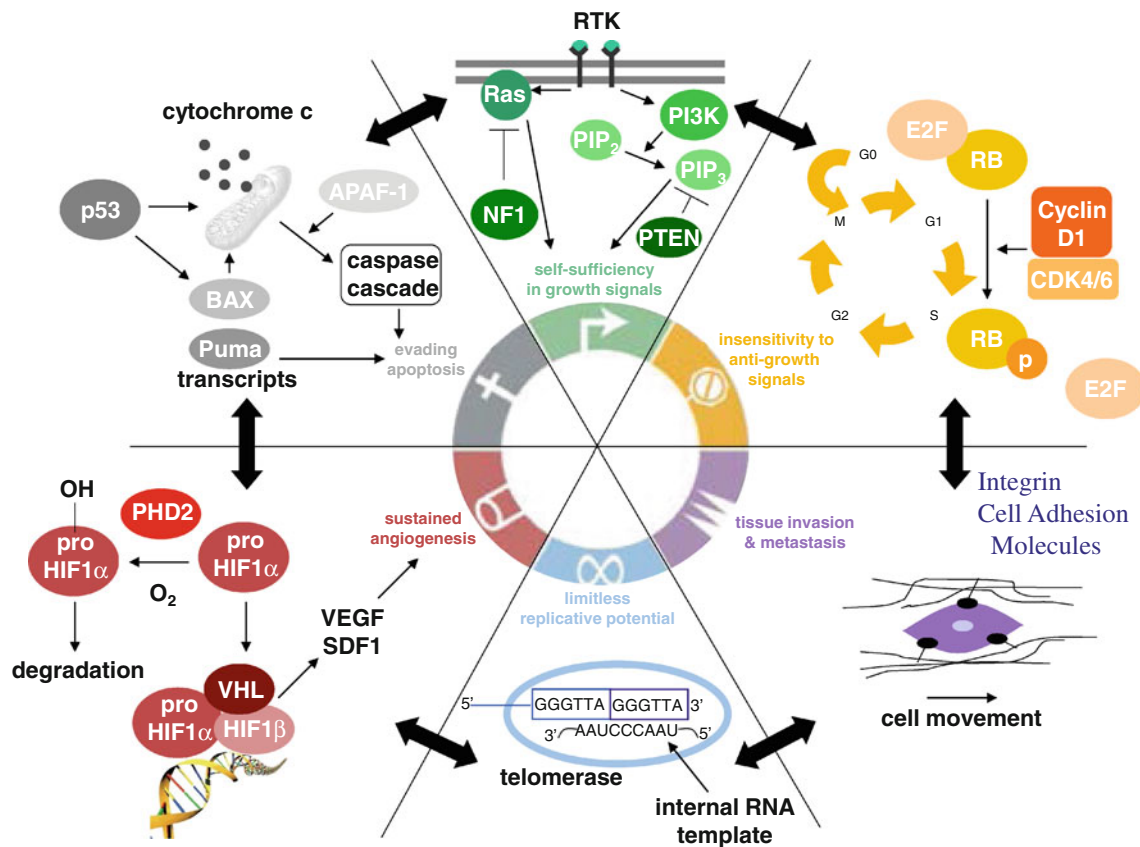


Fig. 1 Hallmarks of the cancer phenotype. TCGA revealed mutations that contributed to the cancer phenotype as proposed by Hanahan and Weinberg, including evading apoptosis, self-sufficiency in growth

signals, insensitivity to anti-growth signals, tissue invasion and metastasis, limitless replicative potentials, and sustained angiogenesis. (Figure adapted from reference [1])

dimerization of the receptor. This dimerization facilitates autophosphorylation of the cytoplasmic domains of the dimerized receptor at select tyrosine residues [10]. The phosphorylated tyrosine residue, in turn, recruits and binds to other signaling proteins on the cell membrane. In some cases, the phospho-tyrosine bound proteins serve as a platform for the recruitment of other effector proteins. In other cases, the bound protein undergoes a conformational change upon binding to the RTK and becomes activated in the process [10].

One of the critical cellular kinases that become activated upon binding to RTK is PI3K [14]. PI3Ks catalyze the phosphorylation of a critical component of the cell surface, phosphatidylinositol-4,5-isphosphate (PI(4,5)P2). This phosphorylation generates phosphatidylinositol-1,4,5-isphosphate (PI(1,4,5)P3), which in turn serves as a docking site for pro-proliferative down-stream effector proteins [11]. Thus, RTK activation transforms the cell membrane into a catalytic surface populated with a high density of pro-mitotic signaling molecules, ultimately leading to cell proliferation. Hydrolysis of (PI(1,4,5)P3) into (PI(4,5)P2) is catalyzed by a phosphatase termed phosphatase and tensin homology (PTEN), in turn, shuts off the RTK–PI3K pathway.

A number of investigations revealed activating mutations in the RTK–PI3K pathway [15–17], validating the importance of this pathway in glioblastoma pathogenesis. The large scale genomic efforts add to this literature in two ways. First, the effort revealed the wide prevalence of RTK–PI3K pathway activation in glioblastomas. In the TCGA report [3], activating mutations in the RTK–PI3K pathway are found in 88% of the 206 glioblastomas sequenced. Efforts by Parsons et. al. [4] revealed that activating mutations in the RTK–PI3K pathway are found in 50% of the 22 glioblastomas sequenced. Second, the TCGA effort revealed mutations in members of the RTK–PI3K pathway that were not previously implicated in glioblastoma pathogenesis. For instance, one of the effector proteins recruited to a phosphorylated RTK is Ras. Ras encodes a monomeric G-protein that cycles between an active form bound to GTP and an inactive form that binds to GDP [18]. It functions as a critical component of the pro-proliferative “catalytic surface”. Through a series of protein–protein interactions, RTK activation catalyzes the exchange of GDP for GTP in Ras, initiating signals required for cellular proliferation. The protein encoded by neurofibromatosis 1 (NF1) functions to catalyze the exchange of GTP for GDP in Ras, consequently preventing cell proliferation. While it is

known that NF1 patients are predisposed to gliomagenesis [19], inactivating mutations in NF1 was not discovered in glioblastoma until recently [3, 4, 20, 21]. The TCGA results showed that approximately 20% of glioblastomas harbor loss of function mutations in NF1 [3, 4]. Importantly, mutations in NF1 appear to define a particular subtype of glioblastoma (see section on [Transcriptomal subtypes](#)). However, the oncogenic role of NF1 loss is complex and does not appear comparable to activation of Ras [3, 4, 13]. Overall, the available data suggest that NF1 is not an ubiquitously relevant Ras regulator.

Insensitivity to anti-growth signals—the RB axis

In addition to receiving pro-growth signals from their environment, cells also receive multiple anti-proliferative signals to prevent cell growth. These anti-growth signals, like their pro-mitotic counterparts, are sensed by the binding of transmembrane receptors to soluble factors, extracellular matrix components, or cell surface components.

Most of these anti-proliferative signals operate at the G₁ phase of the cell cycle to trigger either 1) entry into a transient quiescent (G₀) state or 2) entry into a post-mitotic, differentiated state. At the molecular level, nearly all of these signals converge at the retinoblastoma protein (RB) [1]. In quiescent cells, the RB protein is hyper-phosphorylated. This form of RB binds and sequesters the E2F family of transcription factors [22]. The genes transcribed by these transcription factors are essential for the G₁–S transition of the cell cycle [23]. Phosphorylation of RB releases the sequestered E2F transcription factors and allows for cell growth. During normal cell cycle progression, induction of cyclin D1 and its associated cyclin-dependent kinases, CDK4 and CDK6, at the G₁–S transition is responsible for the phosphorylation of RB. The kinase activity of the CDK4/6-cyclin D complex is under complex regulation, including the critical negative regulators CDKN2A (p16^{Ink4a}), CDKN2B, and CDKN2C.

Pathway inactivating mutations in the RB pathway were described in glioblastomas prior to the large scale genomic efforts [24, 25]. Parsons et. al. [3, 4] and the TCGA validated these results and demonstrated that mutations and gene amplifications disrupting RB function are found in approximately 68–80% of glioblastomas, suggesting the critical importance of escaping anti-growth signals. Additionally, Genome-Wide Association Studies (GWAS) revealed that single nucleotide polymorphisms in the *CDKN2A* and *CDKN2B* have been identified as risk factors for glioma development [26, 27].

Evading apoptosis—the p53 axis

Apoptotic programs are inherent in all normal cells. These programs are activated by a number of physiologic signals

during development and/or in response to cellular stress. Since the tumor state is associated with cellular stress capable of activating apoptosis (e.g., increased oxidative stress, increased DNA damage accumulation), inactivation of these programs constitute a critical step during carcinogenesis.

The regulation of apoptotic pathways is highly complex [28]. Broadly speaking, there are two pathways of apoptosis that converge on the activation of effector proteases (termed caspases), which ultimately trigger the pathognomonic DNA fragmentation, cell shrinkage, and membrane blebbing. The intrinsic cell death pathway (often termed the mitochondrial apoptotic pathway) involves the release of cytochrome c from the mitochondrial membrane space [29]. Binding of cytochrome c to a protein termed apoptosis protease-activating factor 1 (APAF-1), in turn, initiates the caspase cascade. In contrast, the extrinsic apoptotic pathway operates independently of mitochondria and is activated by direct signaling from cell surface receptors to the effector caspase [30].

Both intrinsic and extrinsic apoptotic programs are profoundly influenced by the p53 tumor suppressor protein [31]. *TP53* encodes a transcription factor that regulates gene sets critical for cell cycle progression and apoptosis. Under normal conditions, p53 is a short-lived protein [32]. In response to cellular stress (for instance, DNA damage or oncogene expression), p53 undergoes post-translational modifications and protein–protein interactions that enhance its stability and transcriptional activity [32]. Key among the transcripts regulated by p53 are pro-apoptotic genes (including BAX and Puma) that facilitate both the intrinsic and extrinsic pathway [31]. Additionally, p53 interacts with a number of anti-apoptotic proteins to inhibit their function [31].

There are several lines of evidence that point to the importance of the p53 axis in glioblastoma pathogenesis. As with other critical pathways discussed, there was a body of literature implicating p53 pathway inactivation in glioblastomas [25, 33]. However, these studies implicate p53 pathway inactivation only in a subset of glioblastomas. The TCGA effort and the effort by Parsons et. al. [3, 4] added to the literature by demonstrating that the p53 axis is more broadly impaired in glioblastomas than previously thought. Mutations that inactivate this axis are found in greater than 70% of all glioblastoma specimens as reported by both studies. This understanding has led to more accurate murine modeling of glioblastoma by combined inactivation of p53 and PTEN [34].

Replicative potential

The definition of cancer as a continuous growing entity implies that normal cells exhibit a limited capacity for

proliferation. Indeed, estimates based on tissue culture study suggest that most normal cells have the capacity for 50 doublings [35]. Studies over the past three decades suggest that the main reason for this limited life span involves progressive shortening of chromosomes due to loss of telomeres. Telomeres consist of thousands of six base pair sequence repeat elements that are located at the ends of every chromosome. Because of the inability of DNA polymerases to replicate the 3' ends of chromosomal DNA, approximately 60 base pairs of the telomeric sequence is lost with each replicative cycle [36]. With progressive erosion of the telomeric sequence, the unprotected chromosomal ends participate in aberrant fusion events that inevitably result in cell death [37].

To overcome this inherent limitation, most cancer cells activate an enzyme called telomerase. Telomerase is a reverse transcriptase capable of elongating telomeres [38]. Various mechanisms are employed by tumors to activate telomerase in order to sustain continued cell growth. With regards to glioblastomas, single nucleotide polymorphisms in two genes encoding components of the telomerase (*RTEL1* and *TERT*) have been identified as risk factors for glioma development in two independent GWAS studies [26, 27]. Additionally, elevated expression levels of *TERT* in glioblastoma are associated with decreased patient survival [39]. These studies suggest a critical importance of telomeric biology in glioblastoma growth and survival.

Angiogenesis

The intense proliferation of cancer cells require continued supply of oxygen and nutrients. Due to inherent limitations on the distance that oxygen and macromolecules can travel, virtually all cells in a tissue reside within 100 μm of a capillary. In xenograft model systems, solid tumors can only proliferate up to a size of 1–2 mm without the development of a new blood supply [40]. Thus, angiogenesis is a necessary pre-requisite of solid tumor growth.

One way that cancer cells signal angiogenesis is by secretion of soluble factors that bind to receptors present on the surface endothelial cells. A key soluble factor that functions in such capacity is the vascular endothelial growth factor (VEGF). VEGF binds to RTKs on the surface of endothelial cells to facilitate their proliferation—leading to angiogenesis [41]. Expectedly, clinical studies revealed that the expression level of VEGF correlated with both tumor aggressiveness and poor patient survival [42–44].

In normal cells, transcription of VEGF and other pro-angiogenic signaling factors are under strict regulation. The induction of Hypoxia inducible factor I (HIF1) is a pivotal element in this regulatory network [45]. HIF1 encodes a dimeric transcription factor consisting of two subunits:

HIF1 α and HIF1 β . HIF1 β is constitutively expressed irrespective of oxygen concentration, whereas HIF1 α levels increase dramatically in response to hypoxia. The underlying mechanism for this regulation is that HIF1 α is hydroxylated by HIF Prolyl-4-Hydroxylase (HPH) in the presence of di-oxygen (O_2), iron, and α -ketoglutarate. The hydroxylated HIF1 α is targeted for proteasome degradation. Without molecular oxygen, HIF1 α is not hydroxylated and is free to dimerize with HIF1 β to activate the transcription of downstream pro-angiogenic factors.

Integrated analysis of genomic data in glioblastoma revealed recurrent mutations in the R132 residue of isocitrate dehydrogenase 1 (*IDH1*) [4], a gene largely responsible for the production of α -ketoglutarate. The TCGA data revealed that the *IDH1* mutation is predominantly found in the proneural subtype of glioblastoma [13, 46] (see following section on [Transcriptomal subtypes](#)). The wildtype *IDH1* normally functions as a homodimer that converts isocitrate to α -ketoglutarate [47]. Biochemical characterization of the R132 mutated *IDH1* revealed that it functions to inhibit the process. Expectedly, glioblastoma harboring the R132 *IDH1* mutation harbor decreased levels of α -ketoglutarate.

Given the importance of α -ketoglutarate in HIF1 α degradation, some investigators have proposed that *IDH1* mutations may predispose to increased HIF1 α accumulation and increased VEGF secretion. These predictions were confirmed in a panel of primary glioblastoma specimens [48]. However, there are several issues in narrowly conceptualizing *IDH1* mutations as a pro-angiogenic event. First, *IDH1* mutations are frequently found in low grade gliomas where angiogenesis is essentially never observed [49]. Thus, while *IDH1* mutations may contribute to angiogenesis, it is insufficient to initiate the process. Second, the R132 mutant *IDH1* proteins exhibit a gain-of-function phenotype by generating R(-)-2-hydroxyglutarate, a carcinogenic metabolite [50]. In principle, this metabolite may impact a pleiotropy of cellular processes. Finally, any enzymes requiring α -ketoglutarate are, in principle, affected by the *IDH1* mutations. Interestingly, studies in Acute Myeloid Leukemia (AML) revealed that *IDH1* mutations are found in 15–30% of the afflicted patients [51, 52]. Mutational and epigenetic profiling of these patients revealed that *IDH1* mutations closely associate with a specific hyper-methylation signature. Moreover, expression of *IDH1* mutations induced global DNA hyper-methylation [53]. These results suggest that *IDH1* mutations may lead to dysregulated epigenetic processes. Of note, many proteins required for epigenetic modulation, including the Jumonji-C domain histone demethylases, require α -ketoglutarate as a cofactor [54]. Ultimately, the mechanism of *IDH1* pathogenesis remains unclear given current literature. A thorough discussion of this topic is beyond the

scope of this review but can be found in the above referenced articles.

In glioblastomas without *IDH1* mutation, alternate mechanisms are utilized to facilitate angiogenesis. It is somewhat intuitive that during normal development, periods of cellular proliferation must be coordinated with angiogenesis. Indeed, a large body of study suggests that gene functions that facilitate cell-autonomous growth or insensitivity to growth inhibition and apoptosis also tend to facilitate angiogenesis [55, 56]. It is likely that most glioblastoma cells attain angiogenesis by aberrant activation of such coordinated developmental programs. For instance, EGFR activation has been shown to up-regulate VEGF in both HIF dependent and independent manner [57]. Inactivation of Rb increases VEGF expression and angiogenesis in vivo [55]. Similarly, p53 normally up-regulates thrombospondin 1, an inhibitor of angiogenesis [58]; inactivation of p53 can facilitate angiogenesis by ablation of this up-regulation.

Invasion and metastasis

The ability to invade and metastasize constitutes the fundamental distinction between benign and malignant tumors. It is important to note that invasion refers not just to distortion of normal tissue secondary to tumor growth. Instead, it refers to a coordinated set of cellular activities to destroy and migrate into the surrounding normal tissue. Metastasis refers to the capacity to travel via circulation to a distant tissue site [40]. Glioblastoma is unique in that while it is one of the most invasive of cancers, it rarely metastasizes outside of the central nervous system.

It is a truism that cancer cells generally retain some general properties of the cell of origin. Since glioblastoma originates from astrocytes, which normally possess significant migratory capacity, the invasive nature of glioblastoma would be anticipated. During normal development, astrocytes migrate in a centripetal manner to establish a scaffold for neuro-blasts [59]. Additionally, in response to injury, astrocytes migrate to the affected region to form a gliotic scar [60]. This migratory capacity is the phenotypic expression of carefully orchestrated interactions between cellular cytoskeletal proteins, cell adhesion molecules, and extracellular matrix [40].

To date, the TCGA has not uncovered gain-of function mutations in these proteins. However, enhanced invasive properties have been associated with mutations establishing autonomous growth or suppressing apoptosis. For instance, aberrant EGFR activation results in increased expression and phosphorylation of cell adhesion molecules that ultimately lead to increased invasiveness [61]. Similarly, the p53 mutation drives cancer invasiveness by facilitating the recycling of integrin, a class of cell surface

receptor that interacts with extracellular matrix during cell migration [62].

The aggregate of the data suggest that both angiogenesis and cell migratory properties are intimately integrated into a master circuitry controlled by critical proteins that dictate cellular response to growth or apoptotic signals. In this context, mutations facilitating self-autonomous growth or suppression of apoptosis also contribute to angiogenesis and cell invasion.

Cross-talk between canonical pathways

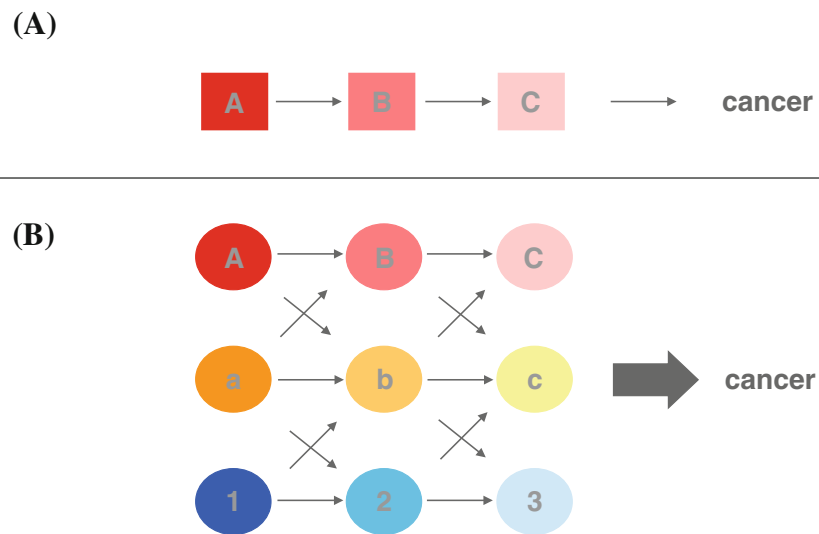
The conceptualization of distinct pathways contributing to the various critical phenotypes constitutes a simplification aimed to consolidate distinct biological concepts. The reality is that pathways mediating the cancer phenotype exhibit high degrees of cross-talk and functional redundancy. For instance, EGFR hyperactivation is associated with increased tumor growth (replicative potential), angiogenesis, and increased tumor motility [63]. Similarly, many genes mediating cell motility, telomere function, and angiogenesis are under transcriptional regulation by p53 and RB associated E2Fs [64].

Pathway of glioblastoma progression

It was previously thought that glioblastoma arises from the acquisition of a defined set of mutations that occur in a particular temporal order. This model is largely grounded on the framework established in colon cancer, where a series of genetic alterations characterizes different phases of neoplastic progression [65]. The framework is supported by the observation that Grade II astrocytomas typically harbor mutations in p53; Grade III astrocytomas harbor activating mutations/amplifications of CDKN2A (p16^{Ink4a}); and Grade IV astrocytomas harbor mutations in PTEN and EGFR [66]. This data was interpreted to mean that glioblastoma results from sequential inactivation of the p53, RB, and RTK/PI3K axes.

While such a paradigm may hold true for a subset of the secondary glioblastomas, the picture emerging from the genomic characterization of primary glioblastomas reveals a much more dynamic process [3, 4]. The profile of somatic mutations in different glioblastomas is highly variable. These results suggest that most glioblastomas, primary or secondary, evolve along a multitude of pathways in response to differing selective pressures to achieve the phenotypes described by Hanahan and Weinberg [67] (Fig. 2). This somewhat stochastic model of cancer progression further implies that mutations critical at one juncture in the neoplastic process may lose relevance as additional mutations are acquired. Thus, while a mutational

Fig. 2 Pathway of glioblastoma pathogenesis. **a** Glioblastoma was previously thought to evolve along a linear progression of mutational accumulation. **b** The TCGA data suggests that most glioblastomas evolve along a multitude of pathways in response to differing selective pressures to achieve the cancer phenotypes



profile constitutes an archeological profile of the history of the neoplasm, extrapolating therapeutic targets from such a profile may be challenging.

Transcriptomal subtypes

Though glioblastoma has been conceptualized as a single disease since Cushing and Bailey's proposed classification scheme, it is widely appreciated that the term encapsulated significant histologic heterogeneity. This heterogeneity suggests distinct subtypes with differing physiologic states that are captured under the umbrella term "glioblastoma". Following up on this hypothesis, there have been many efforts using distinct genomic profiling methods to tease out these subtypes. The number of subtypes varies depending on the study [9, 13, 68–70]. Interpretations of these results are difficult due to methodological differences in profiling platforms, bioinformatic extrapolation, and specimen collection. However, the aggregate of data suggests at least two distinct subtypes that reflect fundamental biologic behavior [9, 13, 70]. Importantly, these subtypes have been validated by independent studies. Further, the transcript signature parallels those obtained during distinct stages in neural development, suggesting the tumor may have arisen from different stages of neurogenesis [70].

The first subtype initially reported by Phillips et al. [70] and subsequently confirmed by the TCGA mRNA [13] and microRNA profiling [71]. The transcript signature resembles those of neuro-blasts and oligodendrocytes derived from fetal and adult brain [70]. The subtype harbors transcriptomal and clinical features that mirror those previously classified as secondary glioblastomas. Molecularly, proneural glioblastomas harbor mutations classically associated with the secondary glioblastomas [13]. Accordingly,

Grade II and III gliomas harbor transcriptomal signatures most reminiscent of the proneural subtype [70]. Clinically, this subtype typically affects younger patients, is associated with improved overall survival [70], and responds poorly to concurrent radiation/temozolomide treatment upon disease progression [13].

Interestingly, *IDH1* mutations that are frequently found in secondary glioblastomas, are found up to 30% of primary proneural glioblastomas [13]. Importantly, methylation profiling, revealed that the nearly 80% of the *IDH1* mutated glioblastoma, whether primary or secondary, harbor a particular global pattern of methylation, termed Glioma CpG Island Methylation Phenotype (G-CIMP) [72]. These studies suggest that the *IDH1* mutated primary proneural glioblastomas may share cell states similar to those observed in secondary glioblastomas. The overall similarity in transcriptomal signature between the primary proneural glioblastomas and secondary glioblastoma appears independent of *IDH1* status, suggesting molecular similarities between these two subclasses extend beyond *IDH1* related physiology.

The second subtype that has emerged is characterized by a gene expression signature that mirror those observed in the neural stem cells of the forebrain [70], cultured astroglial cells [73], and tissue of mesenchymal origin [70]. The subtype is termed "mesenchymal" for the latter correlation. Like the proneural subtype, this second subtype was initially identified by Phillips et al. [70] and subsequently confirmed by the TCGA [13]. Both studies share the observation that patients afflicted with the mesenchymal subtype exhibit poorer clinical prognosis relative to the proneural subtype. In support of these findings, the expression level of key genes in this signature, including VEGF and YKL40, were identified by several independent studies to associate poor prognosis in glioblastoma patients

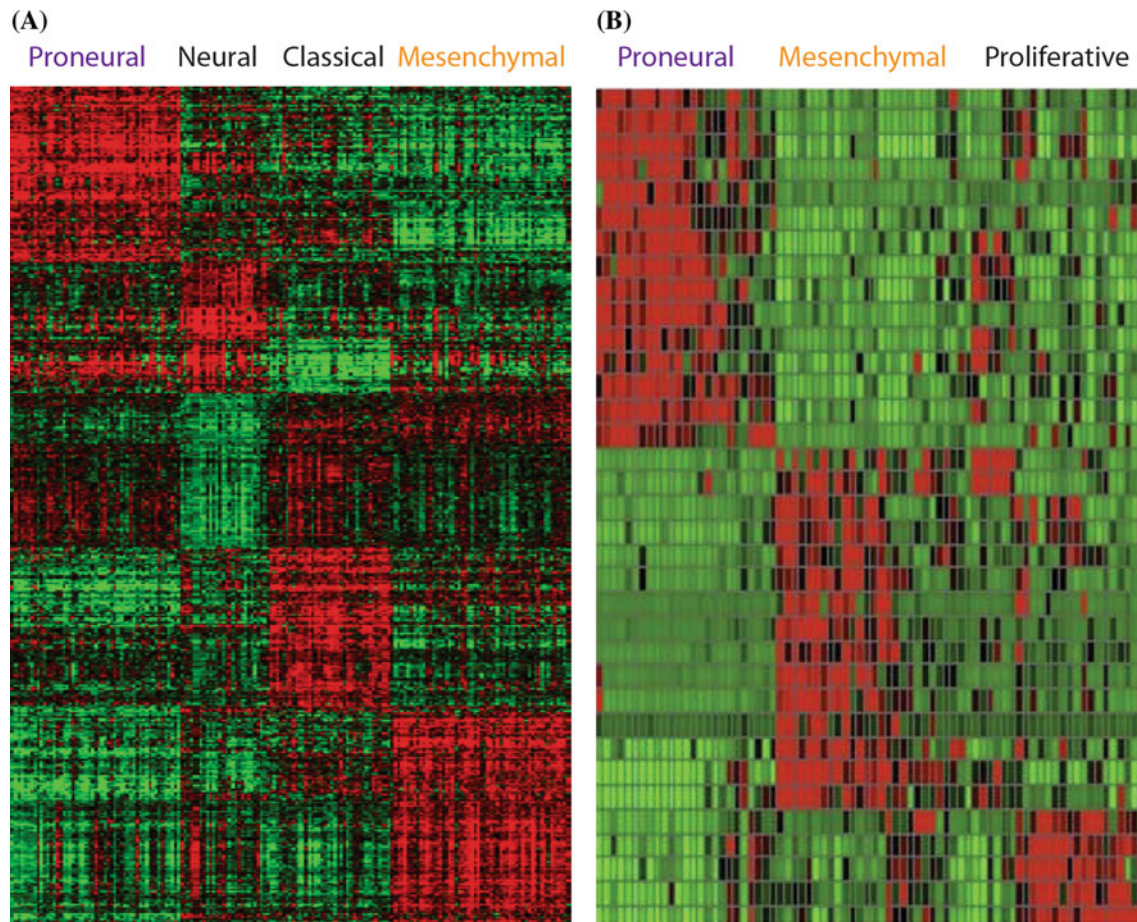


Fig. 3 Transcriptomal subtypes of glioblastoma. Transcriptomal classification of transcriptomal subtypes of glioblastoma reported by, **a** the TCGA (figure adapted from references [13]) and **b** Phillips

et al. (figure adapted from reference [70]). *Red*, high transcript level; *Green*, low transcript level

[42–44, 74]. Three other lines of evidence support the existence of the mesenchymal glioblastoma subtype. First, this subtype is highly enriched for mutations inactivating NF1, suggesting a common genetic etiology [13]. Second, the mesenchymal signature appear driven a common transcriptional network. Expression of two key critical factors in this network (STAT3 and CEBP β) can establish the mesenchymal signature and enhance tumor aggressiveness in murine models [75]. Finally, microRNA profiling revealed that mesenchymal subtype glioblastoma share common signatures, though the data suggested that further subtypes exist within the mesenchymal class [71].

Other glioblastoma subtypes proposed by the TCGA and Phillips et al. [13] are as reviewed in Fig. 3. However, most (if not all) of these subtypes have not been as rigorously validated as the proneural and the mesenchymal. The emerging literature suggests that the proneural and mesenchymal subtypes define the two poles in the spectrum of molecular glioblastoma physiology [9, 13, 70]. It remains unclear whether the other proposed subtypes constitute a “forced fit” of a set of truly heterogeneous biology, a

gradation of phenotypes between the two extreme poles, or a genuine subtype whose biologic basis remains to be understood.

There is significant debate with regards to the origin of the distinct transcriptomal subtypes. On one extreme is the thought that the subtypes originate from the same cell type with differences driven by distinct signaling pathways. The other extreme suggests that subtypes are determined by the same signaling pathways activated in a different cell of origin. The observation that the same canonical pathways are altered irrespective of subtype would tend to support the latter hypothesis. However, it is conceivable that different genes thought to participate in the same canonical pathway may modulate processes distinct of that pathway. Such functions may contribute to the distinct transcriptomal subtypes. Still, it is conceivable that differences in signaling and cell of origin both contribute to subtype formation. A hybrid stand posits that certain mutations arrest the developmental program at stages that are particularly to oncogenesis by specific molecular events. This critical debate awaits experimental resolution.

Limitation of genomic profiling and clinical impact

There are many critical aspects of cancer biology that fundamentally cannot be addressed by profiling an excised tumor mass. The critical interactions between glioblastoma and its microenvironment [76] and the cross-talk between subclonal heterogeneity inherent within every tumor [77] are but a few examples of the limitations. However, in broad strokes, the genomic profiling efforts have validated and consolidated the clinical pertinence of the fundamental biologic paradigms. Further, these efforts refined the umbrella term of glioblastoma into subtypes of distinct physiology. Unfortunately, clinical grade methodologies for routine transcriptomal subtyping are not yet available. Reports of the association between *IDH1* mutations and favorable prognosis hold promise for biomarker development [4, 13, 46, 72]. These correlations await validation in prospective clinical trials. Similarly, the reported sensitivity of *IDH1* mutation expressing glioblastomas to pharmacologic inhibitors of glutaminase [78] awaits further investigation. Future development of tools for subtyping, biomarker development, and therapeutic strategies grounded in the genomic landscape of the particular glioblastoma will facilitate clinical trial designs. Ultimately, meaningful therapeutic gain can be achieved only when agents are directed toward the Achilles' heel inherent within the distinct physiologies of different glioblastomas.

Summary

The past three decades of study in cancer research has generated a sophisticated conceptual framework for the process of neoplastic transformation. The framework suggests that genetic and epigenetic events inactivating critical pathways that regulate several key aspects of cellular function are an etiology. These cellular functions can be categorized as self-sufficiency in growth signaling, evasion of apoptosis, insensitivity to anti-growth signals, tissue invasion, and limitless replicative potential and angiogenesis. This framework has largely been validated by a large scale, high-throughput characterization of the genomic and epigenomic landscape in glioblastomas. The picture emerging from these analyses suggests that most glioblastomas evolve along a multitude of pathways in response to differing selective pressures to achieve the cancer phenotypes. Transcript based analysis revealed distinct subtypes with potential implications with regards to the cell of origin and developmental neuro-biology. The dynamic interplay of growth dysregulation and the cell of origin during the neoplastic transformation process harbors vital implications with regards to therapeutic development. Our understanding of this interplay will be advanced by

refined resolution of the genomic landscape (using second and third generation technologies) and interpreting these landscapes in the context of fundamental biologic tenets. As such, the escalating cost of genomic technology development/application must be balanced with funding for advancing fundamental biologic understanding. In the context of the economic limitations increasingly imposed on funding for scientific investigations, visionary leadership will be needed in resource allocation when balancing these critical needs.

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