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Molecular Etiology of Glioblastomas: Implication of Genomic Profiling From the Cancer Genome Atlas Project

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1. 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 function of genes critical for attaining these phenotypes. These gene functions are intimately linked to the regulation of developmental processes[2], their aberrant function in tumor inevitably lead to cell states that resemble stages during normal development. These cell states can be captured using genomic technologies to define distinct molecular subtypes. With the advent of The Genome Cancer Atlas project for glioblastoma[3,4], we now have a glimpse of the genetic events underlying glioblastoma pathogenesis as well as distinct molecular subtypes. In this review, the genomic profiles of glioblastoma will be reviewed in the context of the properties described by Hanahan and Weinberg. Molecular subtypes of glioblastoma will be discussed in the context of developmental biology and the cell of origin.

2. 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, the treatments available remain 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 tumorogenesis remains an issue of debate[8], although it is now clear that CNS carcinogenesis requires multiple disruptions to the normal cellular circuitry[3, 4].

3. The Cancer Genome Atlas (TCGA) project

The Cancer Genome Atlas (TCGA) 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 U.S. 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. This article will review the major insights derived from the TCGA in the context of the cancer phenotypes proposed by Hanahan and Weinberg[1].

4. 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. The following section will review the TCGA findings pertinent to these phenotypes.

4.1 Self-sufficiency in growth signals – The Receptor Tyrosine Kinase (RTK)/Phospholnosital 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[1,9]. The

importance of growth factors in biology was recognized by a Nobel Prize in Physiology or Medicine to Stanley Cohen and Rita Levi-Montalcini in 1986. Subsequent identification that many oncogenes participate in cellular signaling related to growth factor function was also awarded a Nobel Prize in Physiology or Medicine (to Michael Bishop and Harold Varmus in 1989).

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[9,10]. RTKs are cell surface receptors that are normally activated only in response to growth factor binding[9]. Results from the TCGA revealed that nearly all glioblastomas harbor activating mutations or amplifications in genes required for this signaling cascade[3,4,11,12]. Epidermal Growth Factor Receptor (EGFR) and Platelet Derived Growth Factor Receptor (PDGFR) are two prototypical members of RTK[3, 4, 12].

For EGFR and PDGFR, binding of the growth factor to the ligand leads to homo- or hetero-dimerization of the receptor. This dimerization facilitates autophosphorylation of the cytoplasmic domains of the dimerized receptor at select tyrosine residues[9]. The phosphorylated tyrosine residue, in turn, recruits and binds to other signaling proteins to 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[9].

One of the critical cellular kinases that become activated upon binding to RTK is PI3K[13]. 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 [10]. 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.

Expectedly, gene functions that inhibit the generation of this pro-proliferative "catalytic surface" function as tumor suppressors. For instance, the hydrolysis of (PI(1,4,5)P3) into (PI(4,5)P2) is catalyzed by a phosphatase termed Phosphatase and Tensin Homology (PTEN). PTEN inactivating mutations have been identified in up to 50% of tumor specimens [14]. Similarly, 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[15]. 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. In this context, it is not surprising that NF1 patients are predisposed to gliomagenesis [16]. The TCGA results showed that approximately 20% of glioblastomas harbor loss of function mutations in NF1[3,4]. TCGA additionally revealed gain of function mutations in K-ras have also been identified in glioblastoma specimens [3].

4.2 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. The importance of cell cycle regulation in biology was recognized by a Nobel Prize in Physiology or Medicine to Leland Hartwell, Tim Hunt, and Sir Paul Nurse in 2001.
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[17]. The genes transcribed by these transcription factors are essential for the G1-S transition of the cell cycle[18]. 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. TCGA results showed that mutations and gene amplifications disrupting RB function are found in approximately 80% of glioblastomas, suggesting the critical importance of escaping anti-growth signals[3,4]. Additionally, single nucleotide polymorphisms in the *CDKN2A* and *CDKN2B* have been identified as risk factors for glioma development[19,20].

4.3 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 importance of apoptosis as a fundamental biologic process was recognized by a Nobel Prize in Physiology or Medicine awarded to Sydney Brenner, Robert Horvitz, and John Sulston in 2002.
The regulation of apoptotic pathways is highly complex[21]. 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[22]. 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[23].
Both intrinsic and extrinsic apoptotic programs are profoundly influenced by the p53 tumor suppressor protein[24]. *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[25]. 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[25]. Key among the transcripts regulated by p53 are pro-apoptotic genes (including BAX and Puma) that facilitate both the intrinsic and extrinsic pathway[24]. Additionally, p53 interact with a number of anti-apoptotic proteins to inhibit their function[24].

There are several lines of evidence that point to the importance of the p53 axis in glioblastoma pathogenesis. In the TCGA database, mutations that inactivate this axis are found in greater than 70% of glioblastoma specimens[3,4]. Patients harboring germ-line mutations in *TP53* are afflicted with cancer predisposition including increased risk for glioblastoma[26]. Finally, inactivation of p53 is required for glioma formation in genetically defined murine models[27].

4.4 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 work suggest that most normal cells have the capacity for 50 doublings [28]. Studies over the past three decades suggest that the main reason for this limited life span involve progressive shortening of chromosomes due to loss of telomeres. Telomeres consist of thousands of six base pair sequence element of repeats 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[29]. With progressive erosion of the telomeric sequence, the unprotected chromosomal ends participate in aberrant fusion events that inevitably result in cell death[30].

To overcome this inherent limitation, most cancer cells activate an enzyme called telomerase. Telomerase is a reverse transcriptase capable of elongating telomeres[31]. Various mechanisms are employed by tumors to activate telomerase in order to sustain continued cell growth. Elizabeth Balckburn, Carol Greider, and Jack Szostak were awarded the Nobel Prize in Physiology or Medicine in 2009 for their discovery of telomerase.

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[19, 20]. Additionally, elevated expression level of *TERT* in glioblastoma is associated with decreased patient survival [32]. 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 with 100 um of a capillary. In xenograft model systems, solid tumors can only proliferate up to a size of 1-2 mm without development of new blood supply[33]. Thus, angiogenesis necessarily constitutes a pre-requisite during solid tumor progression.

One way by which 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[34]. 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[35]. HIF1 encodes a dimeric transcription factor consisting of two subunits: HIF1α and HIF1β. HIF1β is constitutively expressed irrespective of oxygen concentration, whereas HIF1α levels increase dramatically in response to hypoxia. The underlying mechanism for this regulation is that HIF1α is hydroxylated by HIF Prolyl-4-Hydroxylase (HPH) in the presence of di-oxygen (O2), iron, and α-ketoglutarate. The hydroxylated HIF1α is targeted for proteasome degradation. Without molecular oxygen,

HIF1α is not hydroxylated and is free to dimerize with HIF1β to activate the transcription of downstream pro-angiogenetic 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 one particular molecular subtype of glioblastoma[12, 36] (see following section on **molecular subtypes**). The wildtype IDH1 normally functions as a homodimer that converts isocitrate to α-ketoglutarate[37]. Biochemical characterization of the R132 mutated IDH1 revealed that it functions in a dominantly negative fashion to inhibit the process. Expectedly, glioblastoma harboring the *R132 IDH1* mutation harbor decreased levels of α -ketoglutarate. Given the importance of α -ketoglutarate in HIF1α degradation, one would anticipate increased HIF1α accumulation and increased VEGF secretion in glioblastoma harboring the *IDH1* mutation. These observations were confirmed in a panel of primary glioblastoma specimens[38]. Thus, the *IDH1* mutation constitutes an example of how glioblastoma subverts the endogenous molecular circuit to facilitate angiogenesis. It should be noted that the effect of the *IDH1* mutation appears pleiotropic. Another study revealed that the R132 mutant IDH1 proteins exhibits a gain-of-function phenotype by generating R(-)-2-hydroxyglutarate, a carcinogenic metabolite[39].

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 angioogenesis. Indeed, a large body of work suggests that gene functions that facilitate cell-autonomous growth or insensitivity to growth inhibition and apoptosis also tend to facilitate angiogenesis[40, 41]. 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[42]. Inactivation of Rb increases VEGF expression and angiogenesis *in vivo*[40]. Similarly, p53 normally up-regulates thrombospondin 1, an inhibitor of angiogenesis[43]; inactivation of p53 can facilitate angiogenesis by ablation of this up-regulation.

4.5 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[33]. 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 neuroblasts[44]. Additionally, in response to injury, astrocytes migrate to the affected region to form a gliotic scar[45]. This migratory capacity is the phenotypic expression of carefully orchestrated interactions between cellular cytoskeletal proteins, cell adhesion molecules, and extracellular matrix[33].

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[46]. 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[47].

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.

4.6 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 [48]. Similarly, many genes mediating cell motility, telomere function, and angiogenesis are under transcriptional regulation by p53 and RB associated E2Fs[49].

5. 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[50]. 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[51]. 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 evolve along a multitude of pathways in response to differing selective pressures to achieve the phenotypes described by Hanahan and Weinberg[52]. 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 profile constitutes an archeological profile of the history of the neoplasm, extrapolating therapeutic targets from such a profile may be challenging.

6. Molecular subtypes

Genome-scale gene expression profiling using microarray technology have revealed distinct molecular subtypes within tumors previously classified as glioblastomas [12, 53-55]. The number

of subtypes varies depending on the study, however, three subtypes consistently appear across independent studies and reflect distinct biologic and clinical behaviors [12, 55, 56]. Importantly, the transcript signature parallels those obtained during distinct stages in neural development, suggesting the tumor may have arisen from different stages of neurogenesis[55].

The first subtype is termed pro-neural. The transcript signature resembles those of neuroblasts and oligodendrocytes derived from fetal and adult brain[55]. This subtype harbors molecular and clinical features that closely mirror those previously classified as secondary glioblastomas. Molecularly, pro-neural glioblastomas harbor mutations classically associated with the secondary subtype, including p53 and PDGFR[12]. Accordingly, grade II and III gliomas harbor molecular signatures most reminiscent of the pro-neural subtype[55]. Clinically, this subtype typically affects younger patients, is associated with improved overall survival[55], and responds poorly to concurrent radiation/temozolomide treatment upon disease progression[12]. Interestingly, mutations in the isocitrate **de**h**y**drogenase 1 gene (*IDH1*), a metabolic protein required for conversion of isocitrate to a-ketoglutamate during the citric acid cycle, is frequently observed in pro-neural glioblastomas (see section on glioblastoma predisposition syndromes). The molecular basis of how this mutation contributes to the cancer phenotype remains an active area of investigation.

Classical (also termed proliferative by some authors) constitutes the second molecular subtype. Transcript signature in the classical subtype resembles those observed in transit amplifying neural progenitor cells[55] and murine astrocytes[12]. This subtype is exclusively found in WHO grade IV tumors and constitutes a form of primary glioblastoma[57]. Molecularly, this subtype is characterized by amplification of (or activating mutations in) EGFR and CDKN2A ($p16^{Ink4a}$). Genes involved in pathways highly active in neural stem and progenitor cells (including the Notch and Sonic hedgehog pathway) are highly expressed in the classical subtype).[58] The patients afflicted are typically older than those with the pro-neural subtype. Relative to the other subtype, patients afflicted with the classical subtype exhibit the worst prognosis, but the best therapeutic response to concurrent radiation/temozolomide treatment.

The mesenchymal subtype makes up the final category. The transcript signature in the mesenchymal subtype mirrors those observed in the neural stem cells of the forebrain[55] and cultured astroglial cells[59]. Most cultured glioblastoma cell lines exhibit transcript signatures that fall into this subtype. Molecularly, the subtype is characterized by inactivating NF1 and PTEN mutations[12]. This subgroup also has the highest expression of angiogenesis markers including VEGF (Vascular Epithelial Growth Factor) transcripts and highest density of microvascular proliferation[12]. The patients afflicted are typically older than those with the pro-neural subtype. Relative to the other subtypes, mesenchymal glioblastomas exhibit clinical response similar to the classical subtype, and a trend toward slightly improved prognosis and response to radiation/temozolomide therapy[12].

There is significant debate with regards to the origin of the distinct molecular 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 molecular subtypes. Still, it is conceivable that differences in signaling and cell of origin both contribute to subtype formation. This critical debate awaits experimental resolution.

7. Summary

The past three decades of work 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. The dynamic interplay of growth dysregulation and the cell of origin during the neoplastic transformation process harbors vital implications with regards to therapeutic development.

8. References

[1] Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell.* Jan 7 2000;100(1):57-70.
[2] Zelenka PS. Proto-oncogenes in cell differentiation. *Bioessays.* Jan 1990;12(1):22-26.
[3] TCGA. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature.* Oct 23 2008;455(7216):1061-1068.
[4] Parsons DW, Jones S, Zhang X, et al. An integrated genomic analysis of human glioblastoma multiforme. *Science.* Sep 26 2008;321(5897):1807-1812.
[5] Wen PY, Kesari S. Malignant gliomas in adults. *N Engl J Med.* Jul 31 2008;359(5):492-507.
[6] Walker MD, Alexander E, Jr., Hunt WE, et al. Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas. A cooperative clinical trial. *J Neurosurg.* Sep 1978;49(3):333-343.
[7] Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med.* Mar 10 2005;352(10):987-996.
[8] Stratton MR, Campbell PJ, Futreal PA. The cancer genome. *Nature.* Apr 9 2009;458(7239):719-724.
[9] Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell.* Oct 13 2000;103(2):211-225.
[10] Hennessy BT, Smith DL, Ram PT, Lu Y, Mills GB. Exploiting the PI3K/AKT pathway for cancer drug discovery. *Nat Rev Drug Discov.* Dec 2005;4(12):988-1004.
[11] Stommel JM, Kimmelman AC, Ying H, et al. Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. *Science.* Oct 12 2007;318(5848):287-290.
[12] Verhaak RGW, Hoadley KA, Purdom E, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell.* Jan 19 2010;17(1):98-110.

[13] Cantley LC. The phosphoinositide 3-kinase pathway. *Science.* May 31 2002;296(5573):1655-1657.
[14] Li J, Yen C, Liaw D, et al. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science.* Mar 28 1997;275(5308):1943-1947.
[15] Downward J. Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer.* Jan 2003;3(1):11-22.
[16] Walker L, Thompson D, Easton D, et al. A prospective study of neurofibromatosis type 1 cancer incidence in the UK. *Br J Cancer.* Jul 17 2006;95(2):233-238.
[17] Knudsen ES, Wang JY. Targeting the RB-pathway in cancer therapy. *Clin Cancer Res.* Feb 15 2010;16(4):1094-1099.
[18] Buchkovich K, Duffy LA, Harlow E. The retinoblastoma protein is phosphorylated during specific phases of the cell cycle. *Cell.* Sep 22 1989;58(6):1097-1105.
[19] Shete S, Hosking FJ, Robertson LB, et al. Genome-wide association study identifies five susceptibility loci for glioma. *Nat Genet.* Aug 2009;41(8):899-904.
[20] Wrensch M, Jenkins RB, Chang JS, et al. Variants in the CDKN2B and RTEL1 regions are associated with high-grade glioma susceptibility. *Nat Genet.* Aug 2009;41(8):905-908.
[21] Fan TJ, Han LH, Cong RS, Liang J. Caspase family proteases and apoptosis. *Acta Biochim Biophys Sin (Shanghai).* Nov 2005;37(11):719-727.
[22] Lowe SW, Cepero E, Evan G. Intrinsic tumour suppression. *Nature.* Nov 18 2004;432(7015):307-315.
[23] Ashkenazi A, Dixit VM. Apoptosis control by death and decoy receptors. *Curr Opin Cell Biol.* Apr 1999;11(2):255-260.
[24] Haupt S, Berger M, Goldberg Z, Haupt Y. Apoptosis - the p53 network. *J Cell Sci.* Oct 15 2003;116(Pt 20):4077-4085.
[25] Harris CC. p53 tumor suppressor gene: from the basic research laboratory to the clinic--an abridged historical perspective. *Carcinogenesis.* Jun 1996;17(6):1187-1198.
[26] Li FP, Fraumeni JF, Jr. Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? *Ann Intern Med.* Oct 1969;71(4):747-752.
[27] Holland EC. Gliomagenesis: genetic alterations and mouse models. *Nat Rev Genet.* Feb 2001;2(2):120-129.
[28] Hayflick L. The Limited in Vitro Lifetime of Human Diploid Cell Strains. *Exp Cell Res.* Mar 1965;37:614-636.
[29] Zhang X, Mar V, Zhou W, Harrington L, Robinson MO. Telomere shortening and apoptosis in telomerase-inhibited human tumor cells. *Genes Dev.* Sep 15 1999;13(18):2388-2399.
[30] Artandi SE, Chang S, Lee SL, et al. Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. *Nature.* Aug 10 2000;406(6796):641-645.
[31] Cohen SB, Graham ME, Lovrecz GO, Bache N, Robinson PJ, Reddel RR. Protein composition of catalytically active human telomerase from immortal cells. *Science.* Mar 30 2007;315(5820):1850-1853.
[32] Alonso MM, Fueyo J, Shay JW, et al. Expression of transcription factor E2F1 and telomerase in glioblastomas: mechanistic linkage and prognostic significance. *J Natl Cancer Inst.* Nov 2 2005;97(21):1589-1600.

[33] Leber MF, Efferth T. Molecular principles of cancer invasion and metastasis (review). *Int J Oncol.* Apr 2009;34(4):881-895.
[34] Veikkola T, Alitalo K. VEGFs, receptors and angiogenesis. *Semin Cancer Biol.* Jun 1999;9(3):211-220.
[35] Sharp FR, Bernaudin M. HIF1 and oxygen sensing in the brain. *Nat Rev Neurosci.* Jun 2004;5(6):437-448.
[36] Yan H, Parsons DW, Jin G, et al. IDH1 and IDH2 mutations in gliomas. *N Engl J Med.* Feb 19 2009;360(8):765-773.
[37] Yan H, Bigner DD, Velculescu V, Parsons DW. Mutant metabolic enzymes are at the origin of gliomas. *Cancer Res.* Dec 15 2009;69(24):9157-9159.
[38] Zhao S, Lin Y, Xu W, et al. Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1alpha. *Science.* Apr 10 2009;324(5924):261-265.
[39] Dang L, White DW, Gross S, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature.* Dec 10 2009;462(7274):739-744.
[40] Gabellini C, Del Bufalo D, Zupi G. Involvement of RB gene family in tumor angiogenesis. *Oncogene.* Aug 28 2006;25(38):5326-5332.
[41] Hoeben A, Landuyt B, Highley MS, Wildiers H, Van Oosterom AT, De Bruijn EA. Vascular endothelial growth factor and angiogenesis. *Pharmacol Rev.* Dec 2004;56(4):549-580.
[42] Haley JD, Gullick WJ, eds. *EGFR Signaling Networks in Cancer Therapy*. New York: Humana Press; 2008.
[43] Rak J, Mitsuhashi Y, Sheehan C, et al. Oncogenes and tumor angiogenesis: differential modes of vascular endothelial growth factor up-regulation in ras-transformed epithelial cells and fibroblasts. *Cancer Res.* Jan 15 2000;60(2):490-498.
[44] Jacobsen CT, Miller RH. Control of astrocyte migration in the developing cerebral cortex. *Dev Neurosci.* Mar-Aug 2003;25(2-4):207-216.
[45] Ridet JL, Malhotra SK, Privat A, Gage FH. Reactive astrocytes: cellular and molecular cues to biological function. *Trends Neurosci.* Dec 1997;20(12):570-577.
[46] Micallef J, Taccone M, Mukherjee J, et al. Epidermal growth factor receptor variant III-induced glioma invasion is mediated through myristoylated alanine-rich protein kinase C substrate overexpression. *Cancer Res.* Oct 1 2009;69(19):7548-7556.
[47] Muller PA, Caswell PT, Doyle B, et al. Mutant p53 drives invasion by promoting integrin recycling. *Cell.* Dec 24 2009;139(7):1327-1341.
[48] Nakamura JL. The epidermal growth factor receptor in malignant gliomas: pathogenesis and therapeutic implications. *Expert Opin Ther Targets.* Apr 2007;11(4):463-472.
[49] Sherr CJ, McCormick F. The RB and p53 pathways in cancer. *Cancer Cell.* Aug 2002;2(2):103-112.
[50] Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. *N Engl J Med.* Sep 1 1988;319(9):525-532.
[51] Gladson CL, Prayson RA, Liu WM. The pathobiology of glioma tumors. *Annu Rev Pathol.* 2010;5:33-50.
[52] Salk JJ, Fox EJ, Loeb LA. Mutational heterogeneity in human cancers: origin and consequences. *Annu Rev Pathol.* 2010;5:51-75.

[53] Nutt CL, Mani DR, Betensky RA, et al. Gene expression-based classification of malignant gliomas correlates better with survival than histological classification. *Cancer Res.* Apr 1 2003;63(7):1602-1607.

[54] Liang Y, Diehn M, Watson N, et al. Gene expression profiling reveals molecularly and clinically distinct subtypes of glioblastoma multiforme. *Proc Natl Acad Sci U S A.* Apr 19 2005;102(16):5814-5819.

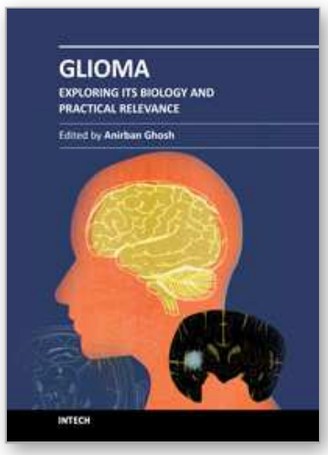
[55] Phillips HS, Kharbanda S, Chen R, et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell.* Mar 2006;9(3):157-173.

[56] Brennan C, Momota H, Hambardzumyan D, et al. Glioblastoma subclasses can be defined by activity among signal transduction pathways and associated genomic alterations. *PLoS One.* 2009;4(11):e7752.

[57] Mischel PS, Shai R, Shi T, et al. Identification of molecular subtypes of glioblastoma by gene expression profiling. *Oncogene.* Apr 17 2003;22(15):2361-2373.

[58] Bar EE, Chaudhry A, Lin A, et al. Cyclopamine-mediated hedgehog pathway inhibition depletes stem-like cancer cells in glioblastoma. *Stem Cells.* Oct 2007;25(10):2524-2533.

[59] Gunther HS, Schmidt NO, Phillips HS, et al. Glioblastoma-derived stem cell-enriched cultures form distinct subgroups according to molecular and phenotypic criteria. *Oncogene.* May 1 2008;27(20):2897-2909.



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The tittle 'Glioma - Exploring Its Biology and Practical Relevance' is indicative of its content. This volume contains 21 chapters basically intended to explore glioma biology and discussing the experimental model systems for the purpose. It is hoped that the present volume will provide supportive and relevant awareness and understanding on the fundamental advances of the subject to the professionals from any sphere interested about glioma.

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