Key concepts in glioblastoma therapy

Bartek Jiri Jr., M.D.[1], Kimberly Ng[2], Bartek Jiri M.D., Ph.D. [3,4], Walter Fischer, Ph.D.[5], Bob, Carter[6], and Clark C. Chen M.D., Ph.D.[1, 7*]

Affiliations:
[1]Department of Neurosurgery, Karolinska University Hospital, Stockholm, Sweden
[2]Department of Radiation Oncology, Dana-Farber Cancer Institute, Boston, MA, USA
[3]Danish Cancer Society Research Center, Copenhagen, Denmark
[4]Laboratory of Genome Integrity and Institute of Molecular and Translational Medicine, Palacky University, Olomouc, Czech Republic
[5]Department of Neurosurgery, Rigshospital, Copenhagen, Denmark
[6]Center for Theoretical and Applied Neurosurgery, UCSD, San Diego, CA, USA
[7]Division of Neurosurgery, Beth Israel Deaconess Medical Center, Boston, MA, USA

***Correspondence:**
Director of Clinical Neuro-oncology
Attending Neurosurgeon
Beth Israel Deaconess Medical Center

Department of Radiation Oncology
Division of Genomic Stability and DNA Repair
Dana-Farber Cancer Institute
Jimmy Fund 620A
44 Binney Street
Boston, Massachusetts 02115-6084
617 582 8643 tel, 617 582 8213

Key words: Glioblastoma, cancer, targeted therapy, oncogene addiction, non-oncogene addiction, tumor-initiating cells, microenvironment, non-coding sequences, DNA damage response

ABSTRACT

Glioblastoma is the most common form of primary brain cancer and remains one of the most aggressive forms of human cancer. Current standard of care involves maximal surgical resection followed by concurrent therapy with radiation and the DNA alkylating agent, temozolomide. Despite this aggressive regimen, the median survival remains approximately 14 months. Meaningful strategies for therapeutic intervention are desperately needed. Development of such strategies will require an understanding of the framework of therapeutic concepts that have evolved over the past three decades. This article will review the key principles that drive the formulation of therapeutic strategies in glioblastoma. Specifically, the concepts of tumor heterogeneity, oncogene addiction, non-oncogene addiction, tumor initiating cells, tumor micro-environment, non-coding sequences, and DNA damage response will be reviewed.

INTRODUCTION

Glioblastoma is the most common form of primary brain tumor. The incidence of this tumor is fairly low, with 2-3 cases per 100,000 people in Europe and North America [1]. It is one of the most aggressive forms of human cancer [2]. Without treatment, the median survival is approximately 3 months [3]. The current standard of treatment involves maximal surgical resection followed by concurrent radiation therapy and chemotherapy with the DNA alkylating agent, temozolomide [4, 5]. With this regimen, the median survival is approximately 14 months. For nearly all affected, the treatment remains palliative.

The best available evidence suggests that glioblastomas originate from cells that give rise to glial cells [6, 7]. These glial derived tumors are graded by the World Health Organization (WHO) into 4 categories, termed WHO grade 1 to grade 4. The higher grade denotes histologic features of increased malignancy. WHO 4 glioma is essentially synonymous with glioblastoma [8].

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 functions in ways that deregulate cellular signaling pathways leading to the cancer phenotype [9]. The exact number and nature of genetic alterations and deregulated signaling pathways required for tumorigenesis remains an issue of debate [10], although it is now clear that CNS carcinogenesis requires multiple disruptions to the normal cellular circuitry. These genetic alterations result in either activation or inactivation of specific gene functions that contribute to the process of carcinogenesis [10]. Genes, that when activated, contribute to the carcinogenesis are generally termed proto-oncogenes. The mutated

forms of these genes are referred to as oncogenes. Genes, that when inactivated, contribute to the carcinogenesis are termed tumor suppressor genes.

Recent research in the area of experimental and clinical oncology has identified the key signaling pathways, critical regulatory nodes, genes and their protein products, as well as their mutual cross-talks, thereby providing a solid molecular basis for selection of candidate therapeutic targets and drug discovery programs. These lines of investigation complement the recent efforts to sequence entire genomes of a growing number of human tumors including glioblastoma. The efforts have led to the formulation of new concepts and principles in tumor cell biology. Exploitation of these major advances have begun to provide exciting leads to conceptual framework that afford innovative therapeutic strategies. This article will aim to review these critical concepts and their relevance for glioblastoma therapeutic development.

CONCEPT 1: GLIOBLASTOMA SUBTYPES

There is an old adage that cancer is a hundred diseases masquerading in one. While this adage is based on clinical and pathologic observations, systemic genomic characterization of a large number of glioblastoma specimens confirms the notion that subtypes with distinct pathologic molecular events and therapeutic responses exist.

The Cancer Genome Atlas project (TCGA) is a major NIH initiative involving institutions spanning the continental U.S. with the goal of tumor specimen collection and molecular characterization [11]. Glioblastoma was one of the first tumor types characterized in this effort. This vast wealth of data is unprecedented, and despite the enormous challenge to process and analyze this incoming information, correlations of such emerging ‘genetic and expression profiles’ or ‘tumor landscapes’ with tumor

biology and clinico-pathological features of the patients including therapeutic responses are beginning to impact oncology.

These studies [12] have led to the understanding of glioblastoma as an umbrella term that encapsulates subtypes characterized by distinct molecular properties. Based on global transcript profiling, glioblastoma can be divided into three to four distinct subtypes [12, 13]. Interestingly, each subtype harbors distinct genetic aberrations [13] and proteomic profiles [14]. The recognition that glioblastoma consists of subtypes varying in molecular circuitry and biologic behavior suggests that no therapy can be universally efficacious. The major importance of this concept of heterogeneity is that meaningful therapeutic gain can only be attained by customizing the therapy to the underlying molecular circuit. One subtype (termed classical by the TCGA and proliferative by Philips et al.) is characterized by frequent amplification or mutations in the Epidermal Growth Factor Receptor (EGFR) gene [11, 12]. In contrast, in another subtype, termed proneural by both groups, harbors frequent mutations in p53, Platelet Derived Growth Factor Receptor A (PDGFRA), and Isocitrate Dehydrogenase 1 (IDH1) [13]. A third type, termed mesenchymal, is characterized by frequent mutations in the Neurofibromatosis type 1 gene (NF-1).

Importantly, these transcriptomal subtypes appear to differ in their clinical courses and therapeutic responses. In terms of prognosis, studies by Philips [12] and Verhaak [13] both demonstrated increased overall survival in patients afflicted with pro-neural glioblastoma relative to other molecular subtypes. In terms of therapeutic response, Verhaak et al.[13] explored this issue by stratifying the patients with various molecular subtypes into two groups: 1) those that received concurrent chemo-radiation therapy or received >3 cycles of chemotherapy and 2) those that did not receive concurrent chemo-radiation therapy or received <4 cycles of

chemotherapy. When stratified this way, the authors found that the two groups exhibited comparable survival in the pro-neural group. In contrast, for other molecular subtypes, patients in group one exhibited improved survival relative to group 2.[13] Since the analysis combined the survival effect of concurrent chemo-radiation therapy and prolonged chemotherapy, it is difficult to assess whether the effect is due to the former or the latter. Taken as a whole, these data sets suggest that the patients with the pro-neural glioblastomas tend to survive longer but are less responsive to conventional chemotherapy or chemo-radiation therapy.

CONCEPT 2: ONCOGENE ADDICTION

The term "oncogene addiction" was initially coined by Dr. Bernard Weinstein to describe the phenomenon that some tumors exhibit exquisite dependence on a single oncogenic protein (or pathway) for sustaining growth and proliferation [15]. Such dependence has been convincingly demonstrated in both tissue culture and transgenic mice systems for oncogenic versions of MYC [16-18] and RAS [19]. Application of this concept to the clinical setting has achieved variable success in various cancer types, including chronic myelogeneous leukemia (CML) harboring the BCR-ABL translocation, Erb2 over-expressing breast cancer, and Non-Small Cell Lung Cancer (NSCLC) harboring a subset of EGFR mutations [20, 21]. A simplistic application of this concept in glioblastoma would involve identification of the critical "addicted" oncogene followed by the inhibition of such oncogene(s). Unfortunately, the actual biology of glioblastoma is far more complex.

To understand this complexity, a careful analysis of the fundamental notion of oncogenic addiction is needed. In some ways, the observation that tumors exhibit

dependence on a particular oncogenic pathway at some point in its history is not surprising. However, considering the plethora of dynamic genetic changes that accumulated during cancer progression [22], it is somewhat counter-intuitive to suspect that any particular pathway would play a prominent role in maintaining cell viability. Moreover, inactivation of the normal counterpart of the addicted oncogenic protein is often tolerated in normal tissue. These observations suggest that the genetic circuitry of the cancer cell have been extensively re-programmed to result in this "addicted" state [15].

The molecular nature of this re-programming remains poorly understood. Several hypotheses have been put forward. One hypothesis involves the notion of "genetic streamlining", where genetic instability in cancer cells is thought to mutationally or epigenetically inactivate certain signaling pathways that are operational in a normal cell but not required for growth in the cancer cell. In this "streamlined" state, the tumor cell becomes hyper-dependent on the oncogene driven processes [23]. A more generalized form of this explanation involves the notion of synthetic lethality. Two genes are considered synthetically lethal if cells remain viable with inactivation of either gene. Simultaneous inactivation of both genes, on the other hand, results in cell death [24]. It is thought that the cancer cells have accumulated mutations that are synthetically lethal with the absence of critical oncogenes. The main difference between this hypothesis and the "streamline" hypothesis is that the mutation in the former can result in a gain or loss of function, whereas the later specifically proposes a loss of function. A third hypothesis suggests that oncogenes reprogram the tumor cell by both pro-survival and pro-apoptotic signaling [23, 25, 26]. With acute inactivation, the pro-survival signaling decayed faster than the pro-

apoptotic signaling, resulting in tumor death. This thesis has been coined the "oncogene shock" hypothesis [23, 25, 26].

The main reason for revisiting the framework of oncogene addiction is to discuss the mechanism by which the cells can evolve to avoid such addiction. For instance, in the context of synthetic lethality, EGFR inhibition may be cytotoxic to glioblastoma cells only in the appropriate genetic context. Indeed, therapeutic effects of EGFR inhibition were observed only in patients with tumors expressing an oncogenic form of EGFR and an intact PTEN tumor suppressor gene [27]. To complicate the matter, recent studies demonstrate that glioblastomas harbor activation of multiple oncogenic Receptor Tyrosine Kinases (RTKs), such that inactivation of any single oncogene merely diverts signaling through other active oncogenes [28]. In these contexts, it is evident that meaningful therapy will require simultaneous inhibition of multiple oncogenes or identification of the fitting genetic context.

CONCEPT 3: NON-ONCOGENE ADDICTION

Emerging literature suggests an alternative strategy to the multi-target approach. These studies reveal that oncogene activation introduces secondary physiologic changes that stress cellular capacity for survival. Consequently, tumor cells becomes more dependent (or hyper-dependent) on processes required to compensate for these stressful conditions [29, 30]. This phenomenon is termed "non-oncogene addiction" since the compensatory processes required for tumor survival do not directly contribute to the cancer formation. In other words, even the genes that are not themselves targeted by tumorigenic mutations may well become essential for the tumor to survive the stressful environment and fuel the demanding process of tumor

progression. Consequently, interfering with the function of such genes could cause tumor kill while sparing the normal counterpart. [29, 30]

There are several examples of such critical non-oncogenic pro-survival functions required for the maintenance of the tumorigenic state in glioblastoma. EGFR is a critical proto-oncogene in glioblastoma pathogenesis [11, 31]. Our laboratory has demonstrated that EGFR hyperactivation results in an increased accumulation of reactive oxygen species (ROS), which in turn cause cytotoxic DNA damage. To compensate for the deleterious effect of ROS, EGFR hyperactive glioblastomas exhibit increased reliance on DNA repair process required for the repair ROS related DNA damage [32]. Selective targeting of EGFR hyperactive glioblastomas can, thus, be achieved by inhibition of these repair process. Other groups have demonstrated that EGFR hyperactivation in glioblastoma cell lines heightens requirement for lipogenesis [33, 34]. Additional examples of such critical non-oncogenic pro-survival functions required for maintenance of the tumorigenic state include dependency on mechanism for compensating mitotic and proteotoxic stress and interplay with the tumor microenvironment including the immune system [29].

The principle of non-oncogene addiction suggests that there is a wider spectrum of therapeutic options than afforded under the paradigm of "oncogene addiction". In many cases, compensatory processes involved in "non-oncogene addiction" are the same as those that basic scientists have studied for years (for instance, DNA repair). Mechanistic investigations into these biologic processes by the basic scientists have yielded a rich database of inhibitors. Thus, identifying gene functions that compensate for oncogene induced cellular stress should afford opportunities to tap into this rich database and expand the denominator of drugs

available for combinatorial therapy. Identifying genes that are synthetically lethal with oncogenes constitute an attractive means to this end.

It is important to note that effects of therapies designed based on the principles of "oncogene addiction" and of "non-oncogene addiction" are inherently antagonistic. For instance, EGFR inhibition leads to a reduction of ROS, obviating the need for DNA repair [32]. In this context, the combination of DNA repair inhibition and EGFR inhibition would not be desirable. Rational strategies for synthesizing the two therapeutic paradigms remains a major intellectual challenge.

CONCEPT 4: TUMOR INITIATING CELLS

Another advance that may profoundly change our thinking about solid tumors including glioblastoma involves the concept of tumor initiating cells. The experimental observation is that within a total population of glioblastoma cells, there appears to be a small sub-population of cells that are highly tumorigenic (hence the term "tumor initiating cells" or "TICs"), with capacity for self-renewal [35, 36]. In some studies where severely immune-compromised are used as assay for melanoma xenograft formation, the proportion of TICs within a tumor has been reported as high as 27% [37]. To the extent that glioblastoma tumor initiating cells share many common properties when compared to neural stem cells, it is proposed that the TICs originated from stem cells. While there are some data supporting this hypothesis [6], the universality of this hypothesis remain controversial.

Protein markers to prospectively identify and isolate these putative TICs have been reported, such as the transmembrane glycoprotein CD133 (prominin-1) in glioblastomas [6]. However, the value of CD133 as a single marker of glioblastoma TICs remains controversial, partly because also CD133-negative glioblastoma cells

could give rise to tumors in an intracranial mouse xenograft model [38-40]. These uncertainties motivate an ongoing search for additional candidate TIC markers. Candidate cell surface molecules suggested in this context include the adhesion glycoprotein L1CAM [41], surface carbohydrate antigen CD15 (SSEA-1) [42], surface marker A2B5 [43], and integrin α6 [44]. Currently, there are no generally accepted cell surface markers for defining TIC. The definition of TICs remains a functional one as defined by the ability of a tumor cell to sustain self-renewal and initiate glioblastoma formation in immuno-compromised xenograft models.

Arguably, the most important aspect of the concept of TICs is that this population appeared particularly resistant to conventional radiation and chemotherapy [35]. In this context, TICs may be responsible for glioblastoma recurrence after conventional therapy. Given such properties, it is understandable that glioblastoma research has recently focused on identification and development of potential anti-TIC therapies. Two of these strategies, namely targeting the TICs as part of a vascular niche, and attempts to overcome their therapeutic resistance, will be discussed in the following sections on glioblastoma angiogenesis and the role of DNA damage response pathways, respectively. Here, we briefly consider strategies that are emerging as potentially fruitful approaches to treat glioblastoma through targeting TICs.

The first strategy reflects the efforts to identify suitable cell surface markers to reliably identify glioblastoma TICs – with the hope of conjugating the corresponding antibody to cytotoxic compounds as therapeutic agents. The second strategy is based on observations that some TICs, like neural stem cells, can be induced into a differentiated state whereby the self-renewal properties are lost. Among the suggested agents to induce such TIC differentiation, the bone morphogenetic proteins (BMPs)

appear promising [45]. The third strategy involves modulating specific signaling pathways required for maintaining the TIC state. Pathways targeted include those mediated by EGFR, Wnt-beta catenin, STAT3, Sonic Hedgehog-Gli, and Notch pathways [46]. Finally, normal neural stem cells have been shown to migrate toward and track TICs. Based on this principle, neural stem cells have been used as delivery vehicles to increase local concentration of therapeutic agents in the vicinity of TICs [47].

CONCEPT 5: TUMOR MICROENVIRONMENT

Over the past two decades, conceptualization of glioblastomas has evolved from a collection of relatively homogenous cells to the recognition of distinct sub-populations of tumor cells to that of a complex organ, with constant interactions between tumor cells and aberrant stromal elements. Analogous to the distinct functions of different tissues in an organ, genomic characterization using cells derived from distinct regions of the tumor revealed genetic heterogeneity [48]. A major concept in oncology has emerged that reciprocal signaling between the distinct subpopulations of neoplastic cells and the aberrant stromal elements serve to sustain progressive neoplastic transformation and possibly functional specialization [49]. Understanding of these interactions has afforded novel therapeutic targets. For the purpose of this review, distinct subpopulation of neoplastic cells (tumor heterogeneity) and aberrant stromal interactions will both be considered as components of the microenvironment.

Studies of EGFR revealed a beautiful illustration of signaling between subpopulation of genetically distinct neoplastic cells in glioblastoma. EGFRvIII is a variant of EGFR that arose from spontaneous deletion of exons 2-7 [50]. This variant is present in about 20% of glioblastomas [51] and results in constitutive hyper-activation

of EGFR [52]. Clinical studies suggest that glioblastoma patients harboring this variant tend to exhibit worse prognosis [53, 54]. Interestingly, the vIII variant is rarely found in the absence of EGFR over-expression [54, 55]. Further, when found, the variant is typically present in only a subset of the total tumor mass [54, 55]. Investigations into the molecular mechanism underlying these observations revealed that EGFRvIII over-expression increased the secretion of interleukin 6 (IL6) and Leukemia Inhibitory Factor (LIF), two soluble cytokines. These cytokines trigger phosphorylation of gp130 in the non-EGFRvIII expressing cells, which in turn activate EGFR of these cells [56]. This activation increases the tumorigenicity and aggressiveness of the cancer. Such signaling may serve to actively maintain tumor cell heterogeneity.

In addition to the signaling between distinct and genetically defined subpopulations of tumor cells, normal cells without genetic alterations associated with carcinogenesis are often recruited to the foci of tumor cells [49]. In the process, these normal cells undergo phenotypic changes in response to direct physical interaction with cell surface proteins on the tumor cells or through interaction with secreted soluble factors. These changes result in the release of growth factors that further enable and sustain neoplastic transformation [57] or lead to new blood vessel formation [58]. Cycles of such reciprocal interaction facilitate stepwise progression in neoplastic progression [49].

In terms of the non-neoplastic cell types shown to facilitate neoplastic information, they can generally be divided into three categories. The first category involves endothelial cells or endothelial cell precursors. These cells are critical for tumor growth since there are inherent limitations on the distance that oxygen and macromolecules can travel. In xenograft models, solid tumors can only proliferate up to a size of 1-2 mm without the development of a new blood supply [59]. Quiescent

endothelial cells in proximity of the neoplastic foci may be induced to initiate biologic programs that lead to blood vessel formation by secreted factors such as Vascular Endothelial Growth Factor (VEGR, see below)[60]. Alternatively, endothelial cell precursors in the blood stream may be recruited into the tumor foci [58, 61]. A final mechanism involves the trans-differentiation of TICs to become endothelial cells [62].

The second class of non-neoplastic cells that actively participate in tumor progression is fibroblasts. There is good evidence that these otherwise genetically normal fibroblasts, when in proximity of tumor cells, can become "re-programmed" to promote/sustain neoplastic transformation. Transplantation experiments mixing cancer-associated fibroblast with cancer cells lead to a more aggressive tumor phenotype than "normal" tumor cells. This tumor promoting activity is largely thought to be the combined effect of cell-to-cell interaction as well as cytokine release [57]. Non-neoplastic astrocytes perform many of the functions associated with fibroblasts. Thus, the interactions between glioblastoma cells and non-neoplastic astrocytes warrant further investigations.

The final class of non-neoplastic cells recruited are cells that mediate immune function. In general, these cells may possess tumor antagonizing activity or tumor promoting activity [49]. These divergent properties may be rationalized by understanding that the immune system is required for both the destruction of foreign cells as well as facilitating wound healing. Properties associated with the former will likely lead to tumor ablation [63]. On the other hand, cytokines and growth factors associated with wound healing may promote tumor growth [64].

The glioblastoma cells have evolved a large number of mechanisms that allow escape from immune detection and ablation, including release of immunosuppressive

cytokines, such as interleukin (IL)-10, CTLA-4, and transforming growth factor-ß (TGF-ß) [65, 66] or expression of cell surface molecules that facilitate immunosuppression, such as B7-H1 [67]. These events, in turn, lead to the induction of regulatory T cells (Treg), down modulation of antigen-presenting cell, with concomitant loss of T-cell effector function [68]; or loss of functional MHC class I receptors [69].

These factors contribute to a "hostile" microenvironment that compromises the immune cells ability to achieve tumor eradication. For instance, primed $CD8^+$ cytotoxic T cells can penetrate the blood-brain barrier and access the central nervous system [70]. However, they are incapable of tumor eradication. Indeed, in patients with glioblastoma, tumor progression is seen despite the presence of tumor-infiltrating lymphocytes (TIL)[71].

Interestingly, glioblastoma's capacity to suppress immune response appears intimately associated with the process of neoplastic transformation. Phosphatase and TENsin homologue (PTEN) encodes a tumor suppressing phosphatase that is frequently mutated during glioblastoma pathogenesis. The translation of many immune-suppressive cytokines and molecules, including IL10 and B7-H1, are under the regulation of PTEN. Thus, PTEN loss during neoplastic transformation leads to increased expression of immune-suppressive cytokines and cell surface molecules [67]. This expression, in turn, creates a hostile environment for immune cells that otherwise target tumor for ablation.

Understanding the interaction between the genetically distinct subpopulation of glioblastoma cells and their microenvironment has yielded novel therapeutic developments. The endeavor most frequently cited in this regard involves

angiogenesis inhibitors. Realizing that VEGF is critical in angiogenesis, bevacizumab, a humanized antibody against VEGF, was developed [72]. While there has not been a randomized control trial to assess the efficacy of bevacizumab, phase II clinical trial demonstrated improved progression free survival in recurrent glioblastomas (after concurrent temozolomide/radiation treatment) relative to historical data of patients who received temozolomide at recurrence [73-75]. However, no overall survival benefit has been demonstrated with bevacizumab treatment. Clearly, angiogenesis inhibition is but one of the many strategies can be developed based on the concept of tumor microenvironment.

CONCEPT 6: NON-CODING DNA SEQUENCES

Classically, coding sequences are defined as the strand of DNA that has the same base sequence as the RNA transcript produced (with the caveat that thymines are replaced by uracil). While the identification of nucleotide alterations within the coding sequences of proto-oncogene or tumor suppressor genes has significantly contributed to our understanding of carcinogenesis, there is an emerging appreciation that alterations in non-coding sequences similarly contribute to carcinogenesis [76]. A notable example involves the regulation of gene transcription by reversible modification of gene promoter regions – a phenomenon sometimes referred to as "epigenetic regulation" [77]. Similarly, we are beginning to appreciate the importance of transcripts that do not encode for proteins but are transcribed, such as microRNAs [78] and Long non-coding RNAs (or LincRNAs) [79, 80], in terms of both transcriptional and post-transcriptional modifications. The concept that non-coding DNA sequences regulate gene function and impact carcinogenesis has significantly

expanded the repertoire of strategies available for glioblastoma therapeutics. To review this concept, we will discuss illustrative examples of epigenetic regulation, microRNAs, and LincRNAs.

The term "epigenetic regulation" has been coined to describe the phenomenon that heritable changes in gene expression can occur in the absence of changes in the DNA sequences encoding for gene function [77]. The mechanism underlying this regulation involves cytosine methylation [81] or histone modifications that, in turn, modulate the accessibility of gene promoter regions to transcriptional factors [82]. Cytosine methylation typically occurs in the context of CpG di-nucleotide repeats, or CpG islands [81]. Promoters harboring heavily methylated CpG islands are typically transcriptionally silenced. There are two types of promoter methylation that are particularly pertinent to glioblastoma therapy: methylation in the promoter region of the DNA repair gene, Methyl-Guanine Methyl Transferase (MGMT) and the glioma-CpG Island Methylator (G-CIMP) phenotype.

MGMT encodes an enzyme that removes alkyl adducts at the O6-position of guanine [83]. Because alkyl modification at this position is highly toxic and constitute the primary mechanism for the tumoricidal activity of the chemotherapeutic agent, temozolomide (TMZ), MGMT expression level correlates well with TMZ response in glioblastoma patients [84]. The human MGMT gene possesses a CpG island that spans approximately 1,000 bases around the transcriptional start site. Detailed analysis of this region revealed 108 CpG sites [85] that are methylated. Methylation of a subset of these CpGs has been associated with transcriptional silencing of MGMT [86, 87] and is associated with improved clinical outcome in glioblastoma patients receiving TMZ therapy. Interestingly, MGMT promoter methylation is also associated with improved survival in patients who did not receive TMZ therapy [88, 89]. While the mechanism

underlying this observation remains unclear, it seems likely that MGMT may participate in detoxifying the accumulation of endogenous DNA damage that is typically associated with the oncogenic state [32]. As discussed in concept 7, glioblastoma cells accumulate endogenous DNA damage in the absence of DNA damaging agents [32]. These endogenous DNA damages are not unlike those induce by temozolomide or radiation in that they could trigger cell death if unrepaired. Thus, tumors with high levels of MGMT may grow more robustly since MGMT is capable of detoxifying these endogenous DNA damages. If the tumor cells grow more robustly, the patient will survive for a shorter duration. In contrast, the glioblastoma cells with low MGMT may be more susceptible to the deleterious effects of the endogenous DNA damages. These tumors may grow less robustly, resulting in longer patient survival.

The G-CIMP phenotype refers to the observation that a subset of glioblastomas exhibits concerted CpG island methylation at a large number of loci [90]. Since genes required for tumor growth are located at many of these loci, glioblastomas harboring the G-CIMP phenotype tend to be more benign. Correspondingly, patients with G-CIMP glioblastomas experienced significantly improved outcome. Understanding the concept that the patterns of CpG island methylation directly impact outcomes in glioblastoma patients open the door to therapeutic strategies aimed at enhancing promoter methylation at select promoter loci. Importantly, recent studies suggest that promoter methylation at distinct loci may be affected by specific chromatin modulating factors [91].

MicroRNAs (miRNAs) are small non-coding RNAs of 20-22 nucleotides that, through imperfect pairing, bind to the 3' untranslated regions (UTR) of protein-

coding mRNAs. Typically, this binding leads to mRNA degradation or inhibition of protein translation to suppress the expression of the target proteins [78]. Bioinformatic analysis predicts that a single miRNA can potentially regulate hundreds of target onco- or tumor suppressor proteins. Expectedly, miRNAs have been implicated in carcinogenesis and resistance to chemotherapy [78]. As one illustrative example, our laboratory recently demonstrated that the protein MGMT is under the regulation of miR-181d [92]. Cell biologic studies revealed that binding of miR-181d to the 3'UTR of MGMT caused decreased MGMT expression. This inverse relationship was validated in glioblastoma specimens. Importantly, patients with high miR-181d expression (hence low MGMT) are more likely to respond to TMZ chemotherapy.

LincRNAs are transcripts > 5 kb that are evolutionarily conserved across mammalian genomes. These RNAs are transcribed by Polymerase II but do not encode proteins. The LincRNAs serve to suppress transcription by targeting chromatin-modifying complexes to specific genomic loci [79, 80]. While the role of LincRNA in glioblastoma awaits careful scrutiny, LincRNA have been shown to mediate the function of tumor suppressor genes pertinent to glioblastoma pathogenesis. As one example, *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 [93]. 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 [93]. One of the down-stream effectors of p53 is a LincRNA. This LincRNA serves as a key mediator to suppress transcription of other p53 effectors [94]. Such mechanisms may be operational in glioblastomas.

Understanding the concept that non-coding sequences play critical roles in glioblastoma pathogenesis and resistance to chemotherapy offer novel strategies for biomarker development and therapy. For instance, direct introduction of select miRNAs into glioblastoma has been shown to inhibit growth and proliferation [95]. Similarly, incorporation of miR-181d expression level may further augment the predictive value of MGMT promoter methylation. Importantly, the concept predicts certain situations where the effects of an oncogenic mutation can be voided by the effects of non-coding sequences. Integrating the biology of non-coding sequences in the context of mutational profile will be critical in understanding tumor physiology and meaningful therapeutic development.

CONCEPT 7: DNA DAMAGE RESPONSE

From a broader perspective, the status of the molecular machinery that detects, signals and repairs DNA damage, and overall orchestrates the multifaceted cellular response to genotoxic insults (here referred to as the DNA damage response: 'DDR'[96]) critically impacts both tumor development and clinical outcome. While this is arguably relevant for any type of tumor to some extent, the DDR concept is particularly important for glioblastomas for the following reasons. First, the standard-of-care nonsurgical modalities used to treat glioblastomas, namely ionizing radiation and TMZ-based chemotherapy, operate through their genotoxic effects by causing mainly DNA double strand breaks (DSB) and alkylated DNA lesions, respectively. Therefore, each patient's germ-line disposition of the DDR-related genes, along with any somatic alterations within the DDR machinery that have been selectively acquired by the tumor dictate (along with other factors such as the tumor microenvironment

discussed above) the response of individual glioblastoma patients to such therapies. Second, among the hallmarks of glioblastomas is their resistance to radiotherapy and chemotherapy [97]. These phenomena highlight the intimate involvement of the cellular DDR network, particularly DNA damage signaling, cell-cycle checkpoints and DNA repair pathways, in the pathobiology of glioblastomas. Third, the harmful side-effects of the standard therapies, including brain damage and consequently cognitive changes, are also attributable to DNA damage and the cellular and tissue responses to such treatments. Fourth, genetic and/or epigenetic aberrations of a range of DDR factors, including the above mentioned p53 tumor suppressor or DNA repair genes such as MGMT, occur commonly during glioblastoma pathogenesis and/or upon treatment. This aspect of gliomagenesis has been suspected and partly known for years, however, it has only been validated by the recent insights gained through comprehensive analyses by complete tumor genome sequencing within the framework of the TCGA initiative[11]. Finally, the TICs (see Concept 4), appear to be particularly resistant to DNA-damaging therapies. This resistance is, at least in part, due to enhanced DNA damage signaling and checkpoint machinery [35].

Conceptually very relevant for such DDR-related features of gliomas is the recently described strong, constitutive activation of the DDR signaling pathways, observed from the early stages (grade II gliomas) of gliomagenesis up to glioblastomas [98]. This spontaneous DDR activation precedes any genotoxic treatment, and it appears to be even more pronounced in gliomagenesis than in early lesions of major epithelial tumor types, where this phenomenon represents a candidate intrinsic barrier against activated oncogenes and tumor progression [98-102]. A major source of such DDR activation in early lesions including low-grade gliomas appears to be oncogene-induced replication stress, while in later stages

of tumor progression, particularly in glioblastomas, the constitutive DNA damage signaling is fueled by both continued replication stress and also by enhanced oxidative stress [32, 98, 100, 103]. Biologically, such oncogene-evoked DDR activation often leads to cell death or permanent proliferation arrest known as cellular senescence. This activation eliminates nascent tumor cells from the proliferative pool, thereby delaying or preventing tumor progression [99, 102]. Those lesions that do progress in the face of such constitutively activated DDR often do so due to selection of various defects along the DDR signaling or effector pathways, such as mutations in the ATM-Chk2-p53 DDR pathway [99, 102, 104]. Importantly, while such selected DDR aberrations facilitate tumor progression by allowing escape from DDR-induced senescence or apoptosis, the very same defects may create tumor-specific vulnerabilities that can be exploited by therapeutic strategies based on the synthetic lethality (see Concept 2 above) principle [29, 32, 96].

In terms of exploiting the status of the DDR machinery for glioblastoma therapies, two major avenues are under intensive research and validation. First, there are promising attempts to sensitize glioblastoma cells (including the more resistant TICs) to conventional genotoxic therapy, such as ionizing radiation, by concomitantly inhibiting the DNA damage signaling to downstream checkpoint and repair effectors. This strategy relies mainly on small molecule inhibitors of DDR kinases ATM; ATR, Chk1 and Chk2. This strategy appears particularly suitable for tumors with mutant p53. Such cancer cells lack the major p53-dependent G1/S checkpoint, and upon inhibition of the DDR kinases (whose activity underlies the still operational G2/M checkpoint) enter mitosis with an overload of unrepaired DNA damage, both endogenous and therapy-induced,

followed by cell death [29, 105]. An analogous strategy to overload glioblastoma cells with unrepaired DNA damage involves temozolomide treatment with concurrent inhibition of MGMT in those cases where the MGMT gene promoter is not methylated [106].

An emerging alternative treatment strategy that takes advantage of the synthetic lethality and the accumulated knowledge about the DDR mechanisms [29, 107]. This strategy exploits tumor-selective defects in certain DNA repair pathways, such as the DSB repair by homologous recombination (HR). HR is a mechanism to copy a DNA sequence from an intact DNA molecule (mainly from the newly synthesized sister chromatid) in order to bypass or repair replication-associated DNA lesions. The promising strategy exploiting HR defects that are found in some tumors. These HR deficient tumors are particularly dependent on other repair processes to avoid the generation of DSBs. These tumor cells are, thus, particularly sensitive to inhibition of these other repair processes. Such strategy has shown promise in preclinical studies where breast tumor cells defective to HR appear hypersensitive to inhibition of base excision repair by a small molecule inhibitors of poly(ADP-ribose) polymerase (PARP). [96, 108]. Of note, PARP inhibition has shown promise in glioblastoma treatment in cell culture models [109], and several PARP inhibitors are under investigation in clinical glioblastoma trials [110].

SUMMARY

In this review, we have discussed key principles underlying current development of glioblastoma therapeutics. Emphasis was placed on conceptual

framework rather than specific drugs or targets. These frameworks should serve as the basis for translating fundamental biologic tenets into clinically useful therapeutic strategies.

Acknowledgements

This work was supported by the Doris Duke Charitable Foundation Clinical Scientist Development Award, the Sontag Foundation Distinguished Scientist Award, the Burroughs Wellcome Fund Career Awards for Medical Sciences, the Kimmel Scholar award, a Discovery Grant from the American Brain Tumor Association, and an National Cancer Institute K12 award. the Danish National Research Foundation, the Czech Ministry of Health (NT/11065-5/2010), and the European Commission (projects DDResponse, CZ.1.05/2.1.00/01.0030, and Infla-Care)

Conflict of Interest: the authors have no conflicts of interest to declare

Contributorship Statement: CCC and KN authored the sections on tumor heterogeneity (Concept 1), oncogene addiction (Concept 2), non-oncogene addiction (Concept 3), non-coding RNAs (Concept 6). CCC, BJ and BJ Jr. authored the section on Tumor Initiating Cells (Concept 4) and DNA damage response (Concept 7) and. CCC, WF, BC, and BJ Jr. authored the section on tumor microenvironment (Concept 5). The content of the final manuscript was reviewed by all authors.

REFERENCES

1. Jemal A, Siegel R, Xu J, et al. Cancer statistics, 2010. CA Cancer J Clin. 2010 Sep-Oct;60(5):277-300.
2. Wen PY, Kesari S. Malignant gliomas in adults. N Engl J Med. 2008 Jul 31;359(5):492-507.
3. 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. 1978 Sep;49(3):333-43.
4. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial.[see comment]. Lancet Oncology. 2009;10(5):459-66.
5. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005 Mar 10;352(10):987-96.
6. Alcantara Llaguno S, Chen J, Kwon CH, et al. Malignant astrocytomas originate from neural stem/progenitor cells in a somatic tumor suppressor mouse model. Cancer Cell. 2009 Jan 6;15(1):45-56.
7. Ignatova TN, Kukekov VG, Laywell ED, et al. Human cortical glial tumors contain neural stem-like cells expressing astroglial and neuronal markers in vitro. Glia. 2002 Sep;39(3):193-206.
8. Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol. 2007 Aug;114(2):97-109.
9. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000 Jan 7;100(1):57-70.
10. Stratton MR, Campbell PJ, Futreal PA. The cancer genome. Nature. 2009 Apr 9;458(7239):719-24.
11. TCGA. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 2008 Oct 23;455(7216):1061-8.
12. 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. 2006 Mar;9(3):157-73.
13. 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. 2010 Jan 19;17(1):98-110.
14. 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.
15. Weinstein IB. Cancer. Addiction to oncogenes--the Achilles heal of cancer. Science. 2002 Jul 5;297(5578):63-4.

16. Felsher DW, Bishop JM. Reversible tumorigenesis by MYC in hematopoietic lineages. Mol Cell. 1999 Aug;4(2):199-207.
17. Felsher DW, Bishop JM. Transient excess of MYC activity can elicit genomic instability and tumorigenesis. Proc Natl Acad Sci U S A. 1999 Mar 30;96(7):3940-4.
18. Yokoyama K, Imamoto F. Transcriptional control of the endogenous MYC protooncogene by antisense RNA. Proc Natl Acad Sci U S A. 1987 Nov;84(21):7363-7.
19. Chin L, Tam A, Pomerantz J, et al. Essential role for oncogenic Ras in tumour maintenance. Nature. 1999 Jul 29;400(6743):468-72.
20. Druker BJ. Inhibition of the Bcr-Abl tyrosine kinase as a therapeutic strategy for CML. Oncogene. 2002 Dec 9;21(56):8541-6.
21. Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene. 2007 May 14;26(22):3291-310.
22. Greenman C, Stephens P, Smith R, et al. Patterns of somatic mutation in human cancer genomes. Nature. 2007 Mar 8;446(7132):153-8.
23. Sharma SV, Settleman J. Oncogene addiction: setting the stage for molecularly targeted cancer therapy. Genes Dev. 2007 Dec 15;21(24):3214-31.
24. Kaelin WG, Jr. The concept of synthetic lethality in the context of anticancer therapy. Nat Rev Cancer. 2005 Sep;5(9):689-98.
25. Sharma SV, Fischbach MA, Haber DA, et al. "Oncogenic shock": explaining oncogene addiction through differential signal attenuation. Clin Cancer Res. 2006 Jul 15;12(14 Pt 2):4392s-5s.
26. Sharma SV, Settleman J. Oncogenic shock: turning an activated kinase against the tumor cell. Cell Cycle. 2006 Dec;5(24):2878-80.
27. Mellinghoff IK, Wang MY, Vivanco I, et al. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors.[see comment][erratum appears in N Engl J Med. 2006 Feb 23;354(8):884]. New England Journal of Medicine. 2005;353(19):2012-24.
28. Stommel JM, Kimmelman AC, Ying H, et al. Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. Science. 2007;318(5848):287-90.
29. Luo J, Solimini NL, Elledge SJ. Principles of cancer therapy: oncogene and non-oncogene addiction.[erratum appears in Cell. 2009 Aug 21;138(4):807]. Cell. 2009;136(5):823-37.
30. Luo J, Emanuele MJ, Li D, et al. A genome-wide RNAi screen identifies multiple synthetic lethal interactions with the Ras oncogene. Cell. 2009;137(5):835-48.
31. Parsons DW, Jones S, Zhang X, et al. An integrated genomic analysis of human glioblastoma multiforme. Science. 2008 Sep 26;321(5897):1807-12.
32. Nitta M, Kozono D, Kennedy R, et al. Targeting EGFR induced oxidative stress by PARP1 inhibition in glioblastoma therapy. PLoS One. 2010;5(5):e10767.
33. Guo D, Hildebrandt IJ, Prins RM, et al. The AMPK agonist AICAR inhibits the growth of EGFRvIII-expressing glioblastomas by inhibiting lipogenesis. Proc Natl Acad Sci U S A. 2009 Aug 4;106(31):12932-7.

34. Guo D, Prins RM, Dang J, et al. EGFR signaling through an Akt-SREBP-1-dependent, rapamycin-resistant pathway sensitizes glioblastomas to antilipogenic therapy. Sci Signal. 2009;2(101):ra82.
35. Bao S, Wu Q, McLendon RE, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature. 2006 Dec 7;444(7120):756-60.
36. Singh SK, Hawkins C, Clarke ID, et al. Identification of human brain tumour initiating cells. Nature. 2004 Nov 18;432(7015):396-401.
37. Quintana E, Shackleton M, Sabel MS, et al. Efficient tumour formation by single human melanoma cells. Nature. 2008 Dec 4;456(7222):593-8.
38. Chen R, Nishimura MC, Bumbaca SM, et al. A hierarchy of self-renewing tumor-initiating cell types in glioblastoma. Cancer Cell. 2010 Apr 13;17(4):362-75.
39. Sun Y, Kong W, Falk A, et al. CD133 (Prominin) negative human neural stem cells are clonogenic and tripotent. PLoS One. 2009;4(5):e5498.
40. Wang J, Sakariassen PO, Tsinkalovsky O, et al. CD133 negative glioma cells form tumors in nude rats and give rise to CD133 positive cells. Int J Cancer. 2008 Feb 15;122(4):761-8.
41. Bao S, Wu Q, Li Z, et al. Targeting cancer stem cells through L1CAM suppresses glioma growth. Cancer Res. 2008 Aug 1;68(15):6043-8.
42. Son MJ, Woolard K, Nam DH, et al. SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma. Cell Stem Cell. 2009 May 8;4(5):440-52.
43. Ogden AT, Waziri AE, Lochhead RA, et al. Identification of A2B5+CD133-tumor-initiating cells in adult human gliomas. Neurosurgery. 2008 Feb;62(2):505-14; discussion 14-5.
44. Lathia JD, Gallagher J, Heddleston JM, et al. Integrin alpha 6 regulates glioblastoma stem cells. Cell Stem Cell. 2010 May 7;6(5):421-32.
45. Lee J, Son MJ, Woolard K, et al. Epigenetic-mediated dysfunction of the bone morphogenetic protein pathway inhibits differentiation of glioblastoma-initiating cells. Cancer Cell. 2008 Jan;13(1):69-80.
46. Ebben JD, Treisman DM, Zorniak M, et al. The cancer stem cell paradigm: a new understanding of tumor development and treatment. Expert Opin Ther Targets. 2010 Jun;14(6):621-32.
47. Frank RT, Najbauer J, Aboody KS. Concise review: stem cells as an emerging platform for antibody therapy of cancer. Stem Cells. 2010 Nov;28(11):2084-7.
48. Jung V, Romeike BF, Henn W, et al. Evidence of focal genetic microheterogeneity in glioblastoma multiforme by area-specific CGH on microdissected tumor cells. J Neuropathol Exp Neurol. 1999 Sep;58(9):993-9.
49. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011 Mar 4;144(5):646-74.
50. Nishikawa R, Ji XD, Harmon RC, et al. A mutant epidermal growth factor receptor common in human glioma confers enhanced tumorigenicity. Proc Natl Acad Sci U S A. 1994;91(16):7727-31.
51. Halatsch ME, Schmidt U, Behnke-Mursch J, et al. Epidermal growth factor receptor inhibition for the treatment of glioblastoma multiforme and other malignant brain tumours. Cancer Treat Rev. 2006 Apr;32(2):74-89.

52. Huang HS, Nagane M, Klingbeil CK, et al. The enhanced tumorigenic activity of a mutant epidermal growth factor receptor common in human cancers is mediated by threshold levels of constitutive tyrosine phosphorylation and unattenuated signaling. J Biol Chem. 1997 Jan 31;272(5):2927-35.
53. Heimberger AB, Hlatky R, Suki D, et al. Prognostic effect of epidermal growth factor receptor and EGFRvIII in glioblastoma multiforme patients. Clin Cancer Res. 2005 Feb 15;11(4):1462-6.
54. Shinojima N, Tada K, Shiraishi S, et al. Prognostic value of epidermal growth factor receptor in patients with glioblastoma multiforme. Cancer Res. 2003 Oct 15;63(20):6962-70.
55. Biernat W, Huang H, Yokoo H, et al. Predominant expression of mutant EGFR (EGFRvIII) is rare in primary glioblastomas. Brain Pathol. 2004 Apr;14(2):131-6.
56. Inda MM, Bonavia R, Mukasa A, et al. Tumor heterogeneity is an active process maintained by a mutant EGFR-induced cytokine circuit in glioblastoma. Genes Dev. 2010 Aug 15;24(16):1731-45.
57. Bhowmick NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. Nature. 2004 Nov 18;432(7015):332-7.
58. Kasper G, Dankert N, Tuischer J, et al. Mesenchymal stem cells regulate angiogenesis according to their mechanical environment. Stem Cells. 2007 Apr;25(4):903-10.
59. Leber MF, Efferth T. Molecular principles of cancer invasion and metastasis (review). Int J Oncol. 2009 Apr;34(4):881-95.
60. Ribatti D, Nico B, Crivellato E, et al. The history of the angiogenic switch concept. Leukemia. 2007 Jan;21(1):44-52.
61. Kioi M, Vogel H, Schultz G, et al. Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice. J Clin Invest. 2010 Mar 1;120(3):694-705.
62. Soda Y, Marumoto T, Friedmann-Morvinski D, et al. Transdifferentiation of glioblastoma cells into vascular endothelial cells. Proc Natl Acad Sci U S A. 2011 Mar 15;108(11):4274-80.
63. Kurpad SN, Zhao XG, Wikstrand CJ, et al. Tumor antigens in astrocytic gliomas. Glia. 1995 Nov;15(3):244-56.
64. Schafer M, Werner S. Cancer as an overhealing wound: an old hypothesis revisited. Nat Rev Mol Cell Biol. 2008 Aug;9(8):628-38.
65. Maxwell M, Galanopoulos T, Neville-Golden J, et al. Effect of the expression of transforming growth factor-beta 2 in primary human glioblastomas on immunosuppression and loss of immune surveillance. J Neurosurg. 1992 May;76(5):799-804.
66. Sonabend AM, Rolle CE, Lesniak MS. The role of regulatory T cells in malignant glioma. Anticancer Res. 2008 Mar-Apr;28(2B):1143-50.
67. Parsa AT, Waldron JS, Panner A, et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. Nat Med. 2007 Jan;13(1):84-8.
68. Roszman T, Elliott L, Brooks W. Modulation of T-cell function by gliomas. Immunol Today. 1991 Oct;12(10):370-4.
69. Gu Y, Wang C, Roifman CM, et al. Role of MHC class I in immune surveillance of mitochondrial DNA integrity. J Immunol. 2003 Apr 1;170(7):3603-7.

70. Hickey WF, Hsu BL, Kimura H. T-lymphocyte entry into the central nervous system. J Neurosci Res. 1991 Feb;28(2):254-60.
71. Gajewski TF, Meng Y, Blank C, et al. Immune resistance orchestrated by the tumor microenvironment. Immunol Rev. 2006 Oct;213:131-45.
72. Chi AS, Sorensen AG, Jain RK, et al. Angiogenesis as a therapeutic target in malignant gliomas. Oncologist. 2009 Jun;14(6):621-36.
73. Friedman HS, Prados MD, Wen PY, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol. 2009 Oct 1;27(28):4733-40.
74. Kreisl TN, Kim L, Moore K, et al. Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. J Clin Oncol. 2009 Feb 10;27(5):740-5.
75. Vredenburgh JJ, Desjardins A, Herndon JE, 2nd, et al. Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. J Clin Oncol. 2007 Oct 20;25(30):4722-9.
76. Mattick JS, Makunin IV. Non-coding RNA. Hum Mol Genet. 2006 Apr 15;15 Spec No 1:R17-29.
77. Nagarajan RP, Costello JF. Epigenetic mechanisms in glioblastoma multiforme. Semin Cancer Biol. 2009 Jun;19(3):188-97.
78. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer. 2006 Nov;6(11):857-66.
79. Guttman M, Amit I, Garber M, et al. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature. 2009 Mar 12;458(7235):223-7.
80. Khalil AM, Guttman M, Huarte M, et al. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. Proc Natl Acad Sci U S A. 2009 Jul 14;106(28):11667-72.
81. Clark SJ, Harrison J, Frommer M. CpNpG methylation in mammalian cells. Nat Genet. 1995 May;10(1):20-7.
82. Turner BM. Reading signals on the nucleosome with a new nomenclature for modified histones. Nat Struct Mol Biol. 2005 Feb;12(2):110-2.
83. Tano K, Shiota S, Collier J, et al. Isolation and structural characterization of a cDNA clone encoding the human DNA repair protein for O6-alkylguanine. Proc Natl Acad Sci U S A. 1990 Jan;87(2):686-90.
84. Hegi ME, Liu L, Herman JG, et al. Correlation of O6-methylguanine methyltransferase (MGMT) promoter methylation with clinical outcomes in glioblastoma and clinical strategies to modulate MGMT activity. J Clin Oncol. 2008 Sep 1;26(25):4189-99.
85. Mikeska T, Bock C, El-Maarri O, et al. Optimization of quantitative MGMT promoter methylation analysis using pyrosequencing and combined bisulfite restriction analysis. J Mol Diagn. 2007;9(3):368-81.
86. Herfarth KK, Brent TP, Danam RP, et al. A specific CpG methylation pattern of the MGMT promoter region associated with reduced MGMT expression in primary colorectal cancers. Mol Carcinog. 1999;24(2):90-8.
87. Watts GS, Pieper RO, Costello JF, et al. Methylation of discrete regions of the O6-methylguanine DNA methyltransferase (MGMT) CpG island is associated with heterochromatinization of the MGMT transcription start site and silencing of the gene. Mol Cell Biol. 1997;17(9):5612-9.

88. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med. 2005;352(10):997-1003.
89. Rivera AL, Pelloski CE, Gilbert MR, et al. MGMT promoter methylation is predictive of response to radiotherapy and prognostic in the absence of adjuvant alkylating chemotherapy for glioblastoma. Neuro Oncol. 2009 Feb;12(2):116-21.
90. Noushmehr H, Weisenberger DJ, Diefes K, et al. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell. 2010 May 18;17(5):510-22.
91. Orkin SH, Hochedlinger K. Chromatin connections to pluripotency and cellular reprogramming. Cell. 2011 Jun 10;145(6):835-50.
92. Zhang W, Zhang J, Hoadley K, et al., editors. A prognostic and predictive microRNA (miRNA) that down-regulate MGMT in glioblastoma. Congress of Neurological Surgeons; 2011; Washington D.C.: Congress of Neurological Surgeons.
93. Harris CC. p53 tumor suppressor gene: from the basic research laboratory to the clinic--an abridged historical perspective. Carcinogenesis. 1996 Jun;17(6):1187-98.
94. Huarte M, Guttman M, Feldser D, et al. A large intergenic noncoding RNA induced by p53 mediates global gene repression in the p53 response. Cell. 2010 Aug 6;142(3):409-19.
95. Tong AW, Nemunaitis J. Modulation of miRNA activity in human cancer: a new paradigm for cancer gene therapy? Cancer Gene Ther. 2008 Jun;15(6):341-55.
96. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature. 2009 Oct 22;461(7267):1071-8.
97. Furnari FB, Fenton T, Bachoo RM, et al. Malignant astrocytic glioma: genetics, biology, and paths to treatment. Genes Dev. 2007 Nov 1;21(21):2683-710.
98. Bartkova J, Hamerlik P, Stockhausen MT, et al. Replication stress and oxidative damage contribute to aberrant constitutive activation of DNA damage signalling in human gliomas. Oncogene. 2010 Sep 9;29(36):5095-102.
99. Bartek J, Bartkova J, Lukas J. DNA damage signalling guards against activated oncogenes and tumour progression. Oncogene. 2007 Dec 10;26(56):7773-9.
100. Bartkova J, Horejsi Z, Koed K, et al. DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. Nature. 2005 Apr 14;434(7035):864-70.
101. Gorgoulis VG, Vassiliou LV, Karakaidos P, et al. Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature. 2005 Apr 14;434(7035):907-13.
102. Halazonetis TD, Gorgoulis VG, Bartek J. An oncogene-induced DNA damage model for cancer development. Science. 2008 Mar 7;319(5868):1352-5.
103. Di Micco R, Fumagalli M, Cicalese A, et al. Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. Nature. 2006 Nov 30;444(7119):638-42.
104. Squatrito M, Brennan CW, Helmy K, et al. Loss of ATM/Chk2/p53 pathway components accelerates tumor development and contributes to radiation resistance in gliomas. Cancer Cell. Dec 14;18(6):619-29.

105. Zhou BB, Bartek J. Targeting the checkpoint kinases: chemosensitization versus chemoprotection. Nat Rev Cancer. 2004 Mar;4(3):216-25.
106. Kaina B, Margison GP, Christmann M. Targeting O-methylguanine-DNA methyltransferase with specific inhibitors as a strategy in cancer therapy. Cell Mol Life Sci. Nov;67(21):3663-81.
107. Martin SA, Lord CJ, Ashworth A. DNA repair deficiency as a therapeutic target in cancer. Curr Opin Genet Dev. 2008 Feb;18(1):80-6.
108. Fong PC, Boss DS, Yap TA, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med. 2009 Jul 9;361(2):123-34.
109. Dungey FA, Caldecott KW, Chalmers AJ. Enhanced radiosensitization of human glioma cells by combining inhibition of poly(ADP-ribose) polymerase with inhibition of heat shock protein 90. Mol Cancer Ther. 2009 Aug;8(8):2243-54.
110. Chalmers AJ, Lakshman M, Chan N, et al. Poly(ADP-ribose) polymerase inhibition as a model for synthetic lethality in developing radiation oncology targets. Semin Radiat Oncol. 2010 Oct;20(4):274-81.