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Genomic instability of surgical sample and cancer-initiating cell lines from human glioblastoma

Arantxa Perez-Garcia[1], Josefa Carrion-Navarro[1,2], Minerva Bosch-Fortea[1], Elisa Lazaro-Ibanez[1], Ricardo Prat-Acin[3], Angel Ayuso-Sacido[1,2]

[1]Regenerative Medicine Program, Principe Felipe Research Center, AVDA Autopista del Saler 16, 46012, Valencia, Spain, [2]CIBERNED, AVDA Autopista del Saler 16, 46012, Valencia, Spain, [3]Neurosurgery Department, Hospital Universitario La Fe, AVDA de Campanar 21, 46009 Valencia, Spain

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1. ABSTRACT

Glioblastoma multiforme (GBM) is the most aggressive brain tumor in the adult human, with an average survival of 16 months. A small population of cells within the GBM termed cancer-initiating cells is responsible for the initiation and maintenance of the tumor mass. The traditional glioblastoma cancer cells, grown with serum containing media, display increased rate of genomic instability events, which in turn renders the cell cultures with little resembling to the original tumor, making doubtful their use as preclinical models for screening therapeutic agents. On the contrary, the cancer-initiating cells grown in serum-free media seems to show lower rate of genomic instability processes. However, considering the diversity of genetic and/or epigenetic background, we will need to evaluate the possibility of using different culture conditions to allow for the isolation and culture of such cancer-initiating cells diversity, keeping, at the same time, the genomic instability rate as the original tumor. We summarized the main genetic and epigenetic mechanisms that are driving genomic instability in cancer-initiating cells from human glioblastoma.

2. INTRODUCTION

Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor and is characterized by uncontrolled cell proliferation, diffused infiltration, propensity for necrosis, robust angiogenesis, intense resistance to apoptosis, and rampant genomic instability (1). Despite advances in diagnosis and multimodal therapies, including surgical resection, radiation, and chemotherapy, the life expectancy of GBM patients is less than 16 months (1,2).

Current therapies are directed to avoid tumor progression. The first-line treatment for GBM includes radiation with alkylating chemotherapy (temozolomide) given concurrently and then continued after radiation (3). However, differential response to this traditional therapy is commonly observed, probably due to the existence of different subclasses of GBM, likely representing different diseases. Interestingly, a plethora of genetic and epigenetic alterations as well as variability in mRNA expression patterns have been reported in gliomas, which opens the door to new therapeutic approaches including the discovery

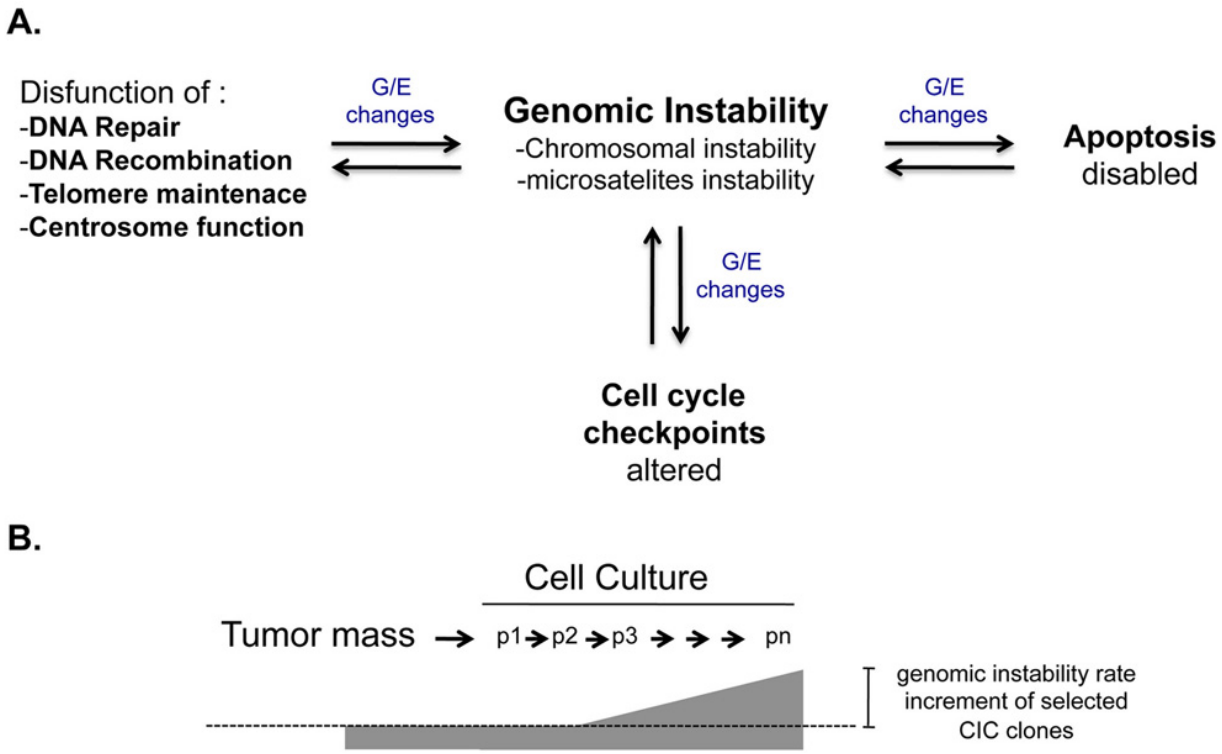

Figure 1. Schematic representation of major genetic and epigenetic mechanisms that influence the genomic instability rate. A, Different mechanisms participate in generating genomic instability proceses which, in tuns, altere the function of these mechanisms. B, The culture condition might also increase the genomic instability rate of glioma derived cells above their specific level in the original tumor acting through previous mechanisms. Key Words: G, genetic; E, epigenetic; p, passage

of new molecules targeting specific oncogenic signaling pathways (for an extensive review see Sathornsumette and Rich, 2008) (4-8). To evaluate the potential of these new generation drugs for the treatment of GBM, they have to be tested on a suitable target cell that better mirrors the original glioma subtype. In this sense, traditionally established GBM cell lines have been used to characterize the biology of primary tumors and to evaluate a number of drugs in preclinical trials. However, in the last decade, some authors have identified a small subpopulation regarded as cancer-initiating cells (CICs) responsible for the initiation and maintenance of GBMs and more closely related to the original tumor when cultured *in vitro* (4, 5). Therefore, this subpopulation of cells has become the most promising target for the new generation of drugs directed at specific intermediates of oncogenic pathways.

One of the fundamental characteristics of cancer cells is that they are subjected to a higher rate of genomic instability events due to deficiencies in pathways including DNA repair and recombination, cell cycle checkpoints, and apoptosis (Figure 1). Thus, differential genetic and/or epigenetic alterations background in CICs affecting these processes gives rise to a diversity of CICs with different rates of genomic instability either intra- or mostly inter-tumor. Once these cells are isolated from the tumor mass, the isolation processes themselves, and more importantly, the culture conditions also affect the rate of genomic instability. The traditional GBM cancer cells grown with serum containing media display increased rate of genomic instability events, which in turn renders cell cultures with little resemblance to the original tumor, making doubtful their use as preclinical models for screening therapeutic agents. On the contrary, the CIC grown in serum-free media seems to show lower rate of genomic instability processes. However, considering the diversity of genetic and/or epigenetic background, we need to evaluate the possibility of using different culture conditions to allow for the isolation and culture of such CIC diversity, keeping, at the same time, the genomic instability rate as the original tumor.

In this review, we have summarized the main genetic and epigenetic mechanisms that drive genomic instability in both established cell lines and primary CIC cultures in the context of the new classification based on integrative criteria including mRNA expression profiles and genetic and epigenetic alterations. Additionally, we will discuss how culture conditions can disturb these intrinsic mechanisms by trying to establish the optimal conditions to maintain the genetic characteristics of the isolated cells as similar as possible to original GBM tumors.

3. CLASSIFICATION OF TUMOR MASSES AND CANCER-INITIATING CELLS FROM GLIOBLASTOMA

The genetic mechanisms described in the gliomagenesis process are different, depending on the GBM subtype. GBMs are traditionally classified into

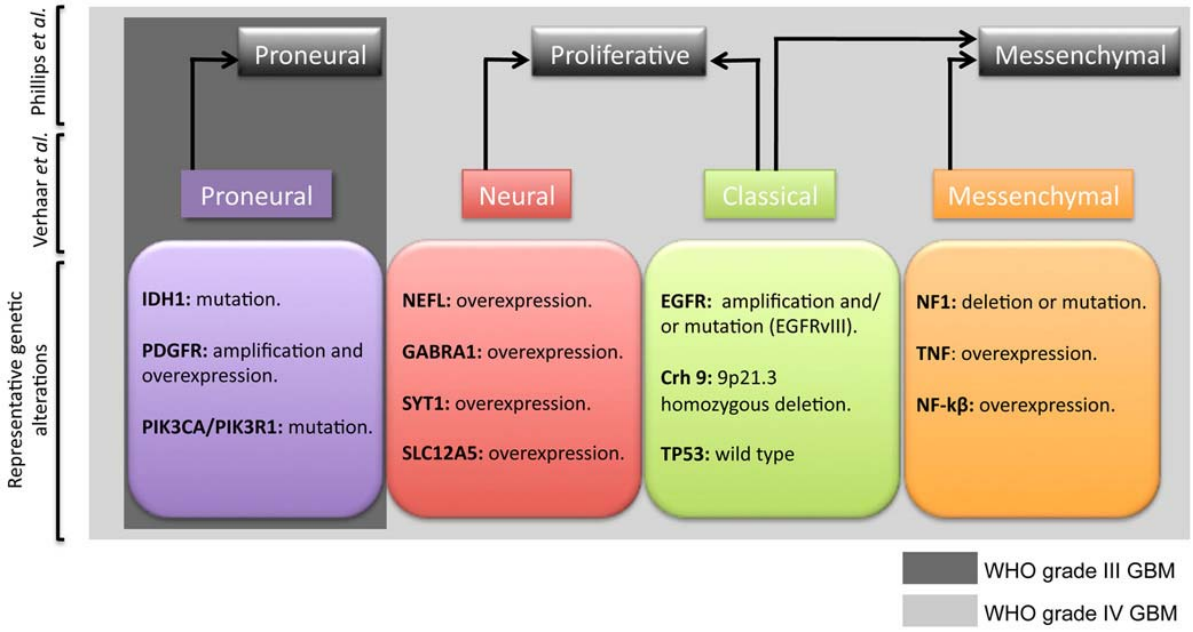

Figure 2. Integrative representation of GBM classification including the main alterations present in the different GBM subtype.

primary and secondary. Despite their histopathological similitude, they constitute distinct disease entities that affect patients of different age, develop through different genetic pathways (8, 9) (9, 10), show different RNA and protein expression profiles (11-13), and may differ in their response to radio- and chemo-therapy. The EGFR/PTEN/Akt/mTOR pathway is a key signaling route in the development of primary GBM. However, in secondary GBM, the TP53 pathway plays a crucial role in the development of tumors (9). In spite of this, both types of tumors also share alterations in pathways such as p16INK4a/RB1 and loss of heterozygosity in chromosome 10q. In addition to this traditional classification, Phillips *et al.*, identified molecular signatures associated with tumor aggressiveness as well as with disease progression. Moreover, they related these signatures to different signaling pathways implicated in gliomagenesis (14). The authors defined 3 subclasses of gliomas related to the process of neurogenesis: proneural class, which express markers found in neuroblasts and immature neurons; mesenchymal class, which recapitulate aspects of neural stem cells; and proliferative class, which are similar to transient-amplifying cells. However, these established classes seem to be dynamic entities with the ability to change unidirectionally from one class to another, from proneural to mesenchymal, suggesting that the tumor subtypes may represent alternate differentiation states of the disease. A recent study has shown the reproducibility of this classification by using an integrative approach and higher number of tumor samples (15). In the new work authored by Verhaak and co-workers, proneural subtype is characterized by alterations of PDGFRA, point mutations in IDH1, as well as high expression of oligodendrocytic development genes, such as Sox genes, DCX, DLL ASCL1, and TCF4. The mesenchymal subtype is defined by focal deletions of a region at 17q11.2 that contains the gene NF1; mutations of NF1 and PTEN as well as high expression of NF-KB pathway are also found in this subclass of GBM. Unlike previous studies, they also found 2 new groups regarded as classical and neural subtypes. The classical subtype is defined by the most common genomic aberrations seen in GBM, such as chromosome 7 amplifications, chromosome 10 deletions, EGFR amplification, and homozygous deletion spanning the INK4a/ARF locus. Finally, the neural subtype is typified by the expression of neuron markers, such as NEFL, GABRA1, SYT1, and SLC12A5 (Figure 2). This new classification, although may not have an immediate application in clinical practice, will be absolutely useful for the recruitment of patients in either preclinical or clinical trials. Nonetheless, there are still at least 2 main issues to address. One of them is the enormous diversity of genetic and/or epigenetic alterations within a specific subclass that may represent different subtypes. The second one is related to the piece of tumor mass randomly taken for the analysis that might not be representative of the whole tumor; in fact, different tumor grades are frequently identified in distinct locations within the same tumor.

Despite the high number of genetic alterations described in GBM, a reduced number of genetic pathways have been found to play a significant role in the biology of CICs (4, 5). Recent studies have shown a tentative classification of CIC lines of GBM by following a similar approach used for GBM tumor samples. Phillips *et al.*, analyzed 16 cell lines; all of them negatively correlated with proneural subclass but showed a wide range of similarities to the mesenchymal and proliferative subclasses (14). Similar studies were performed by Günther *et al.* (2008) (16). In this work, the authors studied 9 CIC lines established under neural stem cell conditions. Four out of 9 cell lines showed an expression pattern associated with

neural development and displayed full stem-like phenotype, corresponding to the proneural subclass. The remaining 5 cell lines presented an expression signature enriched for extracellular matrix-related genes and a restricted stem-like phenotype belonging to the mesenchymal subclass. A recent study also has established 2 types of CICs associated with proneural and mesenchymal phenotype (17). These 2 clearly distinguishable CICs subtypes displayed characteristics of either fetal or adult neural stem cells, suggesting that different cell of origin gives rise to different types of CICs that may account for the heterogeneity of GBMs (17). In the same line of thinking, Klink *et al.*, analyzed the different histological parts of GBMs with oligodendroglial components using CGH and interphase-FISH (18). Although they never isolated CICs from different locations, they observed little genetic variations between cells coming from either oligodendroglial or glioblastoma components. On the basis of these results, the authors suggested a clonal origin for the 2 different histological parts analyzed. This clone acquired different genetic or epigenetic alterations along the course of the disease, giving rise to histologically different tissues within the tumor. Taken together, we could speculate that differential expression profile between CICs may be more related to the cell of origin. Cells derived from this original clone of CICs may acquire distinct alteration with time, affecting specific genes like EGFR or PTEN and giving rise to a family of CICs within the same tumor.

4. GENETIC MECHANISMS LEADING TO GENETIC INSTABILITY IN GLIOBLASTOMA

The cell of origin of the CIC subpopulation and its genetic background will directly affect the CICs' genomic instability rate. Along these lines, a recent study has shown the involvement of DNA damage repair genes in the transition from neural stem cells to immortalized clones of CICs that target important genes related to cancer development such as TP53 (19). This study provides evidence about the emergence of an immortalized cell line from a neural stem cell in culture. The cells bypassed replicative senescence and overcame crisis by expressing telomerase (19). After that, the cells showed an important activation of kinases that participate in the amplification of DNA damage response and checking the entry of cells into mitosis (19). This constitutive kinase activation as well as the loss of TP53, frequently found in tumor cells, led to an increased DNA repair capacity and continuous cell cycling. Besides, other alterations traditionally found in the tumor mass are also frequently found in CICs. NOTCH signaling pathway has been pointed out to play an important role in the pathogenesis of GBM (20, 21). Recent studies have found that NOTCH pathway blockade inhibits GBM neurosphere engraftment *in vivo*, reduces the percentage of cells expressing the stem/progenitor cell markers CD133 and Nestin, and induces the expression of apoptotic markers (22). Hedgehog signaling pathway has been also proposed to be involved in maintaining the GBM growth. Although initially the role of Hedgehog signaling was unclear, recent studies have shown that Hedgehog pathway blockade inhibits glioma neurosphere formation and growth (23, 24). Furthermore, TGF-β signaling pathway also has an important role in cancer (23) (25). In this sense, elevated TGF-β activity in gliomas confers poor prognosis in patients by inducing elevated angiogenesis, cell invasion, and proliferation capacity in these tumors (26). These properties appear as a result of increased self-renewal capacity of CICs in glioma through the induction of LIF and Sox2 (27, 28). In this sense, a new study (29) has shown that in a subpopulation of CICs, the expression of high levels of CD44 and Id1 correlates with poor prognosis in GBM patients. This population is regulated by the TGF-β through Id1 and Id3 that control the expression of LIF and Sox2 and Sox4, respectively. All these elements, regulated by TGF-β, are required for the maintenance of the CD44high/Id1high CICs subpopulation. All these mechanisms, together with previously described, are mainly involved in cell cycle transition and cell growth, leading to increased cell proliferation, which causes an increased genomic instability (30, 31).

Besides genetic alterations described before, interactions between the altered signaling pathways are also frequently found in GBM. In this sense, effects derived from NOTCH pathway blockade were associated with phosphorylation changes in Akt and STAT3, suggesting additional pathways that may have been synergistically targeted. Moreover, mutually exclusive events are also possible. This is the case of mutual exclusivity between NFKBIA deletion and EGFR amplification (28) (32). This type of combination was recently described by Li *et al.* (33). Here, the authors showed that the presence of constitutively activated EGFRvIII as well as PTEN loss causes an increased proliferation, enhanced migration, and invasiveness as well as DNA damage. All these mechanisms, together with those previously described, are mainly involved in cell cycle transition and cell growth, leading to increased cell proliferation that causes an increased genomic instability. Furthermore, genomic instability may also evolve from abnormal chromosome segregation at cell division (34) In this sense, a well-described mechanism that generates mitotic instability by failed chromatid segregation at anaphase due to the formation of a chromatin bridge is the telomerase-dependent anaphase bridging (35). The formation of anaphase bridges is associated with the shortening of telomeric repeat sequences (36) and end-joining of chromosome ends, leading to the formation of dicentric chromosomes (37).

5. EPIGENETIC MECHANISMS COLLABORATE TO INCREASE GENOMIC INSTABILITY IN GLIOBLASTOMA

Epigenetics, defined as mitotically heritable changes in gene expression that are not due to changes in the primary DNA sequence, is increasingly recognized as a source of phenotypic variability. The main epigenetic mechanisms described in GBM include covalent modifications of DNA and associated proteins as well as the presence of micro RNAs (mi-RNAs). Most DNA covalent modifications are due to methylation mechanisms. It results from the addition of a methyl group to cytosine. This modification is regulated by DNA methyltransferases

(DNMT), which may create (DNMT3A; DNMT3B) or maintain (DNMT1) methylation patterns (38, 39). DNA methylation is required for many processes such as controlling the differential expression of the paternal and maternal alleles of imprinted genes (36) (40) and maintaining genome stability (41). Modifications of associated DNA proteins mainly include post-translational modification of N-terminal tails of histone proteins by acetylation, methylation, phosphorylation, ubiquitylation, sumoylation, ADP ribosylation, and biotinylation among other modifications (42). These changes are catalyzed by several families of enzymes. Moreover, a single histone molecule may present several modifications, thereby increasing combinatorial complexity. The spectrum of molecular abnormalities with the potential of driving glioma progression is enriched by the presence of some non-coding RNAs (miRNAs) that can modulate epigenetically the expression of some genes. miRNAs are 20–25 nucleotides long non-coding RNAs derived from larger primary transcripts (pri-miRNAs). These miRNAs can modulate gene expression by downregulating the expression of their targets through interactions with complementary sequences in their 3′-UTRs (43).

All these mechanisms are acting to modulate gene expression in GBM. Global hypomethylation affects both single-copy loci and repetitive sequences, and some experimental evidences emphasize that global DNA hypomethylation is sufficient to initiate tumorigenesis and to modulate tumor incidence in cancer models driven by an increasing genomic instability (44). In these models they observed a high rate of chromosome gain as well as duplicated or deleted chromosome regions were observed (45). In a different study, the Fannelli and coworkers suggest that hypomethylation of repetitive sequences in GBM acts as predisposition factor to chromosomal breakage and copy number alteration, increasing genomic instability (46). On the other hand, locus-specific hypermethylation, mostly at CpG island promoters, is also frequent in GBM, causing gene silencing. This hypermethylation occurs in genes with diverse functions related to tumorigenesis and tumor progression, including cell cycle regulation, DNA repair, apoptosis, angiogenesis, invasion, and drug resistance (47-52). A relevant example of epigenetic silencing by promoter hypermethylation in GBM affects the O6-Methylguanine-DNA methyltransferase (MGMT) gene. MGMT encodes a DNA repair protein ubiquitously expressed in normal human tissues (53), which may protect normal cells from carcinogens, but also from chemotherapeutic alkylating agents in cancer cells. For this reason, MGMT hypermethylation is associated with longer survival in GBMs treated with radiation and alkylating agents (54, 55). This hypermethylation is also correlated in GBMs with mismatch repair deficiencies, which exert a powerful influence on the overall frequency and pattern of somatic point mutations (56). In addition to MGMT, an important number of genes frequently affected by CpG island promoter hypermethylation have been described in GBM, including the retinoblastoma (RB), PI3K, and p53. These genes play an important role in the progression of glioma over the time as well as the recurrence (57, 58).

Aberrant patterns of histone modifications are frequently detected in GBM. These alterations are usually due to deregulation of genes controlling histone modification. In this sense, BMI-1, a member of the polycomb group complex that regulates histone H3K27 methylation, is subjected to frequent copy number alterations (59). Histone deacetylase (HDAC) proteins are also altered in GBM (60). In this case, a negative correlation between HDAC gene expression and the glioma grade have been established. Histone H3 is more acetylated in GBM than normal brain tissue. Furthermore, mutations in many other genes involved in epigenetic modifications have been found, including histone deacetylases, histone demethylases, and histone methyltransferases, suggesting a broad group of defective epigenetic mechanisms acting in GBM (Figure 3).

miRNAs are also potential epigenetic regulators in GBM, where multiple miRNAs are aberrantly expressed or repressed (61). Each miRNA may have hundreds of targets, and many genes are targeted by multiple miRNAs, thus leading to highly complex regulatory networks. However, many miRNAs show a specific expression pattern in GBM (62-65). The miR-21 shows the highest expression levels in GBM, and several miRNAs are weakly expressed compared with normal brain, including miR-124, miR-7, and miR-128 (Review in depth in Zhang *et al.*) (66) (Figure 3).

Although previous data are related to GBM tumor samples, epigenetic mechanisms in the regulation of gene expression may also play an important role in the oncogenic phenotype of CICs from human gliomas. Actually, recent studies describe how some epigenetic mechanisms affect CIC grown and differentiation (67, 68). The delta and notch-like epidermal growth factor-related receptor (DNER) signaling pathway, which is modulated by histone deacetylase proteins, modulates GBM stem-like cell growth, differentiation, and possibly tumor propagation and maintenance. The bone morphogenetic protein receptor (BMPR) pathway, which is epigenetically regulated, is also implicated in glioblastoma-initiating cell differentiation (68). In this study, the authors found that a particular CIC line isolated from a human tumor showed BMPR silencing by hypermethylation of its promoter. This silenced BMPR leads to undifferentiated cells with a high tumorigenic potential. However, this phenotype seems to be restricted to specific genetic backgrounds.

Finally, the presence of altered epigenetic mechanisms is frequently associated with the presence or absence of some genetic mutations. The transforming growth factor (TGF)-beta pathway is regulated by promoter hypermethylation in GBM. High levels of TGF-beta signaling are frequently associated with poor prognosis in GBM, promoting proliferation through the induction of platelet-derived growth factor (PDGF)-B. However, PDGF-B promoter hypermethylation prevents PDGF-B transcriptional activation by TGF-beta-induced Smad proteins (26). In this sense, a recent study has shown a correlation between epigenetic mitogen-activated protein kinase phosphatase (MKP)-2 downregulation and the

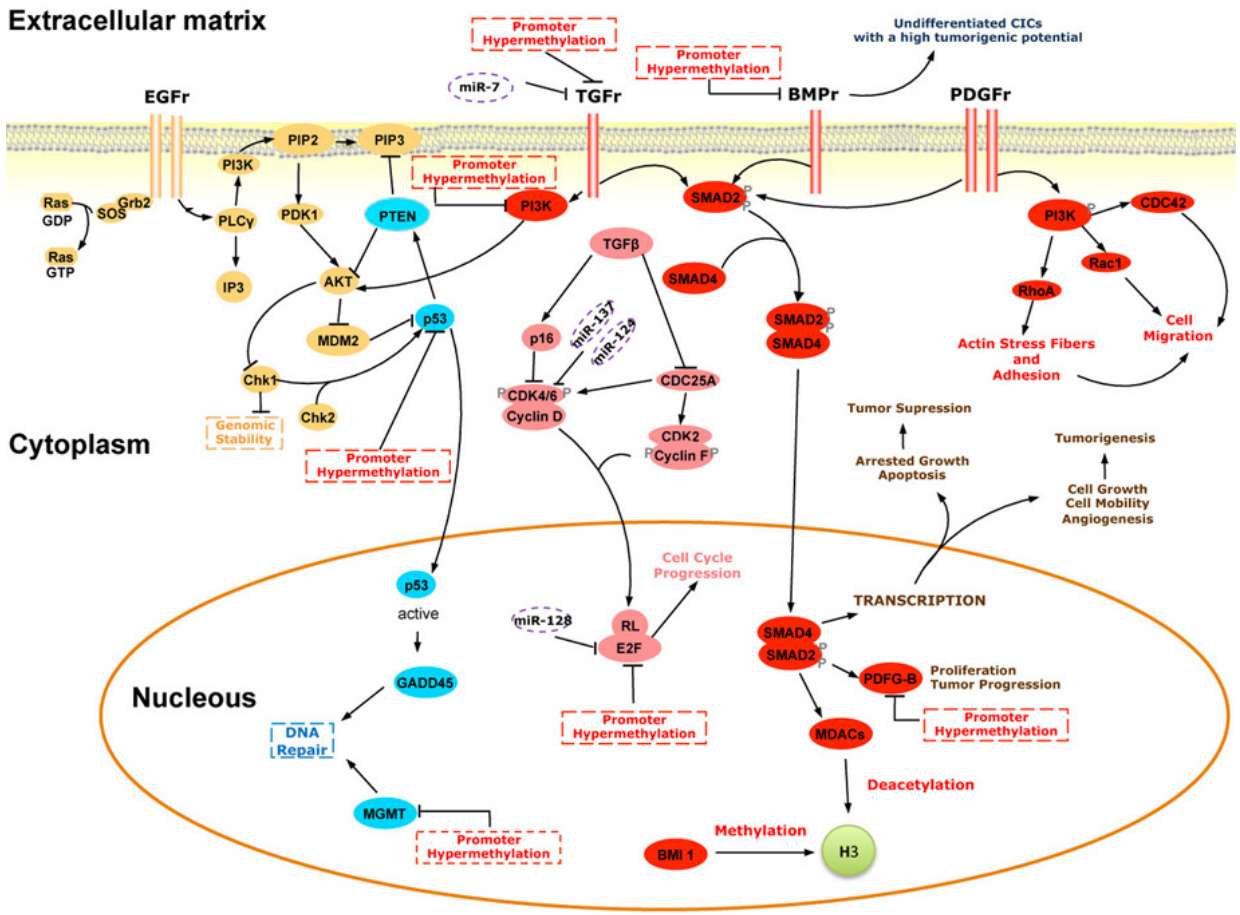

Figure 3. Schematic representation of the main interactions between genetic and epigenetic abnormalities in GBM. Solid colors represent the main molecular pathways where co-interaction between genetic and epigenetic abnormalities have been described in GBM. Red clear boxes, promotor hypermetilation of specific genes related to cell cycle regulation, DNA repair, apoptosis, angiogenesis, invasion, and drug resistance. Purpure clear boxes, microRNA modulating the expression of specific genes.

presence of TP53 mutations and EGFR amplifications (69). Similar interactions have been found between miRNAs and genetic aberrations. This is the case of miR-26, which is amplified in a subset of high-grade gliomas (70). miR-26 amplification has a relatively frequent occurrence in human GBM and is correlated with monoallelic PTEN deletion. These correlations suggest a temporal sequence in the molecular evolution of miR-26 amplified gliomas, with PTEN loss most likely preceding miR-26 copy number gain. The subsequent repression of the remaining PTEN alleles by miR-26 would then, presumably, eliminate the driving force for formal loss of heterozygosity. This last example shows how a particular change can lead to combined alterations that can increase genomic instability (Figure 3).

6. INFLUENCES OF CULTURE CONDITIONS ON GENOMIC INSTABILITY

Traditionally, the enrichment of CICs from brain tumors was performed by neurosphere culture (4, 6, 71-73). In this case, serum-free media and uncoated plates were used as culture conditions. A number of studies have pointed out that CIC population within primary GBM is quickly lost in typical glioma adherent serum culture conditions. Then, cells found following the prolonged *in vitro* passages have de novo genetic and/or epigenetic changes as a result of an outgrowth of low number of cell clones (73, 74). In this sense, de Witt Hamer *et al.*, showed that the genomic profiles are preserved in spheroids cultures in comparison with adherent cultures (75). According to the authors, some reasons that could explain this fact are that during primary cell culture, specific subpopulations may be selected owing to the existence of considerable genetic heterogeneity. In addition to that, the divergent clonal evolution may have been accelerated during primary cell culture because cell proliferation is more pronounced in monolayer cell cultures. Moreover, CICs that presumably drive the cancer cell population are possibly lost early in primary cell culture but maintained in spheroids (75). However, recent studies have pointed out that adherent culture provides uniform access to growth factors, which suppresses differentiation and enables expansion of highly pure populations of stem cells (76). In this study, the authors show that although higher rates of proliferation are observed in comparison with neurosphere

culture, no gross chromosome instability is detected at low passages. Alterations in the whole chromosome copy number do occur following long-term *in vitro* expansion. Therefore, suspension culture is not a requirement for successful long-term propagation of tumor-derived stem cells (73, 77, 78). Nevertheless, the apparently different results obtained from these 2 studies may be because of the low number of CIC lines analyzed. Due to the enormous heterogeneity of GBM, some cells with a higher genomic instability ratio could show detectable changes in early passages, regardless of whether they are cultured in suspension or monolayer. If that is the case, it will be necessary to complement the study with higher number of samples and heterogeneity. In any case, more than growing as adherent or suspension cultures, the presence of serum seems to be a crucial factor that determines cell selection. Although the serum composition is not defined, the presence of growth factors, such as PDGF, IGF, HGF, EGF, or FGF, or hormones, such as insulin or progesterone, may modulate cell response in culture. In particular, as previously mentioned, some pathways such as EGFR and PDGFR are frequently altered in GBM. For this reason, increasing these growth factors may increase the cell proliferation rate, favoring a rise in genomic instability. In this sense, the use of serum-free media may reduce the cell growth rate, reducing genomic instability. On the other hand, it has been shown that some cell lines may grow in the absence of EGF, which suggests the possibility of using different defined media depending on the cell genotype (14).

In addition to culture media, environmental factors such as oxygen tension are involved in all major aspects of cell biology including proliferation, cell death, differentiation, self-renewal, and migration (79). CICs grown under normoxia or hypoxia conditions display profound differences in the level of gene expression of a panel of stem cells and chemoresistance markers (75) (80). Kolenda *et al.*, observed an increase in the expression of genes related to hypoxia, stemness, and chemoresistance and a reduction of proliferation markers under hypoxia conditions. Finally, oxygen levels have been involved in other important mechanisms affecting the biology of GBM. DNA damage response (DDR) machinery is constitutively activated in GBM (81). DDR is an important factor both in pathogenesis and treatment response. In this sense, low oxygen conditions have been found to reduce the constitutive DDR signaling. Although DDR are involved in limiting the expansion of nascent malignant clones with unstable genomes (82, 83), the constitutive activation could give rise to an increased genomic instability. According to these authors, using low oxygen tension in the culture conditions might have a positive influence in order to reduce the possibility of incorporating new genetic or epigenetic alterations.

7. CONCLUSIONS

The CICs' subpopulation has become the most promising target for the new generation drugs directed to specific intermediates of oncogenic pathways. The comparison between massive transcriptome analyses of important number of GBM tumor samples have contributed to clarify the enormous heterogeneity of this kind of tumor, suggesting a classification into 4 different groups. However, the real contribution of the CIC subpopulation to the whole tumor mass transcriptome is still unknown, and it will be necessary to carry out comparative studies between high numbers of CIC lines to classify them. Nonetheless, a pilot study has been able to correlate the expression profile of still low number of CIC lines from human GBM with specific type of neural stem/progenitor cells, which allowed the author to speculate that these cells could be the cell of origin of some glial tumors. In this sense, most authors share the idea of the existence of a clonal origin from which the different type of cells derives in the glial tumor mass. Despite this clonal origin, the CICs may acquire different genetic or epigenetic alterations at distinct locations of the tumor mass, thereby generating different histological parts within the glial tumor.

Therefore, CICs with different genetic and epigenetic backgrounds may cohabit in the same human brain tumor. Their specific genetic or epigenetic alterations influence individually, synergistically, or in a mutually exclusive way their particular genomic instability rate. At the same time, this rate determines the new mutation acquiring frequency, and therefore, the differential evolution of distinct regions within the tumor along the course of the disease. After the CICs' isolation from the tumor mass, it is not possible to keep the genetic/epigenetic background of the CICs unaltered along the passages in culture because of inherent genomic instability. Whether the procedure of acquiring new genetic/epigenetic either *in vitro* or *in vivo* follow the same path is still unknown. New works with CICs isolated from primary surgery and relapse tumors should be done to confirm this point. Additionally, although it has been demonstrated that CICs are quite less unstable that traditionally derived glioma cells, it will be necessary to design new media and culture conditions based on the CICs specific genetic/epigenetic background to keep uniform their genomic instability rate. This will help to get a reliable *in vitro* model resembling the original tumor useful for either studying the biology of CICs or their response in drug discovery programs.

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Abbreviations: microRNA (miRNA), Cancer Initiating Cells (CICs)

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Send correspondence to: Angel Ayuso-Sacido, Regenerative Medicine Program, Principe Felipe Research Center, AVDA, Autopista del Saler 16, 46012, Valencia, Spain, Tel: 34 96 328 96 80, Fax: 34 96 328 97 01, E-mail: aayuso@cipf.es

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