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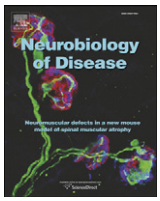

Review

The DNA methylome of glioblastoma multiforme

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ABSTRACT

Glioblastoma multiforme (GBM) is the most frequent brain tumor in adults. This lethal cancer is a challenge in neuro-oncology since patients almost invariably succumb to the disease. Intensive molecular studies have revealed a variety of deregulated genetic pathways involved in DNA repair, apoptosis, cell migration, angiogenesis and cell cycle. Recent investigation of epigenetic lesions in GBM have led to a more comprehensive understanding of this malignancy and even to target therapies, including the milestone of temozolomide chemotherapy, which makes possible a better outcome for GBM patients with hypermethylated *MGMT*. Nevertheless, the whole scenario including global hypomethylation, aberrant promoter hypermethylation, histone modification and chromatin states in GBM has only been partially revealed. We discuss the magnitude of epigenetic alterations in the pathogenesis of GBM and their translational relevance to patient survival.

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Focusing on the challenge of GBM management

Glioblastoma multiforme (GBM) is the most frequent primary brain tumor and accounts for about 69% of all gliomas and 12–15% of all brain tumors (Ohgaki and Kleihues, 2005, 2007). It is one of the most devastating and lethal forms of human cancer despite the significant efforts that have been made to understand its molecular etiology and to improve treatment regimens. In Europe and North America the incidence is three new cases per 100,000 inhabitants per year (Central Brain Tumor Registry of the United States, CBTRUS, www.cbtrus.org). Although GBM can manifest itself at any age, it preferentially occurs in adults, with a peak age of incidence between 45 and 70 years (Aldape et al., 2003). It most frequently involves the brain hemispheres, but it can also affect basal ganglia and the brain stem.

In spite of all the progress in the fields of surgery and radiochemotherapy the prognosis of GBM is still dismal (Aldape et al., 2003; Brandes et al., 2001; Stummer et al., 2006). A recently published meta-analysis reported that, despite multimodal therapy with gross total resection and the combination of radio- and chemotherapy with the drug temozolomide, only a small improvement in median survival time (14.6 months) had been achieved compared to surgery plus radiotherapy alone (12.1 months) (Stupp et al., 2005). As a general

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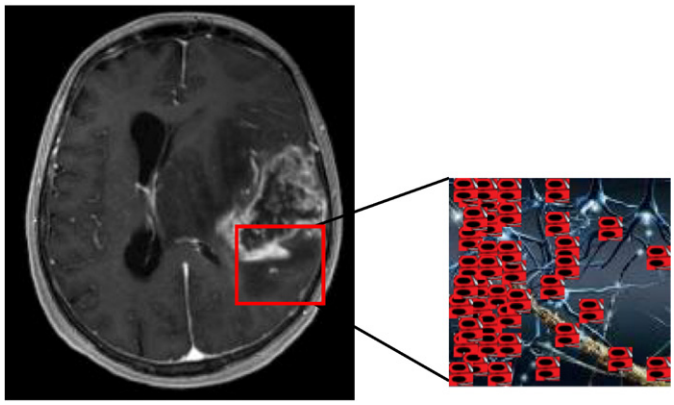

Fig. 1. T1-weighted gadolinium MRI showing a large left temporal glioblastoma with an enhanced peripheral ring, surrounding edema leading to brain shift. Right, schematic representation of the tumor border cells and infiltrating cells beyond invading normal brain.

rule, recurrence develops after a short relapse-free period, signaling a survival time of only a few months (Brandes et al., 2001).

Additional important features of malignant glioma include its great tendency to infiltrate into adjacent normal brain tissue, rendering the condition incurable by surgery alone. The outer GBM border usually observed in T1-weighted gadolinium MRI do not delineate the true dimension of the tumor. Moreover, tumor cells diffusely invade the normal brain and may be detected beyond that border (Fig. 1). Therefore, GBM cannot actually be completely removed from a surgical point of view. Modern microscopic surgical technologies, such as multiphoton microscopy and coherence confocal tomography microscopy (Kantelhardt et al., 2007), which should allow a more exact identification of the invasion border of the tumor, could serve in the future to improve the surgical results with respect to the patient's neurological functions.

One side of the coin: genotyping GBM

Two patterns have so far been described in the pathogenesis of GBM through the gatekeeper pathway. These have different molecular profiles. Type 1 GBM typically shows inactivation of the *p53* tumor suppressor gene (Fig. 2). Mutations of *p53*, mostly associated with loss of heterozygosity (LOH) in the 17p chromosome region, can be observed in GBMs originating from WHO grade II or III astrocytomas. Interestingly, *p53* inactivation does not consistently appear at the same time as amplification of the *EGFR* oncogene, which, on the other hand, is identified in those GBMs lacking *p53* inactivation (Benjamin et al., 2003; Collins 2002; Louis 1997; Ohgaki et al., 2004; Ohgaki and Kleihues, 2007). More than 70% of GBM and anaplastic astrocytomas show a deregulated p53 pathway not only by mutation of TP53 but also amplification of *MDM2*, or homozygous deletion/mutation of *p14ARF* (Ichimura et al., 2000; Riemenschneider et al., 1999).

Type 2 GBM shows overexpression or amplification of the *EGFR* oncogene (Collins 2002; Benjamin et al., 2003). It affects patients who have not previously had a lower grade glioma (von Deimling et al., 1992). These findings suggest two independent GBM pathogenetic pathways (von Deimling et al., 1995). Moreover, EGFR amplification is almost always consistent with LOH in chromosome region 10q (von Deimling et al., 1992). The tumor suppressor gene *PTEN*, in the 10q23 region, is mutated in approximately one-third of type 2 GBMs (Boström et al., 1998). Mutations in this gene have been described only in malignant gliomas and are rarely associated with *p53* mutations. Other frequent mutations in type 2 GBM affect the CDK cell-cycle-regulator genes. Amplification of *CDK4* and *CDK6* was observed in 15% of type 2 GBMs (Ichimura et al., 2000). Mutations of the cell-cycle-regulator genes *CDKN2A*/*CDKN2B* (in chromosome region 9p21 localized) have been observed in 40% of all GBMs. Moreover, a functional loss of expression of the *CDKN2A* gene by promoter hypermethylation was found in 15% of GBMs (Nakamura et al., 2001a,b).

Recently, mutations of the *IDH1* gene have observed in those GBMs progressing from less malignant precursor lesions, mostly in young GBM patients. These were associated with a better outcome (Parsons et al., 2008; Yan et al., 2009). In addition to type 1 and type 2 GBMs, there are other forms, whose molecular profiles do not identify them as belonging to either of the two classic pathways (Benjamin et al., 2003; Lang et al., 1994; Louis 1997). A small subgroup of these GBMs are found to harbor LOH on chromosomes 1p and 19q, which have been suspected for a long time of containing tumor suppressor genes (Benjamin et al., 2003; Kraus et al., 2000).

The other side of the coin: epigenotyping GBM

In addition to genetic alterations, epigenetic abnormalities, such as changes in genomic DNA cytosine methylation patterns, are associated with all cancer types. The spectrum of alterations includes both gain and loss of DNA methylation involving multi-copy elements as well as single-copy genes. Many of the changes, particularly those resulting from DNA methylation and histone deacetylation, affect gene expression and genome stability through the inappropriate regulation of local chromatin structure. Furthermore, recent data suggest that epigenetic changes are involved in the earliest phases of tumorigenesis, and that they may predispose stem/progenitor cells to subsequent genetic and epigenetic changes that are involved in tumor promotion (Widschwendter et al., 2007; Zhao et al., 2007). Given the observed frequency of DNA methylation changes in tumorigenesis and the inherent stability of these molecular abnormalities, these events may provide ideal biomarkers for molecular diagnostics and early detection of cancer (Brena et al., 2006).

In cancer cells, a wide-ranging process operates to change their methylation pattern. For example, regulation of the activity of methylating DNA methyltransferases 1, 3A and 3B is altered. Furthermore, there is general hypomethylation due to demethylation in the CpG islands of a wide variety of genes and also severe hypermethylation that locally affects normally unmethylated chromosome regions. In general, densely methylated DNA is associated with deacetylated histones and compacted chromatin, which is refractory to transcription (Jones and Baylin, 2007; Esteller, 2007). Epigenetic lesions in DNA without mutations in the coding regions have been shown to be common phenomena in the pathogenesis of a wide range of cancers, especially the methylation-mediated silencing of tumor suppressor genes such as *VHL*, $p16^{INK4a}$, *E-cadherin*, *hMLH1*, *BRCA1* and *LKB1* (Esteller, 2002; Jones and Baylin, 2002). Moreover, promoter hypermethylation has been linked with a large number of genes involved in tumor pathogenesis, including $p15^{INK4b}$ (hypermethylated in hematological malignancies), *p73* (hypermethylated in lymphomas) and *ER* (receptor for estrogen-induced transcriptional activation), the DNA repair genes *MGMT* and *GSTP1* (related to the prevention of oxidative DNA damage), *TIMP3* and *DAPK1* (Herman et al., 1994, 1995; Bachman et al., 1999). *MGMT* is another good example of a regulator gene undergoing methylation-mediated inactivation in human cancer. *MGMT* removes mutagenic and cytotoxic adducts from O^6-guanine in DNA. Alkylation of DNA at the O^6 position of guanine is a primordial step in tumor formation, primarily due to the tendency of the O^6-methylguanine to mispair with thymine during DNA replication, leading to the conversion of guanine–cytosine to adenine–thymine pairs in DNA. Furthermore, most common mutations caused by alkylating drugs are G:C to A:T transitions. *MGMT* protects cells against these sequence alterations, transferring the alkyl group from the O^6-guanine in DNA to an active cysteine within its own sequence (Pegg et al., 1995). Recently, a link was observed in GBM between *MGMT* promoter methylation and a hypermutator phenotype as a