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DNA METHYLATION IN BRAIN DEVELOPMENT AND GLIOMAGENESIS

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1. ABSTRACT

The underlying basis of human tumors involves genomic alterations that activate oncogenes and inactivate tumor suppressor genes, culminating in unregulated cell growth. The existing models of tumorigenesis, the selection of subsets of genes contributing to this process and many cancer therapies are based on this genomic approach. However, epigenomic alterations such as gene silencing by aberrant methylation of promoters are also involved in tumorigenesis, but because methylation is not detected by standard genome screening many of these genes have remained undiscovered. Advances in our ability to observe the methylation status of the entire cancer cell genome have led to the unmistakable conclusion that methylation abnormalities are far more prevalent than expected, particularly in low-grade gliomas where genomic changes are less common. In addition, the aberrant methylation of particular genes can influence tumor response to therapy and is strongly associated with patient survival. Thus it is likely that our understanding of tumor genomes is far from complete, and that a significant number of prognostic indicators and therapeutic targets remain to be explored. These studies raise important questions regarding the relative contribution of genomic and epigenomic mechanisms in the genesis and malignant progression of gliomas. Here I begin with the evidence supporting a role for methylation in normal brain cell function and the consequence of its dysfunction, and conclude with an effort to integrate epigenomic views of the genesis of sporadic brain tumors with the traditional genomic model.

2. INTRODUCTION

DNA methylation is essential for a properly functioning genome (1-6). DNA methylation involves transfer of a methyl-group to cytosines that are primarily in a CpG dinucleotide. CpGs are five times more abundant in regions termed CpG islands, relative to the majority of the genome (7-8). CpG islands are approximately 500 bp to several kb in length and nearly always encompass gene promoters and/or exons (9-10). CpGs within CpG islands from imprinted genes**,** genes on the inactive X chromosome in females, and the cell type-specific MAGE and SERPINB5 genes can be differentially methylated in normal cells, while the majority of CpG islands are thought to be entirely unmethylated in all tissues (11-15). In peripheral blood T cells however, Zhu et al have identified extensive clonal CpG island methylation heterogeneity, and Kuromitsu et al have shown that specific CpG islands change methylation status during blast formation in lymphocytes (16-17). Conversely, most CpGs outside of CpG islands are methylated. These heritable patterns of methylation stabilize chromosomes and may also compartmentalize the genome into transcriptionally active and inactive zones (18-19).

Methylation influences gene expression by suppressing the function of gene regulatory elements such as promoters, enhancers, insulators and repressors (11, 20-22). Methylation can directly inhibit the binding of transcription regulatory proteins to DNA, or can indirectly influence transcription by attracting proteins that bind specifically to methylated DNA. These methyl-binding proteins in turn attract histone deacetylases that ultimately form transcriptionally inactive chromatin (21). Although the naturally unmethylated state of promoter-associated CpG islands is independent of transcription activity, it is required for maintaining a transcriptionally permissive state of genes (21-24).

The patterns of DNA methylation are established in defined phases during development of an organism. In general, oocytes are less methylated than

sperm, but these patterns are erased by genome-wide demethylation near the 8-cell stage of blastocyst formation (25-26). During implantation of the embryo, there is a wave of de novo methylation. A long-standing hypothesis is that methylation might be an on-off switch for developmentally regulated and tissue-specific gene expression, but this has not been well supported by examples (27-28). However, in the adult, the amount and pattern of methylation are in fact tissue and cell type-specific, though the role of the majority of this specificity remains unknown. During aging, there is both aberrant methylation of CpG islands in the promoter of genes, including the estrogen receptor gene and *MYOD1*, as well as a progressive decrease in overall 5-methylcytosine in several tissues including the brain (29-30). Methylation patterns of certain genomic regions appear polymorphic between individuals and can be inherited, suggesting either the persistence of certain methylation at all stages of development, or the encryption of methylation pattern information (31). At each stage of life, having the correct amount and pattern of DNA methylation is important to keep cells functioning properly. It is therefore of significant interest to understand the mechanisms that reproducibly establish and maintain methylation patterns.

Methylation patterns are established and maintained by at least three DNA methyltransferases, DNMT1, DNMT3A, and DNMT3B (1, 32-33). DNMT1 appears to be the primary maintenance methyltransferase and is active on hemimethylated substrates, allowing faithful transmission of methylation patterns to newly synthesized DNA following replication. DNMT1 also interacts with RB, E2F1 and HDAC1, suggesting a possible mechanism for sequence-specific effects of DNMTs (34). Mouse embryos having homozygous deletion of *DNMT1* die between embryonic day 8 and 10.5, and exhibit a significant number of apoptotic cells in many tissues including the brain (35). Conditional deletion of *DNMT1* from mouse fibroblasts results in p53-dependent apoptosis and massive dysregulation of gene expression (36). In contrast to DNMT1, DNMT3A and DNMT3B are de novo methyltransferases (1). *DNMT3B* -/- mice also die prior to birth and exhibit growth impairment and rostral neural tube defects, while *DNMT3A* -/- mice are normal appearing at birth but die approximately four weeks later. Mice that are heterozygous mutant for any one of the DNA methyltransferases appear normal and are fertile (1, 35). A fourth gene, DNMT3L was identified based on similarity to DNMT3A and DNMT3B, but lacks the motifs involved in activation of the cytosine, binding of the methyl-group donor and sequence recognition (37). Nevertheless, DNMT3L is essential for establishment of maternal-specific methylation and imprinted gene expression. It is thought that DNMT3L may act as a regulator of imprint establishment, rather than as a DNA methyltransferase (38). Furthermore, heterozygous progeny of DNMT3L homozygous mutant mothers do not develop past 9.5 days postcoitum and exhibit exencephaly and other neural tube defects. These studies show that DNMTs and DNA methylation are essential for life. Although brain development is impaired in the DNMT mutants, it is unclear from these studies alone if methylation plays a primary role in brain development.

3. METHYLATION IN THE DEVELOPING BRAIN

Very little is known of the exact role of methylation in brain development and normal functioning of brain cells in the adult. Perinatally, there is a dramatic change in overall DNA methylation in the brain (39). During several embryonic and postnatal stages there are also single-copy sequences in the brain that become methylated or specifically demethylated (40-41). These changes could result from programmed methylation/demethylation or could reflect, in part, the differing cell type composition of the developing brain. For example, a CpG in the promoter of the mouse GFAP gene appears to be methylated in postmitotic neurons, but unmethylated in neural precursor cells and astrocytes (42-45). The differential methylation involves a critical STAT3 binding site, preventing STAT3 binding and blocking GFAP expression (42). If GFAP expression is controlled in a similar fashion in human brain, it is possible that the apparent astrocytic lineages (GFAP positive cells) within normal brain and astrocytic gliomas may be defined by a single methylation event. Regional differences within the brain, as well as cell type-specific differences in methylation patterns are also suspected, but poorly understood. The discovery that postmitotic neurons continue to express DNMT1 is particularly intriguing given that a major role of DNMT1 is to maintain methylation patterns following DNA replication (46). Recently described conditional deletions of DNMT1 in mouse brain cells are yielding fascinating new insight into this area.

To understand the role of methylation in neural development, Fan et al, have used the *cre/loxP* system to produce conditional mutants of DNMT1 in CNS cells at different developmental stages (47). Homozygous deletion of DNMT1 from cultured postmitotic cerebellar neurons did not alter their global methylation level, morphology or survival. DNMT1 deletion in *in vivo* postmitotic neurons from the perinatal stage did not affect animal viability or brain structure. Furthermore, the percentage of DNMT mutant cells remained constant during postnatal life in all areas except the olfactory bulb. From these and other experiments it appears that DNMT1 is not necessary for maintaining DNA methylation in these postmitotic cells and it's loss is not detrimental to long-term neuronal survival.

DNMT1 null alleles were also created at an earlier developmental stage using CRE driven by a nestin promoter (47). In CNS cells, DNMT1 mutant cells were detected by embryonic day 10.5, deletion was complete by E12.5, and by E19.5, 95% of the CNS cells were mutant and exhibited significant hypomethylation. Although these mutant cells survived throughout embryogenesis, the DNMT1 mutant mice were either not observed in live births, or were delivered by E19.5 and subsequently died of respiratory failure within one hour. This phenotype was not attributable to failure of the peripheral respiratory system, and further data suggested that abnormal neural control of respiration might be involved. A potential role for DNMT1 in gliogenesis, which occurs postnatally, was not tested in these animals.

To investigate the role of methylation in postnatal brain cells, Fan studied animals in which only 30% of E12 CNS cells were mutant (as a result of a fortuitous maternal transmission effect). The mutant cells were not selected against during embryogenesis or early postnatal development. By three weeks however, all mutant cells have been eliminated. Thus, DNMT1 and DNA methylation are critical for the function and survival of postnatal CNS neurons, but not for postmitotic neurons in the mouse.

4. METHYLATION IN HUMAN BRAIN

Brain has one of the highest levels of 5-methylcytosine in human and mouse tissues (39, 48). Human thymus has a slightly greater amount, and lymphocytes, spleen, lungs, liver, heart, sperm, and placenta have progressively lesser amounts relative to brain. These tissue-specific differences are conserved between different individuals. While repetitive sequences account for the largest fraction of 5-methylcytosine, the tissue-type differences are found in both unique and repetitive sequence fractions of the genome. Methylation differences can also be cell type-specific (14-15, 48), though a detailed analysis of different brain cells in vivo has not been reported. The function of these conserved tissue differences is unknown.

Several genetic mutations that affect DNA methylation and brain function in humans have been identified. One such disorder is ICF syndrome, which is linked to mutations in the DNA methyltransferase, DNMT3B (1, 5, 6). ICF syndrome is an autosomal recessive disorder characterized by immunodeficiency, centromere instability, facial anomalies and variable mental retardation. The centromeric instability is most evident on chromosomes 1,9 and 16, which have severely demethylated pericentromeric satellite sequences, and these chromosomes become highly unstable in mitogen-stimulated lymphocytes. A second disorder that affects methylation and brain function is Rett syndrome. Rett syndrome is a neurodevelopmental disorder that affects females exclusively and although development proceeds normally until 6 to 18 months of age, these individuals gradually develop mental retardation and autistic behavior (49). In two-thirds of the sporadic cases and 45% of familial cases the syndrome is linked to mutations in MeCP2, an X chromosome gene that encodes a methylated DNA binding protein (50-51). This protein may be important in mediating methylation-associated gene silencing, possibly through alterations of histone H4 acetylation (52). Conditional deletion of the mouse MECP2 results in a phenotype that is similar in onset and dysfunction to Rett syndrome (53-54). Taken together, the mouse and human data suggest that MECP2 may be more important for stability of brain function than for brain development. Individuals with a third disorder, Fragile X syndrome, also exhibit mental retardation and have mutations and aberrant methylation and silencing of the FMR gene (21). Further evaluation of the mutations found in each of these syndromes will be required to determine how these methylation defects impact normal brain cell function.

Defects in methylation may underlie or contribute to other disorders. Because of the heritable and reversible nature of methylation, intriguing theories have been proposed regarding a role that epigenetics (possibly aberrant methylation) might play in complex, non-Mendelian disorders such as schizophrenia and affective disorders (55-57).

Neural tube defects and mental retardation are also linked to mutations in a gene that is indirectly related to DNA methylation, MTHFR (58). MTHFR encodes an enzyme that helps synthesize folate, a ubiquitous one-carbon source for a number of biochemical processes including production of S-adenosylmethionine (SAM). SAM is the donor of methyl-groups for DNA and other molecules. A direct role for DNA hypomethylation in the neural tube defects is uncertain, particularly because of the ubiquitous requirement for folate in cellular biochemistry. However, folate deficient diets are also known to produce global hypomethylation that can be reversed by folate repletion, thus linking folate levels to DNA methylation levels (59). There is also a suspected relationship between MTHFR polymorphisms, folate levels and risk for certain types of cancer (60-61).

Finally, aberrant methylation in combination with genetic alterations contribute to tumorigenesis in the brain. Abnormal methylation was observed in human brain tumors at least 20 years ago (62), but follow-up has been minimal until recently. In the next section the current knowledge of aberrant methylation in human gliomas is reviewed, followed by an attempt to integrate this epigenomic view with the traditional genomic models of gliomagenesis.

5. ABERRANT METHYLATION IN CANCER

In discovering and interpreting methylation defects, researchers have adapted the principles of cancer genomics, including theories of the clonal evolution of tumor cell populations and the two-hit model of tumor suppressor gene inactivation (63-64). Methylation may inactivate one or both alleles of the proven tumor suppressor genes in sporadic cancers, and can potentially act as a second hit during the development of hereditary cancer (65-66). If methylation imbalances contribute directly to tumor initiation, the alterations should occur in early stages of cancer or in premalignant cells. If the imbalance contributes directly to tumor progression, methylation defects should increase in frequency and/or severity coordinately with increasing malignancy grades. One might also expect that cells harboring functionally important methylation abnormalities could be selected in a manner consistent with the clonal evolution of cancer cells. Finally, there should be a mechanistic explanation linking the methylation change to malignant behavior. Available evidence from premalignant tissues, primary human tumors and in vitro and in vivo models of cancer support these suppositions (23-24).

An imbalance in methylation is prevalent in human sporadic cancers. Methylation defects include chromosome-destabilizing demethylation and aberrant methylation of CpG islands (23-24, 67-68). The global hypomethylation and regional CpG island

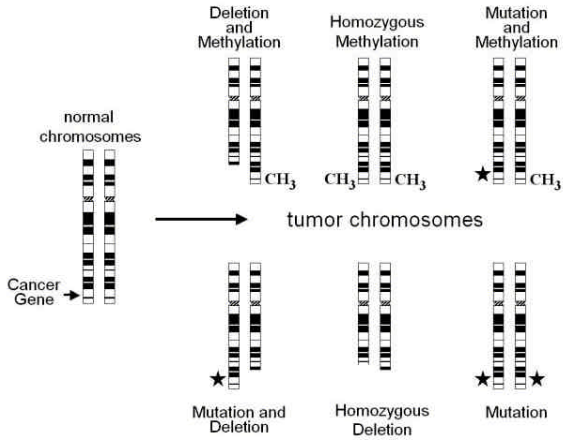

Figure 1. Genomic and epigenomic mechanisms of tumor suppressor gene inactivation. Individual tumor suppressor genes are primarily inactivated by a particular two-hit combination. To date, most suppressor genes involved in gliomagenesis have been isolated through genetic screens which primary detect large deletions, followed by positional cloning and a candidate gene approach that detects small deletions or point mutations. Homozygous deletion has also been very informative in localizing tumor suppressor genes, but except for the CDKN2A locus, it is rare in gliomas. Genes that are primarily inactivated by a two-hit combination involving aberrant methylation of at least one allele (top half of diagram) can be detected by a candidate gene approach, or by using methods that specifically screen the genome for aberrant methylation.

hypermethylation can occur in the same tumor cells but they affect different genomic subregions, and their etiology is unknown. These and other studies have established an important role of aberrant methylation in tumorigenesis. However, there are very few studies of the role of aberrant methylation in human gliomas, the most common form of primary brain cancer.

6. ABERRANT METHYLATION IN HUMAN GLIOMAS

One of the first observations of abnormal methylation in gliomas was a global decrease in 5-methylcytosines in glioblastomas and an astrocytoma relative to normal brain (62). In general, hypomethylation is a common feature of malignant cells and involves both single copy genes and repetitive sequences. The functional consequence of hypomethylation in tumorigenesis is not fully resolved. One hypothesis states that hypomethylation of single copy sequences could activate normally silent oncogenes (27, 69). However, while oncogene overexpression in the absence of gene amplification is fairly common, to date there is no compelling evidence that local hypomethylation causes overexpression. A second hypothesis states that hypomethylation in high copy number juxtacentromeric sequences could destabilize the chromosome, similar to the hypomethylation induced chromosome destabilization observed in ICF syndrome and in cells treated with a DNA demethylating agent (68). These proposed consequences, and subregion localization of hypomethylation have not yet been tested in gliomas.

In contrast to global hypomethylation, gliomas also exhibit extensive regional hypermethylation, primarily at CpG islands. Methylation of CpG island promoters may inactivate both alleles of a proven cancer gene, or may act together with genetic mechanisms including point mutation or deletion (70-73). Methylation of cancer suppressor genes is typically restricted to non-mutated alleles, and demethylating agents (74) are capable of restoring gene activity and tumor suppressor function in cultured tumor cells. A great deal of excitement has come from the possibility that the dormant, but non-mutated genes could be chemically reactivated to restore functional tumor suppressor activity in cancer patients as an alternative to gene-replacement therapy. Currently, the chemically induced demethylation is not specific for tumor suppressor genes. Thus, it can be anticipated that demethylation of other genomic regions will occur, including regions that are normally methylated.

A typical candidate gene approach in gliomas tests for aberrant methylation in the CpG islands of established cancer genes, particularly in tumor samples and on specific alleles that do not harbor genetic alterations of the gene. This approach in gliomas has uncovered aberrant methylation-related gene silencing that suppresses normal cell cycle regulation (e.g. RB, CDKN2A, CDKN2B, p73), DNA repair and drug resistance (MGMT), apoptosis (DAP kinase), angiogenesis (THBS1), and invasion (TIMP3)(75-94). For RB, CDKN2A and CDKN2B, the level of aberrant methylation has been shown to increase over time within individual patients, as the gliomas progress from WHO grade II to WHO grade IV (77-78). Such aberrant methylation may therefore provide a selective growth advantage and is consistent with a significant role for methylation in the clonal evolution of glioma cells. In contrast, MLH1, a gene involved in mismatch repair and frequently methylated in other tumor types, is not methylated in glioblastomas, consistent with the general lack of microsatellite instability in these tumors (81, 95). Only a small number of the candidate gene studies have examined the methylation status of all CpGs within a given promoter, so there is still important follow-up work to be done for these genes, including confirmation of gene silencing in primary tumors. Whether similar gene silencing events are recapitulated in mouse models of gliomas, as they are in models of other tumor types, has not yet been reported. In combination with functional studies of these cancer genes and mechanistic studies linking methylation with gene silencing, there is considerable evidence that CpG island methylation contributes directly to malignancy (21, 23-24).

Cancer genes may be inactivated by a variety of mechanisms, including point mutation, deletion and methylation (Figure 1). For particular genes, it is often one of the mechanisms that predominate in the inactivation. For example, the CDKN2A tumor suppressor gene in brain and breast tumors is inactivated primarily by homozygous deletion. The p53 gene is most frequently affected by

deletion of one allele and point mutation of the other allele, in nearly all tumor types in which it is involved. These observations suggest that there may exist an entirely different set of important cancer genes that are inactivated primarily by aberrant methylation on one or both alleles. In theory, such genes would have remained undiscovered over the past two decades due to the exclusively genetic screening methods employed.

On the foundation set by discovery of aberrantly methylated genes, a number of methods to screen the genome for aberrantly methylated genes have been developed. These include PCR-based methods, array hybridization, and Restriction Landmark Genome Scanning (RLGS)(96-100). Suitable methods for addressing the hypotheses stated above should have a strong bias for 5' CpG islands, cover large numbers of genes, and allow accurate quantification of methylation.

Restriction Landmark Genome Scanning (RLGS) is an approach that is uniquely suited for simultaneously assessing the methylation status of thousands of CpG islands (98). RLGS separates radiolabeled *Not*I fragments in two dimensions and allows distinction of single copy CpG islands from multi-copy CpG rich sequences. The methylation sensitivity of the endonuclease activity of *Not*I provides the basis for differential methylation analysis and *Not*I sites occur primarily in CpG islands and genes. RLGS has been used to identify novel imprinted genes, novel targets of DNA amplification, and methylation in human cancer and to identify deletion, methylation and gene amplification in a mouse model of tumorigenesis (101-112). Additionally, the chromosome of origin of CpG islands displayed on the profiles has been determined, allowing the construction of chromosomal maps of aberrant methylation (113). Such massively parallel analyses are critical for pattern recognition within and between tumor types, and for estimating the overall influence of CpG island methylation on the cancer cell genome. It should be noted that all current methods for assessing methylation genome wide, including RLGS, assess only a fraction of the total possible sites of methylation, and thus, the further development of complementary methods is needed.

Using RLGS, a number of fundamental properties of aberrant methylation patterns in tumors were discovered (108). First, the patterns of aberrant methylation are non-random. This indicates that forces such as growth selection and/or differential susceptibilities must be shaping the patterns, supporting a functional role for methylation in tumorigenesis in humans. Second, aberrant methylation patterns are tumor type-specific, and thus potentially silence specific subsets of genes that contribute to the development of specific tumor types. Third, the total number of aberrantly methylated CpG islands in low-grade gliomas was estimated from RLGS profiles (114). An average of 1544 methylated CpG islands per tumor was estimated, with a range of 0 to 3731. Aberrant methylation of a proportion of these genes correlates with loss of gene expression, though it is unlikely that all methylation events impact transcription or arise through the same mechanism. It should be noted that CpG islands are often bi-directional promoters and can also serve as regulatory elements that act at a distance. Thus, there is potential for aberrant methylation to exert effects that are not confined to, or do not involve the "nearest" gene. How many methylated genes and which ones are integral to gliomagenesis remains an open question. Finally, by applying agglomerative hierarchical clustering to the methylation data, the low-grade tumors form at least three distinct clusters, suggesting there may be methylation subtypes within tumors having similar histology. Central to understanding the impact and importance of CpG island methylation is the extent to which the methylation is capable of silencing the gene and the type of genes that are methylated.

Aberrant CpG island methylation also occurs in pediatric tumors of the nervous system. For example, Caspase 8, a cysteine protease that is part of an apoptosis signaling pathway, is inactivated by aberrant methylation and deletion in primitive neuroectodermal tumors (PNET)/medulloblastomas and the peripheral nervous system tumor, neuroblastoma (115-119). Cells in which Caspase 8 has been inactivated by methylation are resistant to apoptosis induced by chemotherapeutic agents or an apoptosis inducing ligand. Thus, Caspase 8 acts as a tumor suppressor gene. Fruhwald et al have shown that while many classic tumor suppressor genes are not aberrantly methylated in medulloblastomas and other PNETs, other as yet uncharacterized genes do exhibit recurrent methylation in these tumors (120). Furthermore, the patterns of methylation in medulloblastomas are distinct from other PNETs, and a subset of these correlate with poor prognosis. This work suggests that there are a number of important cancer genes remaining to be discovered in these tumor types, and that models of tumorigenesis must begin to integrate traditional genomic changes with epigenomic alterations.

7. INTEGRATING GENOMICS AND EPIGENOMICS OF GLIOMAS

While aberrant CpG island methylation and genetic alterations both fuel tumorigenesis, our current understanding of the interplay of these mechanisms in gene inactivation is meager. For a few candidate genes it has been shown that methylation and deletion, or occasionally point mutation and methylation, can combine to achieve biallelic inactivation (70, 72-73, 121). Two additional observations suggest that convergent methylation and deletion may be an important and perhaps prevalent mechanism for biallelic inactivation of cancer genes. First, there are a significant number of recurrently deleted regions of chromosomes in which the genes on the remaining allele are not genetically altered. Conversely, a large number of genes are methylated in an apparently hemizygous fashion, but are untested for genetic alteration of the other allele. Second, preliminary data using Restriction Landmark Genome Scanning (RLGS) demonstrates that while many of the recurrent methylation sites are not coincident with deletions, a few of the genes are localized to regions that coincide with known, recurrent sites of deletion in gliomas

(114). Currently, genes that are primarily inactivated by the particular two hit mechanism of methylation and deletion are entirely inaccessible to non-integrated approaches, other than on a gene by gene basis.

The interaction of methylation and deletion is particularly important for understanding the genesis and progression of gliomas (122). Aberrant methylation is frequent and widespread in low-grade astrocytomas (WHO grade II), while in tumors that have progressed to high-grade astrocytomas (WHO grade III, IV), large deletions are commonplace. When and where methylation and deletion converge during gliomagenesis will in part determine the subset of genes that are inactivated, and potentially will influence the degree of malignancy. The theoretical models of tumorigenesis that will come from an integrated analysis should then lead to a substantially different approach to therapies and therapeutic decision making.

8. PREDICTORS OF OUTCOME

Patients with gliomas of similar histologic grade can have a remarkably variant length of survival, indicating that current classification schemes lack the ability to identify clinically relevant differences in these tumors. To improve tumor classification and optimize therapeutic decision making, molecular markers are needed to distinguish the indolent from more aggressive tumors at diagnosis. Gene deletion and gene silencing by aberrant CpG island methylation are two major mechanisms of gliomagenesis that can potentially cooperate to increase malignancy. Either type of aberration alone shows promise as a prognostic marker of survival and/or response to therapy. In some cases the alterations may be causally related to the degree of malignancy or response to therapy, or in other cases it may simply be a marker of a particular phenotype. For example, oligodendrogliomas with deletion of chromosome 1p almost always respond to the chemotherapeutic regimen PCV, while tumors without this alteration are less likely to respond and generally have a shorter length of survival (123). Additionally, alterations of PTEN or gains of chromosome 7p and 7q in anaplastic astrocytomas are associated with shorter survival, and p53 overexpression in glioblastomas is associated with longer-term survival (124-126). Aberrant methylation of the MGMT gene is associated with longer survival in malignant gliomas (90), but other studies show that MGMT protein activity does not correlate with patient survival (127), suggesting that MGMT methylation is a marker, but not a cause of longer survival. The presence or absence of some of these alterations is now influencing therapeutic decisions, but significantly more work in this area is desperately needed.

9. PERSPECTIVE

Methylation is essential for normal brain development and aberrant methylation contributes to the development of brain tumors. Although these fields are in their early stages and have not yet crossed paths, we can, with some optimism, anticipate their intersection. For example, determining the exact role of methylation in gliogenesis might help in interpreting the functional consequence of aberrant methylation in gliomagenesis. If the cell of origin of certain gliomas is a neural precursor or stem cell, cells from the Nestin promoter-driven deletions of DNMT1 might be useful for oncogenic transformation studies. Mouse models of brain tumors will also undoubtedly play a significant role in this area. Conversely, learning more about the aberrant methylation in gliomas may yield clues to their cell of origin, or may help determine the function of particular normal methylation patterns in brain cells.

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