Meningiomas, which originate from the arachnoidal cap cells of the leptomeninges, have an incidence of 4.4 per 100,000 person-years and are the most commonly diagnosed primary brain tumor. The peak incidence of meningiomas occurs in the 6th and 7th decades, and women are affected nearly twice as often as men. Meningiomas are often organized with the WHO classification of tumors, with 80% being considered Grade I (benign); 10%–15%, Grade II (atypical); and 2%–5%, Grade III (anaplastic/malignant). Grade III meningiomas are typically associated with brain invasion and recurrence, and the overall 10-year survival rate for patients with these lesions is only 14.2%. Thus, it is of critical clinical significance to accurately determine the characteristics of these tumors in a timely fashion. The WHO pathological grading system cannot always accurately predict the clinical aggressiveness of these tumors, and grading is subject to sampling error and inter-user variability. Additional possible explanations for the difficulty in predicting the behavior of meningiomas have included the observations that there are numerous histological similarities between tumors in each grade and that these tumors exist along a spectrum in which low-grade meningiomas can progress to a higher grade. There is no clear explanation, however, as to why the majority of recurrent meningiomas derive from benign histology even after apparently radical removal.

Other attempts to classify meningiomas have used genomic techniques to study their genesis and progression, as well as to search for genetic mutations and variable gene expression mediated via epigenetic modifications. The use of epigenomics as a clinical tool has become better understood in recent years and research has started to elucidate the importance of epigenetics in meningioma progression. Data suggest that there is an absence of any significant genetic alterations in nearly 40% of meningiomas. One study found that 77% of meningiomas had at least 1 methylated gene, and 25% of samples in another study had 3 or more methylated genes. Epigenetic alterations in the genome are also par-
particularly useful to examine because they are thought to occur in the early stages of tumorigenesis and can function through multiple mechanisms to cause runaway cell growth (Fig. 1).

In this review, we present a systematic analysis of the genes that are known to undergo epigenetic modifications as related to the development, progression, and recurrence of intracranial meningiomas. This article summarizes the literature pertaining to the epigenetic modification of meningiomas and provides a brief discussion for future avenues of molecular classification, prognosis, and epigenetic therapies (epidrugs), that could provide significant clinical value to patients with meningiomas in the future.

**Methods**

A systematic review of the PubMed database was performed to identify all studies published up to August 2012, using all combinations of the search terms “meningioma,” “epigenetics,” “methylation,” “histone,” “sequencing,” and “acetylation.” Only English-language publications were included in our query. Two investigators independently screened all identified abstracts for potential inclusion. To be considered eligible for inclusion, a study looked at the epigenetic changes to meningiomas defined as alterations in levels of gene expression that are not accompanied by changes in the primary DNA sequence.

**Results**

A primary and secondary review of all identified abstracts resulted in 138 studies that met initial inclusion criteria. Of these studies, 112 were discarded due to 1) identified changes in the underlying DNA sequence or 2) publication in a language other than English. The resulting 26 studies from the systematic search were then classified according to gene subtype affected by epigenetic modification.

**Tumor Suppressor Genes**

The best understood of the tumor suppressor genes as pertaining to epigenetic regulation of meningiomas is the **TIMP3** (**TIMP metallopeptidase inhibitor 3** or **tissue inhibitor of metalloproteinase 3**), which encodes for a protein that inhibits matrix metalloproteinases (MMPs) (Table 1). A second function appears to be a unique tumor suppressor–like property that is not related to MMP inhibition. Overexpression of **TIMP3** in vitro has been shown to suppress tumor growth and induce apoptosis. Hypermethylation of the 22q12 gene **TIMP3** and subsequent transcriptional downregulation (gene silencing) has been identified as a marker for an aggressive, high-grade meningioma phenotype. Although there is some minor disagreement in the literature, Grade I tumors have been found to have significantly less aberrant methylation at **TIMP3** than Grade II or III meningiomas.

The urokinase plasminogen activator (uPA) system functions via extracellular matrix proteolysis to facilitate cellular adhesion and migration. Active uPA activates plasminogen to plasmin, which degrades the extracellular matrix and activates various MMPs, intersecting with the pathway of TIMP-3. Increased expression of uPA proteins is associated with higher WHO grade, malignant invasion, and recurrence.

**TP53.** Tumor suppressor p53 (cellular tumor antigen p53, p53) is a ubiquitous component in growth regulation that interacts with multiple other pathways to control the cell cycle. Loss of chromosome 9 is of particular impor-
Epigenetic and gene expression alterations in meningioma

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Product</th>
<th>Modification</th>
<th>Function</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIMP3</td>
<td>22q12.3</td>
<td>metalloproteinase inhibitor 3</td>
<td>hypermethylation</td>
<td>MMP inhibition</td>
<td>higher meningioma grade&lt;sup&gt;4,15&lt;/sup&gt;</td>
</tr>
<tr>
<td>CDK2NA (p14[ARF])</td>
<td>9p21.3</td>
<td>p14[ARF] protein</td>
<td>hypermethylation</td>
<td>cell cycle control</td>
<td>tumorgenesis&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>TP73</td>
<td>1p36</td>
<td>P73 protein</td>
<td>hypermethylation</td>
<td>cell cycle control</td>
<td>tumorgenesis&lt;sup&gt;1,22&lt;/sup&gt;</td>
</tr>
<tr>
<td>TEMEM30B</td>
<td>14q</td>
<td>transmembrane factor</td>
<td>hypermethylation</td>
<td>cell cycle</td>
<td>recurrence&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>HIST1H1C</td>
<td>6p21.1</td>
<td>histone H1.2</td>
<td>upregulation</td>
<td>cell cycle</td>
<td>recurrence&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>MEG3</td>
<td>14q32</td>
<td>noncoding RNA</td>
<td>hypermethylation</td>
<td>cell cycle</td>
<td>tumorgenesis &amp; grade&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The importance of p53 in preventing meningioma development and grade extends to another gene, MEG3 (maternally expressed 3), an imprinted gene with maternal expression that encodes a noncoding RNA. Previous research showed that MEG3 RNA functions by activating p53 target promoters. Hypomethylation of MEG3 has been shown to occur frequently in human brain tumors.<sup>97</sup>

The tumor suppressor protein p73, which shares significant sequence homology with p53, also plays an important role in malignant meningiomas.<sup>14,22</sup> Previous research has indicated that p73 has opposing functions of both cell growth and cell cycle arrest.<sup>38</sup> Epigenetic studies show that p73 plays a similar dual role in meningioma progression. Hypermethylation may cause development of some low-grade forms, whereas enhanced p73 expression characterizes malignant meningiomas.<sup>64</sup>

HIST1H1c. Histone cluster 1, H1c is a gene on chromosome 6 that has been found to be overexpressed in 27% and 89% of original and recurrent meningiomas, respectively, in a study by Pérez-Magán et al.<sup>99</sup> It is hypothesized that the physical interaction of the H1.2 protein can participate in the epigenetic regulation of gene expression by maintaining specific DNA methylation patterns.<sup>24</sup> More specifically, the authors hypothesize that it may suppress p53-dependent p300-mediated chromatin transcription by blocking chromatin acetylation.

The importance of p53 in preventing meningioma development and grade extends to another gene, MEG3 (maternally expressed 3), an imprinted gene with maternal expression that encodes a noncoding RNA. Previous research showed that MEG3 RNA functions by activating p53 target genes and stimulates p53-mediated transcriptional activation.<sup>100</sup> Zhang et al.<sup>98</sup> hypothesized that since MEG3 is located at 14q32, a region that has been previously associated with meningioma progression, it would be an ideal candidate for epigenetic meningioma progression. Their research showed that MEG3 RNA is highly expressed in normal arachnoidal cells but not expressed in the majority of meningiomas, whereas high-grade meningiomas frequently show a higher degree of MEG3 methylation (p = 0.038) as well. Furthermore, when MEG3 cDNA was tested in vitro on meningioma cell lines, the gene was found to suppress colony formation by 80%, similar to the rate of other known growth suppressors. The study illustrates the importance of epigenetic regulation of p53 as a key inhibitor of meningiomas.

Atypical and anaplastic meningiomas show characteristic expression of the GADD45A gene<sup>94</sup> when compared with benign meningiomas, similar to increases previously found in pancreatic carcinomas.<sup>96</sup> Three additional genes were identified by Wrobel et al. through mRNA expression and immunohistochemistry as having a stronger expression profile in atypical and anaplastic meningiomas than benign meningiomas: PCNA, STK15 (AURKA), and CENPF.<sup>94</sup> PCNA interacts with GADD45A (GADD45) and functions as a cofactor for DNA polymerase, causing cell proliferation.<sup>94</sup> STK15 encodes aurora kinase A, a centrosome-associated serine/threonine kinase previously shown to be amplified in colon cancer,<sup>28</sup> while CENPF encodes mitosin, a protein of centromere-kinetochore complex protein that is involved in cell division of somatic cells and involved in a range of oncogenic pathologies.<sup>44,86</sup>

Other tumor suppressor genes that do not involve the p53 pathway include TEMEM30B and GSTP1. TEMEM30B is a gene in the 14q region that encodes a transmembrane factor that participates in the cell cycle.<sup>41</sup> It had previously been found to be expressed in meningiomas<sup>41</sup> and is downregulated in recurrences compared with nonneoplastic tissue.<sup>77</sup> GSTP1 is a member of the glutathione-S-transferase family. Its function is to conjugate carcinogens with glutathione, which neutralizes it and allows its excretion, preventing DNA damage.<sup>33</sup> Hypermethylation of GSTP1 was found with an increasing frequency from 0% in benign to 32% in atypical to 54% in anaplastic variants<sup>49</sup> of meningiomas in one study by Liu et al., and thus GSTP1 promoter region hypermethylation is associated with meningioma grade.<sup>59</sup>

Cell Signaling

The homeobox (HOX) family of genes is a target for gene silencing via DNA demethylation<sup>21,83</sup> and is frequently identified in a wide variety of human tumorigenesis<sup>76,90,95</sup> (Table 2). The concordant methylation of HOXA genes on 7p15.2, including HOXA5, HOXA6, HOXA9, and HOXA11, leads to the downregulation of tumor suppressor targets.<sup>45</sup> Kishida et al.<sup>35</sup> used high-throughput


**TABLE 2: Cell signaling**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Product</th>
<th>Modification</th>
<th>Function</th>
<th>Meningioma Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOXA5, HOXA6, HOXA9, &amp; HOXA11</td>
<td>7p15.2</td>
<td>HOXA5, HOXA6, HOXA9, &amp; HOXA11</td>
<td>unmethylation</td>
<td>transcription factor</td>
<td>tumorigenesis²</td>
</tr>
<tr>
<td>PENK</td>
<td>8q23</td>
<td>preproenkephalin</td>
<td>hypermethylation</td>
<td>apoptosis</td>
<td>tumorigenesis²</td>
</tr>
<tr>
<td>UPK3A</td>
<td>22q13.31</td>
<td>uroplakin-3a</td>
<td>hypermethylation</td>
<td>growth factor</td>
<td>cell growth &amp; tumor aggressiveness³</td>
</tr>
<tr>
<td>IGFBP3</td>
<td>7p13–p12</td>
<td>insulin-like growth factor</td>
<td>upregulation</td>
<td>growth factor</td>
<td>cell growth &amp; tumor aggressiveness³</td>
</tr>
<tr>
<td>IGFBP2</td>
<td>11p15.5</td>
<td>IGF-2 protein</td>
<td>upregulation</td>
<td>growth factor</td>
<td>cell growth &amp; tumor aggressiveness³</td>
</tr>
<tr>
<td>IGF2BP1</td>
<td>17q21.32</td>
<td>RNA binding protein</td>
<td>hypermethylation</td>
<td>transcription factor</td>
<td>tumorigenesis²</td>
</tr>
<tr>
<td>WNK2</td>
<td>9q22.31</td>
<td>WN kinase</td>
<td>hypermethylation</td>
<td>growth factor</td>
<td>tumorigenesis</td>
</tr>
<tr>
<td>NDRG2</td>
<td>14q11.2</td>
<td>NDRG2 protein</td>
<td>hypermethylation</td>
<td>transcription factor</td>
<td>cell growth &amp; tumor aggressiveness³</td>
</tr>
<tr>
<td>LMO4</td>
<td>1p22.3</td>
<td>LIM domain transcription factor</td>
<td>downregulation</td>
<td>transcription factor</td>
<td>tumorigenesis²</td>
</tr>
<tr>
<td>CTGF</td>
<td>6q23.2</td>
<td>CTGF</td>
<td>hypermethylation</td>
<td>growth factor</td>
<td>recurrence²</td>
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<td>CTNNB1</td>
<td>3p21</td>
<td>β-catenin</td>
<td>downregulation</td>
<td>Wnt signaling</td>
<td>cell growth &amp; tumor aggressiveness³</td>
</tr>
<tr>
<td>CDK5R1</td>
<td>17q11.2</td>
<td>CDK5R1 protein</td>
<td>upregulation</td>
<td>Wnt signaling</td>
<td>cell growth &amp; tumor aggressiveness³</td>
</tr>
<tr>
<td>ENC1</td>
<td>5q12–q13.3</td>
<td>ENC 1 protein</td>
<td>upregulation</td>
<td>Wnt signaling</td>
<td>cell growth &amp; tumor aggressiveness³</td>
</tr>
<tr>
<td>CCND1</td>
<td>11q13</td>
<td>CCND1 protein</td>
<td>upregulation</td>
<td>Wnt signaling</td>
<td>cell growth &amp; tumor aggressiveness³</td>
</tr>
<tr>
<td>ALPL</td>
<td>1p36.1–p34</td>
<td>alkaline phosphatase</td>
<td>downregulation</td>
<td>cell cycle control</td>
<td>progress &amp; grade⁹</td>
</tr>
<tr>
<td>THBS1</td>
<td>15q15</td>
<td>thrombospondin 1</td>
<td>methylation</td>
<td>angiogenesis inhibition</td>
<td>angiogenesis¹</td>
</tr>
</tbody>
</table>

Genome-wide DNA methylation analyses of high-grade and high-recurrence meningiomas, and identified several genes potentially implicated in meningioma progression. These genes included HOXA6 and HOXA9 as identified previously by Di Vinci et al., along with genes PENK and UPK3A.

These candidate genes have been identified as methylation targets in various other tumors. PENK has been identified in other genome-wide analysis of various solid tumors. Although its role in oncogenesis is still relatively unknown, a recent study suggested that cellular stress induces PENK to physically bind with p53 and RELA (p65) to regulate stress-induced apoptosis. This would seem in concordance with previous evidence that p53-related pathways play an important role in meningioma epigenetically mediated oncogenesis. UPK3A codes for uroplakin-3a, one of a group of transmembrane proteins that form complexes on the surface of bladder epithelium. DNA methylation of the promoter region of UPK3A is the primary mechanism underlying silencing of the gene, and such alterations have also been reported in metastasis of colorectal neoplasms.

**IGF.** The IGF signaling family and the anomalous expression of IGF, IGF receptor, and IGF-binding proteins have been demonstrated as a key signaling pathway in the tumorigenesis of meningiomas. The literature has consistently revealed that the increased expression of IGFBP3 and IGF2 transcripts, along with decreased expression of IBFBP2, is important in meningioma progression and anaplastic classification. It was reported that IGFBP3 may potentiate IGF1 action by altering B/AKT protein kinase sensitivity to IGF-1 receptor signaling. Furthermore, IGFBP1 is an RNA-binding protein that regulates mRNA stability and translocation. It is hypothesized to play a role in tumorigenesis by stabilizing messenger RNAs of the c-myc oncogene and IGF2 in certain cancers.

WNK2. In meningiomas, Jun et al. found that WNK2 was aberrantly methylated in 83% and 71% of Grade II and III meningiomas, respectively, but rarely methylated in 13 other tumor types. The authors noted that aberrant methylation of the CpG island was associated with decreased gene expression in primary tumors, while expression of exogenous WNK2 inhibited colony formation, implicating it as a potential cell growth suppressor. WNK2 has been shown to inhibit cell proliferation in vitro by negatively modulating the activation of MEK1/ERK1/2 and epidermal growth factor receptor (EGFR) signaling. EGFR is thought to be an important oncogene in meningiomas, and WNK2 silencing could potentially enhance EGFR protein signaling. Jun et al. showed that WNK2 could be reactivated with a methylation inhibitor in IOMM-Lee cells, and thus their findings may be translatable to the bedside.

NDRG2. **N-myc downstream regulated gene 2 (NDRG2)** is normally expressed in brain, heart, and muscle and is one of 4 members of the NDRG family. It has been implicated in cell growth as well as apoptosis, and identified by Lusis et al. as a gene that is consistently downregulated in Grade III meningioma and independent sets of diverse meningiomas. The loss of NDRG2 expression was significantly associated with hypermethylation of the NDRG2 promoter.
**Epigenetic and gene expression alterations in meningioma**

**TGF-β.** The gene LMO4 (1p22.3) is a novel candidate in meningioma epigenetic research. It is silenced more often in original meningiomas (32%) than in normal meningotheelial tissue, although in recurrences of meningiomas the expression is similar.59

LMO4 has previously been identified as a gene that is overexpressed in breast15,61,101 and pancreatic tumors63 and also includes the gene,11 whose product is a growth factor secreted by vascular endothelial cells and has various regulatory functions in cell growth and apoptosis. CTGF has been found to have significantly lower expression in recurrent meningiomas than in original meningiomas.10 The authors of the meningioma study suggest that LMO4 modulates TGF-β signaling through its interaction with receptor-activated SMADs, supporting the finding of the downregulation of the TGF-β pathway in recurrent meningiomas.53

Involvement of the TGF-β–SMAD pathway seems to also include the CTGF gene,11 whose product is a growth factor secreted by vascular endothelial cells and has various regulatory functions in cell growth and apoptosis. CTGF has been found to have significantly lower expression in recurrent meningiomas than in original meningiomas in microarray expression data (p < 0.05).69

**Notch.** The Notch pathway is another signaling cascade that has been implicated in tumorigenesis through epigenetic mechanisms.14,19,99,84 Cuevas et al. found that 45% of meningiomas of all grades have hes family bHLH transcription factor 1 (HES1, previously known as induction of hairy and enhancer of split 1) with overexpression of the Notch receptor or the Jagged ligand, suggesting that there are multiple mechanisms activating the Notch pathway as a critically important genetic alteration in meningioma pathogenesis.16 These findings corroborate prior work showing that HES1 is upregulated in meningioma compared with control dural specimens.42 Deregulated Notch signaling represents a particularly promising therapeutic target for meningioma treatment.

**MAPK.** Findings from an analysis of 10 human meningiomas indicate that MAPK is constitutively expressed in meningioma cells, and upstream signaling factors of MAPK receptors also act as a mitogen in these meningiomas. Subsequent inhibition of MAPK activity also blocked upstream mitogenic stimulation of meningioma proliferation, strongly suggesting that MAPK is an important transducer of cell growth. Furthermore, treatment with PD098059, a MAPK inhibitor, produced progressive growth inhibition, correlating with a marked reduction in MAPK phosphorylation and reduced MAPK activity without changing levels of unphosphorylated MAPK (p44) and MAPK (p42) in most of the cell cultures, indicating that MAPK inhibitors may be useful treatments for meningiomas in humans.34

The LIF locus encodes a factor that acts upstream of the MAPK cascade. Located at the 22q chromosomal region, it is an area that is noted for its importance to meningioma pathogenesis. In one study, all of the tumor specimens were hypomethylated at the LIF locus relative to constitutional DNA from the same patients. The researchers found a novel alternatively spliced LIF mRNA, which suggests that the LIF gene may be near a key tumor suppressor locus associated with meningioma development.70

**Wnt.** The Wnt signaling pathway plays a central role in meningioma tumorigenesis. Immunohistochemical studies have previously demonstrated that anaplastic meningiomas frequently lose E-cadherin expression,79 which are mediated by beta catenin,89 a key Wnt signaling gene. Beta catenin is regulated by the complex of CDK5R1 and CK5.43 When CDK5R1 is upregulated, there seems to be a reduction in beta catenin–regulated cadherin-mediated cell-cell adhesion,29 explaining previous immunohistochemical studies that have shown this very reduction. The levels of beta catenin itself seem to increase a function of this regulatory pathway to also act as a transcription factor in the nucleus for multiple genes for the Wnt signaling pathway, furthering its dysregulation.43 SFRP1, in the family of frizzled-related proteins, is able to downregulate Wnt signaling by forming an inhibitory complex with frizzled receptors. Its tumor suppressor properties have been noted in many other cancers45 and seem to play a role in meningioma recurrence when it is downregulated, as recurrent meningiomas showed significantly lower mRNA levels than in original meningiomas.69 Hypermethylation of the SFPR1 promoter has been noted to have a similar effect in gliomas, adding to the increasing evidence that epigenetic mechanisms may play a key role in the Wnt signaling pathway.

**Histone Acetylation**

Histones and their posttranslational modification system, acetylation, allow for DNA repair and the dynamic regulation of chromatin structure and function.27 N-acyetyltransferases bind various compounds to a primary aromatic amine or hydrazine structure and have been linked to various primary carcinomas of diverse epithelial tissues that include bladder cancer and colon cancer.68 Olivera et al.103 investigated the role of 10 different N-acyetyltransferase-2 (NAT2) alleles for meningioma and astrocytoma growth. Individuals carrying rapid acetylation alleles were found to be at increased risk of developing meningioma and astrocytomas compared with healthy volunteers (OR 1.79, 95% CI 1.05–3.05; p < 0.05). It has been hypothesized that the NAT2 polymorphism may have a local effect on brain tumors by activating carcinogens. Indeed, the rapid acetylator genotype has been linked to patients with glioma.87 The findings in its role in tumorigenesis have not been conclusive,72 however, and thus further studies to investigate its role in meningioma growth are necessary.

**Angiogenesis**

Epigenetic regulation of angiogenic factors may also play an important, yet undefined role in meningioma oncogenesis. The thrombospondin 1 (THBS1) gene normally inhibits angiogenesis by disrupting the motility of endothelial cells and inducing their apoptosis.29 Hypermethylation and subsequent silencing of this gene may therefore promote angiogenesis in tumor cells.52 Bello et al. found that 54% of Grade III meningiomas and 30% of all cases of meningiomas showed hypermethylation of the THBS1 gene,3 respectively, suggesting that its inactivation could lead to neovascularization of atypical meningiomas and contribute to their progression.
Other Mechanisms

Müller et al. found that chromosome 1 alkaline phosphatase (ALPL) was significantly underepressed in meningioma compared with controls and especially in Grade II/III meningiomas compared with Grade I meningioma. To our knowledge, this is the first study to explore genome-scale DNA methylation in malignant, atypical, and benign meningiomas. Additionally, unlike most previous cancer genetics studies that compare DNA methylation patterns between tumor and normal tissue, our goal was to investigate whether benign and malignant tumors differ in DNA methylation patterns and whether these differences have biological and clinical significance. Compared with the benign tumors, the atypical and malignant meningiomas demonstrate increased global DNA hypomethylation. Interestingly, while hierarchical clustering analysis readily separates malignant from atypical and benign tumors, it cannot separate atypical and benign tumors, implicating that DNA methylation patterns may serve as diagnostic biomarkers for malignancy. Additionally, we investigated the correlation between methylation and gene expression. Most genes with hypermethylated CpG islands in promoter regions are suppressed in both malignant and benign meningiomas, suggesting the switching of gene silencing machinery from polycomb repressor complex (PRC) binding to DNA methylation in malignant meningiomas. One exception is the MAL2 gene, which has high expression in levels in benign meningiomas but low expression in malignant tumors. MAL2 has hypermethylation at a CpG island in its promoter region in malignant meningiomas, therefore representing de novo gene silencing induced by DNA methylation.

In addition to methylation, alterations of histone modification and higher-order chromosomal structure represent additional epigenetic mechanisms that may regulate gene expression and function. Because of the availability of high-throughput sequencing, these studies have now become increasingly popular in molecular genetics studies. For example, the ChIP-sequencing (ChIP-seq) technique, which combines chromatin immune-precipitation with next-generation sequencing, can be used to examine histone modification patterns given antibodies for the specific modifications. Similarly, 3C, 4C, 5C, Hi-C and ChIA-PET techniques, were specifically developed for interrogating higher-order chromosomal structures between regulatory elements and target genes that may be located far away (even in different chromosomes). These techniques were becoming increasingly popular in molecular genetics studies of cancer. However, to our knowledge, these types of studies have not yet been reported on meningiomas. It is expected that identification of additional epigenetic changes, such as histone modification and higher-order chromosomal structure, may allow for a more thorough understanding of tumorigenesis and enable future individualized treatment strategies for meningiomas.

Genome-Wide Analysis of Epigenetic Alterations

In addition to candidate gene studies, genome-wide approaches have the advantage of examining all alterations in an unbiased manner. Earlier this year, we published a study comparing genome-wide methylation patterns in benign, atypical, and malignant meningiomas. To our knowledge, this is the first study to explore genome-scale DNA methylation in malignant, atypical, and benign meningiomas. Additionally, unlike most previous cancer genetics studies that compare DNA methylation patterns between tumor and normal tissue, our goal was to investigate whether benign and malignant tumors differ in DNA methylation patterns and whether these differences have biological and clinical significance. Compared with the benign tumors, the atypical and malignant meningiomas demonstrate increased global DNA hypomethylation. Interestingly, while hierarchical clustering analysis readily separates malignant from atypical and benign tumors, it cannot separate atypical and benign tumors, implicating that DNA methylation patterns may serve as diagnostic biomarkers for malignancy. Additionally, we investigated the correlation between methylation and gene expression. Most genes with hypermethylated CpG islands in promoter regions are suppressed in both malignant and benign meningiomas, suggesting the switching of gene silencing machinery from polycomb repressor complex (PRC) binding to DNA methylation in malignant meningiomas. One exception is the MAL2 gene, which has high expression in levels in benign meningiomas but low expression in malignant tumors. MAL2 has hypermethylation at a CpG island in its promoter region in malignant meningiomas, therefore representing de novo gene silencing induced by DNA methylation.

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Discussion

The literature pertaining to epigenetic regulation of meningiomas demonstrates that progressively more methylated loci, in general, are associated with increasing tumor grades. This is likely explained by widespread silencing of tumor-suppressor genes and pathways. This finding is also consistent with findings of previous studies showing that mutations within the genes themselves coincide with increasing tumor grade. In addition, there was a trend for Grade I and II meningiomas to have more hypermethylated loci than genetically mutated loci, highlighting the role that methylation plays in early meningioma tumorigenesis, vis-à-vis somatic mutations. Aberrant DNA methylation is thought to silence genes and reduce expression by blocking transcriptional machinery access to DNA. This suggests that DNA methylation is potentially acquired before phenotypical changes and that aberrant methylation may be a harbinger of malignant transformation and recurrence.

Types of Epigenetic Alteration

Epigenetic alterations in the genome often occur in the early stages of tumorigenesis through aberrant DNA methylation—in particular, CpG island hypermethylation of promoter regions that results in tumor-suppressor gene silencing and tumorigenesis. Normally, many genes have promoter regions that are densely populated with CpG dinucleotides, termed CpG islands, which are maintained in an unmethylated state. Subsequent hypermethylation of these CpG islands at the promoter region has been shown to silence and inactivate gene expression downstream. If the gene is a growth inhibitor or DNA repair enzyme, for example, then growth goes unchecked, often leading to tumorigenesis. In recent years, it has been shown that DNA methylation at non–CpG island sites may play an important role in modulating gene expression as methylation occurring at promoter CpG island sites.

Gene Methylation Detection Methods

In general, researchers use 2 approaches to detect aberrant epigenetic modifications: single-gene (targeted) approaches and genome-wide assessments. The methylation status of single candidate genes can be screened for abnormal epigenetic modifications if they have been previously implicated or prioritized as playing a role in tumorigenesis or transformation, using techniques such as polymerase chain reaction or MethyLight. The single-gene approach, however, is limited, as it only surveys a fraction of the possible modifications to that gene, while also being unable to study the role of additional genes that may contribute to oncogenesis or anaplasia. Larger-scale methods for detecting epigenetic involvement in

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meningiomas involve restriction landmark genome scanning (RLGS), a two-dimensional gel-based method for assessing methylation status in thousands of CpG islands simultaneously.22 Over the past decade, next-generation high-throughput techniques for assessing methylation status across the entire genome have become increasingly used to study meningiomas.26

**Distinction Between Genetic and Epigenetic Mutation Targets**

The most clearly established genetic mutation for meningiomas is inactivation of the neurofibromin 2 (NF2) gene located on 22q, with the associated loss of its merlin protein expression.6,7 NF2 mutations have been detected in more than 50% of all sporadic meningiomas in all pathological grades, clearly showing that mutation of NF2 plays an important early role in meningioma development.8,9 It should be surprising to note, then, that NF2 is not a frequent target of epigenetic modification.23 An analysis of 40 CpG sites within 750 bp of the promoter regions of NF2 showed no methylation of these regions,30 and the NF2 gene itself was methylated in only 1 of 21 tumors in a separate study.31 Other mechanisms involved in loss of function of NF2, such as chromosomal deletions and primary mutations, are more likely to affect NF2 expression than are epigenetic alterations.

Other important genes that have been previously noted as significant in meningioma development but not found as targets of epigenetic target modification include ADAM23,33 MGMT, CDKN2A, CDKN2B, CDKN2C, CDKN2D, and RB1 (RB).32 Esteller et al. previously reported promoter hypermethylation of MGMT in various neoplasms throughout the body,23 with rates ranging from 21% in lung cancers to 34% of all brain tumors. In meningiomas, however, only 6% of MGMT promoters were hypermethylated in 1 group of 48 meningioma cases49 and a separate group of 36 meningioma cases.7 MGMT promoter hypermethylation was also not correlated with grade of the neoplasm.52,53

Our results show that epigenetic and transcriptomic alterations in various tumor suppressors are likely to play an active role in meningioma development. We noted, however, that this mechanism of tumor progression did not seem to develop for RB1. CDKN2A (p16[INK4A]) and CDKN2B (p15[INK4B]) are genes on the 9p21 locus that regulate the transition from G1/S-phase checkpoint and interact with and RB1.3 Loss of CDKN2A, CDKN2B, or RB1 activity through mutation or methylation would, in theory, disrupt the growth-regulatory pathway and allow unrestrained cell proliferation.4 However, the low frequency of methylation of CDKN2B (p15[INK4B]),3,8 CDKN2A (p16[INK4A])3,4,6,8 along with RB1 genes suggest that methylation of these growth control genes does not play a major role in the development of a majority of brain tumors.57 CDKN2C (p18[INK4C]) CDK inhibitors do not seem to have any role in meningioma progression as well, even though they serve a similar cell cycle regulatory function.8 A subset of 67 meningiomas showed only a single tumor with any alterations at the CDKN2C locus. This lack of epigenetic modification applied to CDKN2D at 19q13.37 as well.

Accordingly, Boström et al. proposed that regulation of CDKN transcription in meningiomas might be influenced by as yet unknown mechanisms in addition to hypermethylation. This may explain why genetic mechanisms but not epigenetic mechanisms are crucial in meningioma progression for the RB pathway.8

**Future Directions**

The divergence between genetic and epigenetic manifestations in meningioma progression clearly illustrates the challenge that expression profiles attempt to portray in a phenotypically heterogenous neoplasm. It will be important to investigate expression profiles of more genes in a larger series of tumors with clinical follow-up data to ensure that genes are correlated to grade and clinical outcome as well.

**Conclusions**

We present here a comprehensive review of the epigenetic alterations of genes and corresponding changes in gene expression that may lead to meningioma development, progression, and recurrence. Additional analysis and discovery of other genetic and epigenetic mechanisms involved in meningioma pathogenesis is needed to fully understand this tumor’s biology. Future research into potential primary or adjuvant treatments with demethylating and deacetylating agents will be important in elucidating their potential role in the management of meningiomas.

**Disclosure**

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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**References**


84. Strizzli L, Hardy KM, Seftor EA, Costa FF, Kirschmann DA,

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