The molecular genetics and tumor pathogenesis of meningiomas and the future directions of meningioma treatments

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Meningiomas are mostly benign, slow-growing tumors of the CNS that originate from arachnoidal cap cells. While monosomy 22 is the most frequent genetic abnormality found in meningiomas, a multitude of other aberrant chromosomal alterations, signaling pathways, and growth factors have been implicated in its pathogenesis. Losses on 22q12.2, a region encoding the tumor suppressor gene *merlin*, represent the most common genetic alterations in early meningioma formation. Malignant meningioma progression, however, is associated with more complex karyotypes and greater genetic instability. Cytogenetic studies of atypical and anaplastic meningiomas revealed gains and losses on chromosomes 9, 10, 14, and 18, with amplifications on chromosome 17. However, the specific gene targets in a majority of these chromosomal abnormalities remain elusive.

Studies have also implicated a myriad of aberrant signaling pathways involved with meningioma tumorigenesis, including those involved with proliferation, angiogenesis, and autocrine loops. Understanding these disrupted pathways will aid in deciphering the relationship between various genetic changes and their downstream effects on meningioma pathogenesis.

Despite advancements in our understanding of meningioma pathogenesis, the conventional treatments, including surgery, radiotherapy, and stereotactic radiosurgery, have remained largely stagnant. Surgery and radiation therapy are curative in the majority of lesions, yet treatment remains challenging for meningiomas that are recurrent, aggressive, or refractory to conventional treatments. Future therapies will include combinations of targeted molecular agents as a result of continued progress in the understanding of genetic and biological changes associated with meningiomas. (DOI: 10.3171/2011.2.FOCUS1116)

KEY WORDS • meningioma • signaling pathway • pathogenesis • molecular genetics

M ENINGIOMAS are the second most common adult neoplasm of the CNS, and they are mostly benign, slow-growing tumors originating from the arachnoidal cap cells.[97] The annual incidence of meningiomas is 2.3 per 100,000, increases with age, and peaks in the 7th decade of life.[97,98] Overall incidence is greater in females with a 2:1 ratio, yet higher grade meningiomas are more frequent in males.[36,67] Deletions of the *neurofibromatosis Type 2 (NF2)* gene, ionizing radiation, and head trauma are associated with an increased risk, while the role of sex hormones in meningioma development is still uncertain.[5,30] Meningiomas are categorized according to the WHO classification of tumors as benign (Grade I), atypical (Grade II), and anaplastic (Grade III), comprising 80%, 15%–20%, and 1%–3% of all meningiomas, respectively.[97] Benign meningiomas are slow growing and have a 5-year recurrence rate of 5% following gross-total resection.[97] However, management of the more aggressive and higher grade tumors is difficult. Atypical meningiomas have 5-year recurrence rate of 40%, and anaplastic meningiomas have recurrence rates of up to 80%.[97] Stafford et al. reported 5-year survival rates of 76% and 0%, respectively, for patients with atypical and anaplastic meningiomas who underwent multimodal treatment.[112]

Most meningiomas have a good prognosis, and often surgery and adjuvant radiotherapy are curative. However, gross-total resection is not always achievable because the meningioma may be enveloping sensitive neural or vascular structures, and radiation therapy is limited by neuro-

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Abbreviations used in this paper: IGF = insulin-like growth factor; LOH = loss of heterozygosity; MEF = mouse embryonic fibroblast; NF2 = neurofibromatosis Type 2; PFS = progression-free survival.

toxicity and tumor size. To date, chemotherapy regimens have been minimally effective in treating meningiomas. Thus, treatment of the remaining subset of aggressive, inoperable, or refractory meningiomas remains challenging. In this review, we describe the current understanding of the molecular pathogenesis and future therapies of meningiomas.

Meningioma Development

Chromosome 22 and the NF2 Gene

Meningiomas are the first solid neoplasms to be identified with a characteristic cytogenetic alteration.[94,97] Monosomy 22 is the most frequent genetic abnormality found in meningiomas. This association between the long arm of chromosome 22 (22q) and meningiomas was first studied in patients with NF2. Patients with NF2, a dominantly inherited disorder, commonly present with bilateral vestibular schwannomas, multiple meningiomas, and other nervous system tumors.[59] Roughly 50% of meningiomas have allelic losses in 22q12.2,[59] a region encoding the NF2 gene. Nearly all NF2-associated meningiomas, and 54%–78% of sporadic meningiomas, have deletions in this region.[94]

The NF2 gene encodes the tumor suppressor merlin. Amino acid analysis revealed merlin's similarity to the 4.1 family of proteins (specifically, 3 ERM proteins: ezrin, radixin, and moesin), which link integral membrane proteins to the cortical cytoskeleton.[59] A number of studies suggest that merlin has a critical role in controlling cell growth and motility. Mouse embryonic fibroblasts (MEFs) with merlin defects are associated with abnormal cell growth and motility through the destabilization of adherens junctions.[51,106] Mice heterozygous for NF2 mutations develop a number of motile and metastatic tumors.[73] Both in vivo and in vitro reexpression of wild type merlin leads to reduced tumor growth and decreased cell motility.[24,26,34,80,107] Furthermore, MEFs with inactive NF2 exhibit irregular contact inhibition-dependent growth arrest, suggesting that merlin has a role in curbing growth rate in high cell density environments.[51,90]

Given the structural similarities between merlin and the ERM proteins, merlin has been implicated in regulating various membrane- and cytoskeleton-based cellular processes, including cell migration, cell-cell contact, and cell proliferation.[37,72] Merlin is localized to the cell membrane and consists of 3 major domains, including an amino-terminal protein 4.1 cell surface glycoprotein-binding domain (FERM domain).[97] While the exact mechanisms by which merlin exerts its growth-regulatory functions in human arachnoidal cells is still unclear, merlin's FERM domain allows it to interact with a number of important cytoskeleton-regulating proteins including paxillin, actin, syntenin, and other ERM proteins.[38,39,97,104,127] Merlin also binds important cell surface signaling proteins, including β1-integrin and CD44.[21,80,90] CD44 is a cell surface receptor involved with mediating cell-cell interaction, cell adhesion, and migration.[80] Merlin signals via extracellular growth pathways by forming internal protein complexes through its interaction with the cytoplasmic tail of CD44

and associations with ERM proteins.[80,117] Morrison et al.[80] proposed that merlin and CD44 form a molecular switch between growth permissive and growth inhibiting conformations, where external cues for growth inhibition (for example, increased cell density) lead to consequent merlin activation.

Most recently, merlin was identified as a novel negative regulator of the mammalian target of rapamycin complex 1 (mTORC1), a critical modulator of cell growth and proliferation. The mTORC1, a serine/threonine kinase sensitive to rapamycin inhibition, is dysregulated in hamartoma syndromes and various cancers.[22,37] The mTORC1 pathway is constitutively active in NF2 meningioma patients and NF2 knockout MEFs.[37] Activation of mTORC1 leads to phosphorylation of eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) and S6 kinase 1. Consequently, this results in increased protein translation and ribosome biogenesis.[37] Although the exact mechanism is still unclear, merlin inhibits mTORC1 through a novel pathway, independent from the previously established activators of the mTORC1 pathway, which include phosphoinositide 3-kinase–Akt (PI3K) and mitogen-activated protein kinase/extracellular signal-regulated kinase. Thus, inhibition of the mTORC1 pathway, either through rapamycin or disruption of the PI3K pathway, presents a promising route for targeted therapeutics.[37]

Several studies have linked merlin to the Rho/GTPase and Rac/PAK signaling pathways, which are critical regulators of cytoskeleton organization, intracellular proliferation, and transcription signaling. Active Rac and Cdc42 lead to PAK-mediated merlin inactivation via phosphorylation at serine 518, and merlin expression inhibits the Rac/PAK pathways. Merlin also regulates transcription of cyclin D1, a proto-oncogene that encodes a regulatory subunit of cell cycle regulating cyclin–dependent kinase holoenzymes.[126] Merlin inhibits PAK activity and consequently decreases PAK1-dependent upregulation of cyclin D1 during cell cycle progression. Adenovirus-mediated merlin expression in NF2-inactivated mesothelioma cells inhibits cell proliferation, decreases cyclin D1 expression, inhibits CDK3 activity, and dephosphorylates pRB.[126] Furthermore, this cell cycle arrest at the G_1 phase can be partially overcome by ectopic expression of cyclin D1, suggesting that cyclin D1 is an important mediator of merlin's growth regulation.

The frequency of NF2 inactivation varies between WHO Grade I subtypes. While 70%–80% of fibroblastic and transitional meningiomas have NF2 mutations,[93] only 25% of meningothelial meningiomas and less than 1% of secretory meningiomas possess NF2 mutations.[59] These findings suggest that there may be cytogenetic differences in tumorigenesis between benign subtypes. For higher grade meningiomas, the frequency of NF2 mutations is 70% for both atypical and anaplastic tumors, which is roughly the same as the frequency for benign fibroblastic and transitional meningiomas.[93] This suggests that while NF2 is important for tumor formation, it most likely is not critical in malignant meningioma progression.[59]

Although NF2 losses are frequently present in meningiomas, studies have failed to find chromosome 22 or NF2 abnormalities in 40% of sporadic lesions. As such,

it has been inferred that other anomalous pathways may be responsible for tumorigenesis within this population.[59]

DAL-1

Merlin's role in meningioma tumorigenesis led to several studies of the structurally homologous and functionally similar protein 4.1 family. A number of studies have implicated protein 4.1B as a potential tumor suppressor in meningiomas.[20] The *EPB41L3* or *DAL1* gene is located at 18p11.3 and encodes protein 4.1B, a regulator of proliferation and apoptosis.[52] Like merlin, protein 4.1B contains 3 functional regions, including a highly conserved amino-terminal FERM domain (protein 4.1 and ERM binding), a spectrin-actin binding domain, and a carboxy-terminal domain. Each domain is separated by a unique interspersed region, termed U1, U2, and U3. Differentially expressed in adenocarcinoma of the lung-1 (DAL-1) is a smaller protein fragment of protein 4.1B that retains a tumor suppressive property identical to that of protein 4.1B.[20]

Protein 4.1B loss has been demonstrated in meningiomas at the DNA level through in situ hybridization, at the RNA level through reverse transcription–polymerase chain reaction, and at the protein level through Western blot and immunohistochemical analyses.[90] These studies reported that reexpression of protein 4.1B/DAL-1 suppressed meningioma cell growth. The U2 region is the critical tumor suppressive domain of protein 4.1B/DAL-1, and deletion of the U2 domain weakens its suppression of meningioma cell growth. While the exact mechanisms are still unclear, a study by Gerber et al.[20] provided the first molecular insights underlying protein 4.1B/DAL-1 tumor suppression. The study revealed that protein 4.1B/DAL-1 activated the JNK pathway in a U2 domain–dependent fashion. However, no proteins that specifically bind this important U2 domain have yet been identified. Protein 4.1B growth regulation in meningiomas relies on JNK-mediated activation of the Src, Rac1, and mixed-lineage kinase 3 (MLK3) signaling cascades. JNK activation decreased cell growth through reduced expression of cyclin A, hyperphosphorylation of the retinoblastoma protein (Rb), and G_0–G_1 cell cycle growth arrest.[20]

Protein 4.1B has also been found to associate with the 14-3-3 family of proteins near the plasma membrane. These 14-3-3 proteins are protein 4.1B/DAL-1 specific and do not bind with other members of the protein 4.1 family.[90,130] Past studies have implicated 14-3-3 proteins in regulating signal transduction and apoptosis.[130] Further work is necessary to elucidate the functional significance of 4.1B/DAL-1 and 14-3-3 binding in growth regulation. Additionally, TSLC1 is another protein that interacts with protein 4.1B. Reduced expression levels of TSLC1 are correlated with higher grade meningiomas and worse prognosis, while reexpression of TSLC1 in meningioma cells slows growth.[70]

There have been conflicting reports regarding the role of protein 4.1B/DAL-1 in meningioma tumorigenesis. Gutmann et al.[25] initially reported loss of heterozygosity of *4.1B/DAL-1* in 60% of sporadic meningiomas, and attributed the loss of *DAL-1* as an early event in meningioma pathogenesis. A later study of 63 sporadic meningiomas found a much lower frequency of *DAL-1* inactivation than previously reported, and suggested that the locus had a role in meningioma progression rather than initiation.[84] A study of 83 meningiomas found a very low mutation frequency of *4.1B/DAL-1*, and suggested that epigenetic changes may be responsible for *4.1B/DAL-1* silencing in meningiomas.[33,47,68,90] Furthermore, *4.1B/DAL-1* null mice do not have an increased propensity for developing cancer.[59]
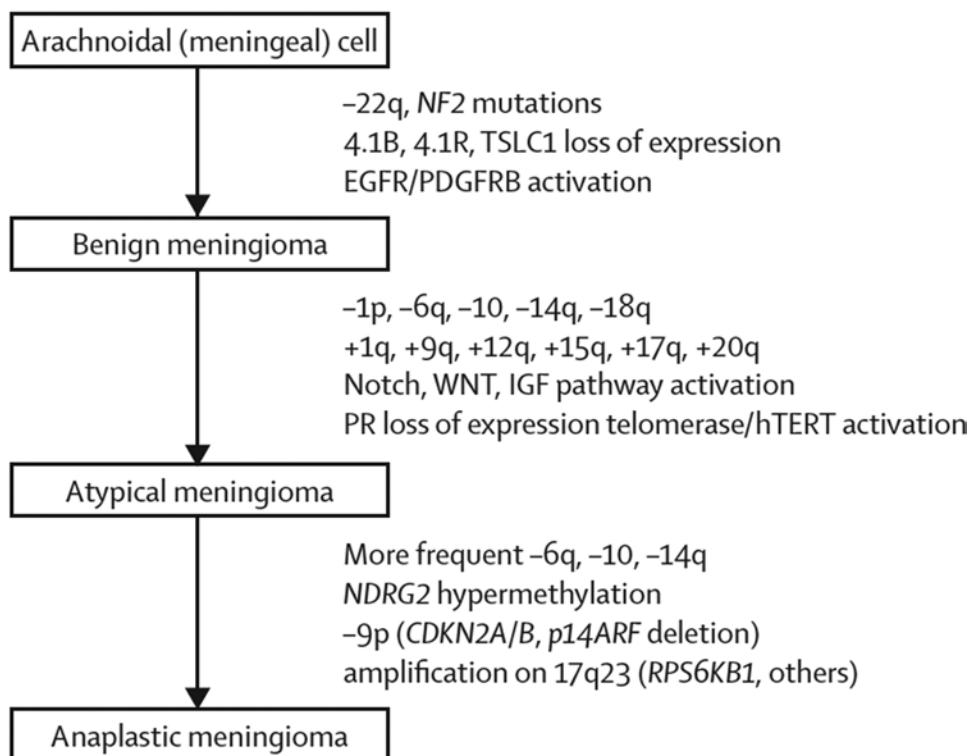
Meningioma Progression

While genetic analysis of losses on chromosome 22 has provided a foundation for understanding meningioma pathogenesis, the previously discussed genetic alterations constitute early events in meningioma development.[59,70,97] Benign meningiomas rarely have chromosomal aberrations beyond chromosome 22 losses. More complex karyotypes are associated with more aggressive meningioma behavior.[59] Malignant progression, thought to follow the theory of clonal evolution, is associated with a stepwise cumulative acquisition of chromosomal gains and losses, leading to more aggressive subclones with greater growth advantage.[63,123,132] Atypical and anaplastic meningiomas exhibit much more complex genetic changes than their benign counterparts, with losses on 1p, 10q, 14q, and less frequent losses on 6q and 18q. Higher grades are also associated with chromosomal gains on 1q, 9q, 12q, 15q, 17q, and 20q.[59,70] In addition to these genetic changes, anaplastic meningiomas exhibit more frequent losses on 6q, 10q, 14q, and 9p, with amplification on 17q23 (Fig. 1).[12,54,86,97,123] Epigenetic alterations, including increased CpG island hypermethylation, have also been associated with malignant meningioma progression.

Most of the data from studies aiming to characterize the stepwise progression of meningioma development have been based on cytogenetic analyses of different meningioma grades in various patients. In a cytogenetic analysis of one group of 11 meningioma patients with tumors that exhibited clear progression from benign to higher grades, the authors found a complex karyotype present in the lower grade tumors prior to progression.[1] Contrary to the model of clonal evolution, these findings suggest that this cohort of meningiomas was destined to be malignant.

Candidate Genes Identified Through Chromosomal Losses

Chromosome 1. Chromosome 1p deletions comprise the second most common chromosomal abnormality in meningiomas and are more frequent in higher grade tumors. 1p losses are found in 13%–26% of Grade I, 40%–76% of Grade II, and 70%–100% of Grade III meningiomas.[52] 1p deletions have also been associated with malignant progression in recurrent meningiomas, suggesting that the loss of 1p is associated with meningioma progression rather than initiation. Loss of 1p is also associated with a 30% recurrence rate, whereas only 4.3% of meningiomas recur when 1p is intact.[94] While a number of candidate targets have been studied on 1p, including *CDKN2C, RAD54 L, EPB41, GADD45A, RAD54 L,* and *ALPL,* no promising tumor suppressor has yet been found.

Studies of *CDKN2C,* a cell cycle control gene encoding p18[INK4C] located at 1p32, found one point mutation and

Fig. 1. The genetic alterations underlying meningioma formation and progression. Meningiomas are thought to arise from arachnoidal cells. Losses of 22q and other family 4.1 proteins represent early events in meningioma formation. Higher grade meningiomas are associated with more complex karyotypes, and progression is characterized by the accumulation of chromosomal losses and gains. Loss of 9p is characteristic of anaplastic meningiomas. PR = progesterone receptor. Reprinted from **The Lancet Neurology**, volume 5, Riemenschneider MJ, Perry A, Reifenberger G: Histological classification and molecular genetics of meningiomas, pp 1045–1054, copyright 2006, with permission from Elsevier.

one homozygous deletion at the *INK4C* locus. Inactivating methylation and loss of cytoplasmic p18 was not found, demonstrating that p18 is rarely mutated in atypical and anaplastic meningiomas and is unlikely to be important in meningioma pathogenesis.[59] A study of 29 meningiomas found no mutations in *RAD54 L*, located on 1p32.[52,74] Loss of heterozygosity and expression analysis failed to find expression losses of *EPB41* and *GADD45A*, located on 1p36.2-p34 and 1p31.2-p31.1 respectively.[59]

ALPL, a gene encoding an alkaline phosphatase, is located on 1p36.1-p34.[52,59,97] *ALPL* has drawn interest as a potential tumor suppressor because 1p loss in meningiomas is strongly associated with loss of alkaline phosphatase activity. However, mutational analysis of *ALPL* is still needed.[52]

Liu et al.[59] found that while many of the candidate genes on 1p lack regular genetic losses, epigenetic changes may have an important role in malignant meningioma progression. Their study found transcriptional silencing via abnormal hypermethylation of various promoter-associated CpG islands of cancer-related genetic regions in atypical and anaplastic meningiomas. For example, *TP73* on 1p26.32 has been examined as a candidate gene. While studies have failed to find significant and consistent *TP73* mutations in meningiomas, one methylation status study found *TP73* methylation-mediated inactivation in 10 of 30 meningiomas with 1p losses, and 3 of 30 meningiomas with intact 1p.[52,60,83] This suggests that assessment of

the methylation status of other candidate genes may be a promising avenue of future study.

Chromosome 14. Similar to 1p losses, deletions on chromosome 14 are important in meningioma progression.[52] Loss of 1p and 14q are frequent in anaplastic meningiomas and are associated with a worse prognosis.[40,61,65] After losses in chromosome 1 and 2, deletions in chromosome 14 represent the third most common chromosomal abnormality and have been found in up to 31% of Grade I, 40%–70% of Grade II, and up to 100% of Grade III meningiomas.[13,52,58,75,109,116,123] Studies have also found losses of 14p to be a prognostic indicator of tumor recurrence.[13,52,66]

Genomic analysis conducted by Lusis and Gutmann[65] identified *NDRG2* as a potential tumor suppressor on 14q. The authors found that *NDRG2* is frequently inactivated in both anaplastic meningiomas and a subset of lower grade yet clinically aggressive atypical meningiomas. Reduction of *NDRG2* expression was associated with promoter hypermethylation in 40% of anaplastic and atypical meningiomas.[59] Additionally, *NDRG2* mRNA is down-regulated in recurrent meningiomas of all grades relative to primary benign meningiomas.[110] Although the mechanism is unknown, NDRG2 is involved with regulating cell growth, differentiation, and apoptosis.[16,62,65,69,111]

Recently, Zhang et al.[134] identified *maternally expressed gene 3 (MEG3)* as a candidate tumor suppressor located at 14q32. Greater loss of *MEG3* expression and al-

lelic loss are associated with higher tumor grades. While MEG3, a noncoding RNA with antiproliferative functions, is robustly expressed in normal arachnoidal cells, it is absent in the IOMM-Lee and CH157-MN meningioma cell lines. Functional studies suggest that MEG3 mediates its tumor suppressive properties by suppressing DNA synthesis and inhibiting colony formation in the meningioma cell lines. Additionally, MEG3 was found to transactivate p53 (TP53), another tumor suppressor involved in an often dysregulated pathway in anaplastic meningiomas.[70] Of note, while mutations of *TP53* (17q) are common in many other cancers, direct alterations in *TP53* are rare in meningiomas;[9,41,52,70,85,121] instead, regulators of the pathway are often mutated.[70,134]

Chromosome 9. Gains and losses on chromosome 9 in meningiomas have led to identification of a number of candidate genes. Losses at 9p are found in 5% of Grade I, 18% of Grade II, and 38% of Grade III meningiomas,[9,52] and are strongly associated with anaplastic, rather than benign or atypical meningiomas.[52,123] While the actual target genes and tumorigenic mechanisms of many chromosomal losses in meningiomas are still unclear, 9p alterations are associated with specific losses of *CDKN2A/p16^INKa* (encoding p16), *p14^ARF* (encoding p14), and *CDKN2B/p15^ARF* (encoding p15).[70,88] All 3 tumor suppressors are located on 9p21. p14 is a tumor suppressor involved with regulating cell apoptosis through modulation of the p53 pathway, and p16 and p15 control cell cycle progression through the G_1/S-phase checkpoint (Fig. 2).[59]

Loss of *CDKN2A*, *p14^ARF*, and *CDKN2B* have been reported in 0% of Grade I, 3% of Grade II, and 38% of Grade III meningiomas.[9,52] Seventy percent of anaplastic meningiomas with 9p21 losses have a considerably shorter survival than 9p21 intact anaplastic meningiomas.[9,28,97]

Similarly, Grade III meningiomas with intact *CDKN2A* have better outcomes than those with *CDKN2A* loss.[52] These findings suggest that loss of cell cycle regulation at the G_1/S-phase checkpoint is associated with clinically aggressive tumors and is a critical component of malignant progression.[59]

Other Chromosomal Alterations. Deletions on chromosome 10 are associated with meningioma progression.[52] Losses on chromosome 10 are found in 5%–12% of Grade I, 29%–40% of Grade II, and 40%–58% of Grade III meningiomas;[52,91,109,120,123] however, some studies have suggested that the true frequencies are higher.[52,77,78] A number of candidate genes have been identified at chromosomal region 10q23-q25, namely *PTEN*, *MXI1*, and *DMBT1*. *PTEN* alterations have been found in Cowden syndrome, but rarely in meningiomas. Studies have also failed to identify mutations of *MXI1* or *DMBT1* in meningiomas.[59]

Similarly, the high frequency of chromosome 17 amplification in malignant meningiomas (42%) compared with lower-grade meningiomas (almost 0%) has led to studies of ribosomal protein S6 kinase (*RPS6K*), a proto-oncogene located at 17q23.[52,123] However, *RPS6K* amplifications only occur in a small subset of higher grade meningiomas, despite robust amplification of adjacent loci.[12,52] While *RPS6K* amplification may be important in the progression of a subset of lesions, *RPS6K* does not appear to be the main target of amplification in meningiomas.

Losses in chromosome 18 are frequent in atypical and anaplastic meningiomas, but rare in benign meningiomas. Büschges et al.[11] examined *MADH2*, *MADH4*, *APM-1*, and *DCC*, tumor suppressor genes on chromosome 18q21. However, mutational and LOH analysis of the four genes found only one missense mutation in *APM-1*, suggesting
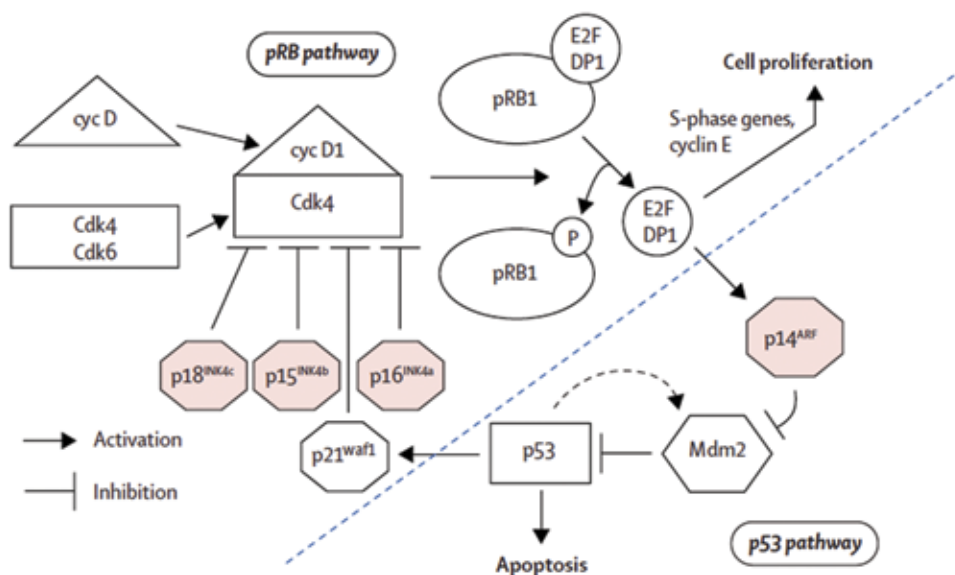
Fig. 2. Cell cycle dysregulation through interrelated p53/pRB pathways. Anaplastic meningiomas characteristically exhibit homozygous deletions and mutations in *p16^INK4a*, *p15^INK4b*, and *p14^ARF*. *p16^INK4a* and *p15^INK4b* prevent S-phase entry by inhibiting the Cdk4/cyclin D complex. p14^ARF negatively regulates MDM2 and removes MDM2-mediated p53 inhibition and degradation. The shaded proteins are affected in meningioma progression. Reprinted from **The Lancet Neurology**, volume 5, Riemenschneider MJ, Perry A, Reifenberger G: Histological classification and molecular genetics of meningiomas, pp 1045–1054, copyright 2006, with permission from Elsevier.

that *MADH2, MADH4, APM-1,* and *DCC* are not the target inactivated genes in 18q losses in meningioma progression.

Telomerase/hTERT

Telomeres comprise repeat DNA sequences at the ends of chromosomes and function to prevent chromosomal deterioration. Telomeres shorten during successive DNA replication and mitoses, eventually limiting cell division through signaling senescence. Telomerase, a reverse transcriptase that rebuilds the lost telomere repeat sequences, is often reactivated in malignant cancers to sustain chromosomal integrity during aggressive growth. Telomerase is made of the telomerase RNA subunit (hTR) and the reverse transcriptase subunit, hTERT. Expression of *hTERT* mRNA, rather than *hTR* in meningiomas is best correlated with telomerase activity.[59]

Telomerase activation is rare in benign meningiomas, found in only 3%–21% of Grade I meningiomas. However, 58%–92% of atypical and 100% of anaplastic meningiomas demonstrate telomerase activity.[19,52,56,108] In addition to higher grade tumors, telomerase activity is also associated with a higher rate of recurrence and greater malignancy in meningiomas, and may serve as a potential prognostic marker.[52,108]

Signaling Pathways

Considerable progress in deciphering the biological mechanisms underlying meningioma development, growth, and malignant progression has been made through cytogenetic studies analyzing chromosomal abnormalities and identifying specific genes involved with tumorigenesis. However, the majority of the actual targets of these chromosomal alterations remain unknown. Understanding the disrupted signaling pathways regulating tumorigenic processes such as growth, angiogenesis, and apoptosis will not only help bridge the link between the various genetic changes and consequent effects on cellular processes involved in meningioma pathogenesis, but will also provide targets for novel therapeutics.

Cell Cycle Dysregulation—the pRB/p53 Pathways. A number of studies have highlighted the importance of aberrant cell cycle pathways in meningioma progression and malignancy.[59] Anaplastic meningiomas characteristically exhibit homozygous deletions and mutations in $p16^{INK4a}$, $p15^{INK4b}$, and $p14^{ARF}$, which are all important modulators in the retinoblastoma protein (pRB)-dependent and p53-dependent pathways.[9,97] pRB inhibits cell cycle progression at the G_1/S-phase checkpoint by binding and inhibiting E2F transcription factors, a dimer comprising the E2F protein and DP protein (E2F-DP).

Cell cycle progression is normally regulated by cyclin D expression. Under mitogenic signals, cyclin D levels increase, and cyclin D binds to either Cdk4 or Cdk6, which leads to the phosphorylation of pRB. When pRB is phosphorylated, it loses its function, releases active E2F-DP, and allows the transcription of genes critical for the S-phase (Fig. 2). $p16^{INK4a}$ and $p15^{INK4b}$, which normally prevent S-phase entry by inhibiting the Cdk4/cyclin D complex, are often mutated in higher-grade meningiomas.[97]

p53 is another important tumor suppressor that can induce cell cycle arrest, DNA repair, and apoptosis. The p53 pathway serves as an important feedback inhibitor of the pRB pathway.[4,97] The mechanism of feedback inhibition occurs via p14ARF. Phosphorylation of pRB and consequent release of E2F-DP not only promote S-phase entry but also leads to increased transcription of *p14ARF*. *p14ARF* promotes p53 activity through negative regulation of the proto-oncogene murine double minute 2 protein (MDM2), which normally binds to p53 and assists in p53 degradation (Fig. 2). However, this regulatory checkpoint is broken in higher-grade meningiomas because of frequent homozygous deletions of *p14ARF*.[4,9,28]

The Hedgehog Pathway. The hedgehog (Hh) signaling pathway is a highly conserved, critical regulator of development during embryogenesis and homeostatic processes.[10,56,118,122] Genetic mutations in the Hh membrane receptor, patched (PTCH), and other downstream proteins have been found in a number of neoplasms including basal cell carcinomas and medulloblastomas.[3,31,44,56] When Hh binds PTCH, PTCH-mediated tonic inhibition of the transmembrane protein Smoothened (SMO) is suppressed.[10,56,118,122] SMO activation initiates a signaling cascade that results in the activation of GLI transcription factors. These include growth activators GLI1 and GLI2, and growth repressor, GLI3, with subsequent transcription of genes implicated in cell growth, proliferation, angiogenesis, matrix remodeling, and stem cell homeostasis.[46,50,56,99] Thus, aberrant Hh pathway activation can have a critical role in tumorigenesis. Recently, Laurendeau et al.[56] examined the mRNA expression patterns of 32 Hh pathway–related genes in 36 meningiomas. The authors found amplifications of 16 genes involved with Hh pathway activation and cell growth, and decreased expression of 7 genes involved with Hh pathway inhibition across all tumor grades. Furthermore, the study identified a number of Hh target genes with significantly increased expression in high-grade meningiomas, in contrast to benign meningiomas. Such targets include *IGF2* and *SPP1* and may serve as potential prognostic markers for malignancy and tumor aggressiveness.[56]

The Notch Pathway. The Notch signaling pathway, involved in intracellular communication, is mediated through the transmembrane proteins Notch1–4. Binding of ligands, which consists of other transmembrane proteins, leads to the proteolytic cleavage of the intracellular domain of Notch.[2,49] This intracellular domain translocates to the nucleus and initiates expression of the Hairy/Enhancer of Split (HES) family of transcriptional regulators. HES then functions as a Notch pathway effector.[2,35] Notch signaling function varies according to cell type and mediates a myriad of cellular processes during embryonic development and later in adult life.[2,8] The role of aberrant Notch signaling in tumor genesis is equally complex and varies from cancer to cancer.[2,14,87,113,131,133]

Gene expression analysis suggests that deregulation of the Notch pathway is a critical component in meningioma development. HES1 expression is increased in all meningioma grades and correlates with increased expression of Notch1, Notch2, and Jagged ligand. TLE2 and TLE3, members of the Groucho/transducin-like enhancer of the Split family of corepressors that modulate HES1 activity,

were specifically upregulated in malignant meningiomas, suggesting that TLE3/HES1 may be associated with more aggressive meningiomas. One study identified tetraploidy and chromosomal instability as possible consequences of notch deregulation in meningiomas.[2] Further studies are necessary to elucidate the mechanism by which abnormal notch activation induces tetraploidy and promotes meningioma pathogenesis.

The PI3K/Akt Pathway and MAPK Pathway. The phosphatidylinositol 3-kinase (PI3K)/Akt pathway and the mitogen-activated protein kinase (MAPK) pathway are also aberrantly activated in meningiomas. Both pathways are involved in numerous cellular processes including differentiation, growth, and apoptosis. Many meningioma growth factors mediate their activity through these signaling pathways. Activation of PI3K leads to Akt phosphorylation and activation of p70^{S6K} via mammalian target of rapamycin (mTOR), a regulator of many cancer-associated cell processes. Administration of a PI3K inhibitor blocks platelet-derived growth factor stimulation and decreases Akt and p70^{S6K} phosphorylation.[124] Mawrin et al.[71] found high levels of phosphorylated Akt in anaplastic and atypical meningiomas, but not in benign meningiomas. Additionally, wortmannin, an Akt inhibitor, reduced malignant meningioma growth and survival, but did not induce apoptosis.

Other studies have found constitutive activation of the MAPK pathway in benign meningiomas.[42,43,71] Upstream activation of this pathway leads to the activation of Ras, with activation and phosphorylation of both Raf and MAPK.[58,71,105] In the same study, Mawrin et al. found that PD98059, a MAPK inhibitor, slowed cell growth and induced apoptosis in malignant meningiomas. However, reduced amounts of activated MAPK were correlated with increased recurrence of meningiomas suggesting that other pathways are involved with meningioma growth. PD98059 also prevents platelet-derived growth factor–mediated meningioma growth.[71] Overall, in malignant meningiomas, PI3K/Akt activation is associated with aggressive growth, and reduced MAPK activation promotes apoptosis at the cost of increased recurrence rates.

WNT/Beta-Catenin. Recent studies have also expanded on the role of the WNT/β-catenin pathway activation in meningiomas, which was first identified through gene expression studies.[90,103] A later study of the *adenomatous polyposis coli (APC)* gene, a tumor suppressor involved in the WNT pathway, found LOH in 15 of 33 meningioma samples; however, only benign meningiomas exhibited this APC loss.[90,115] E-cadherin, another tumor suppressor and modulator of the WNT pathway, is lost in one-third of meningiomas.[90] In aggressive meningiomas, this loss is often associated with increased translocation of β-catenin to the nucleus.[29,76,90] Additionally, E-cadherin expression is associated with a decrease in both invasiveness and recurrence.[29,90]

Growth Factors and Autocrine Loops

A number of autocrine loops and growth factors have been investigated in meningiomas. The activity of various growth hormones is often mediated through the Ras/Raf

MAPK and PI3K/Akt signal transduction pathways. In tumors, alterations in these pathways can contribute to abnormal activation of cellular processes including growth, motility, and angiogenesis without external stimuli.

Platelet-derived growth factor BB (PDGF-BB) and its receptor PDGFR-β are overexpressed in meningiomas.[42,90,129] Expression of PDGF and its receptor are greater in higher grade meningiomas than in benign meningiomas.[124] A recent study suggested that PDGF-BB can mediate its growth regulation through activation of the PI3K/Akt and MAPK pathways.[93] Addition of PDGF-BB to meningioma cells in culture results in increased growth and activation of MAP kinases and c-fos, while anti–PDGF-BB agents can inhibit meningioma cell growth.

A study of 15 meningiomas found expression of epidermal growth factor receptor (EGFR) in all cases, while normal human meningeal tissue did not have detectable EGFR.[90,114] Expression of the EGFR ligands, transforming growth factor alpha (TGF-α), and EGF in meningiomas also contribute to the activation of EGFR through an autocrine loop.[15,27,90] Increased TGFα expression is associated with more aggressive meningioma growth.[124]

Meningiomas also express transforming growth factor-β (TGF-β) and Type I and II TGFβ receptors.[124] Transforming growth factor–β has been demonstrated as a Smad 2/3-mediated inhibitor of meningioma growth. Stromal cell derived factor 1 (SDF1) and its receptor CXCR have been found in human meningiomas, and human SDF1α stimulates growth in meningioma cell cultures. Bone morphogenetic proteins (BMPs) and their receptors (BMPR) have been found to form another autocrine loop involved in proliferation in meningiomas.[93] Other growth factors and receptors investigated in meningiomas include IGF, fibroblast growth factor, placental growth factor, HER2, and somatostatin.[59,64,67]

Angiogenic Pathways

Meningiomas are highly variable with respect to peritumoral brain edema and vascularity.[52] While meningiomas are mainly supplied by meningeal vessels through the external carotid artery, more than half of meningiomas receive additional supply from cerebral-pial vessels.[6,52] Vascular endothelial growth factor A (VEGF-A) and the receptor VEGFR-1 regulate development of neovascularity and peritumoral edema in brain tumors. Studies have found that in meningiomas, 84% expressed VEGF and 67% expressed VEGFR.[93]

While two small studies have suggested that VEGF-A mRNA expression may correlate with meningioma vascularity,[52,101] a larger study by Lamszus et al.[55] of 69 meningiomas did not find such a link between VEGF-A expression and either microvascularity or invasiveness. Despite this result, they did note an association between meningioma grade and VEGF-A levels, with Grade III meningiomas showing a 10-fold and Grade II meningiomas showing a 2-fold increase in VEGF-A relative to benign meningiomas.[52] However, other authors have shown conflicting results for the association between tumor grade and VEGF expression.[93]

Expression levels of VEGF-A are associated with recurrence rates in benign meningiomas.[52,128] While the

mechanisms regulating VEGF in meningiomas are unknown, studies have suggested that increased epidermal growth factor and platelet-derived growth factor can induce VEGF expression. Additionally, both VEGF and hypoxia inducible factor-1 (HIF-1), a transcription factor that regulates VEGF, are expressed in higher levels in meningiomas that have undergone preoperative embolization. Endothelins, peptides involved in angiogenesis, vasoconstriction, and vasodilation, have also been implicated in meningioma angiogenesis and growth.[93]

Sex Steroids

A number of observations have suggested a role for sex hormones in meningioma tumorigenesis. The incidence of meningiomas is more than 2-fold greater in women than in men. Meningiomas have also been reported to undergo increased growth during pregnancy and the luteal phase of the menstrual cycle.[52,102] Additionally, the incidence of meningiomas is increased in patients with breast cancer.[124] While estrogen and androgen receptors are both found in meningiomas, expression of the progesterone receptor is most frequently observed. The progesterone receptor is expressed in 81% of women and 40% of men with meningiomas,[7] and is minimally present in normal arachnoidal cells. Progesterone receptor expression is highest in benign meningiomas (50%–80%), and it is inversely proportional to tumor proliferation and grade.[32,48,89,90,119]

Cyclooxygenase-2

Investigators have begun to examine the potential role of the inflammatory response in meningiomas as a result of numerous case reports and epidemiological studies that suggested head trauma as a risk factor for meningioma formation.[95] Within the inflammatory pathway, cyclooxygenase serves as the rate-limiting catalyst for the biosynthesis of prostaglandins from arachidonic acid. Prostaglandins, a member of the eicosanoid family of biologically active lipid mediators, regulate a variety of critical cellular processes involved with proliferation, adhesion, angiogenesis, suppression of apoptosis, and inflammation.[81] Cyclooxygenase-2 (Cox-2) is an inducible enzyme important in mediating inflammatory responses.[95] The role of Cox-2 in tumorigenesis has also previously been demonstrated through its overexpression in colon, lung, and breast cancers. Ragel et al.[95] found that Cox-2 is highly and universally expressed in meningiomas. Thus, aberrant activity of eicosanoids may mediate tumor development and growth.

Targeted Therapies

The past decade has seen considerable progress in deciphering the myriad of aberrant signaling pathways underlying cell growth, proliferation, and angiogenesis in meningioma development. This progress has helped identify a number of potential therapeutic targets.

A number of drugs targeting growth hormones, growth receptors, and their associated intracellular signaling pathways have been investigated. The North American Brain Tumor Consortium (NABTC) conducted a Phase II study of imatinib mesylate, a PDGF-α and PDGF-β receptor inhibitor, for recurrent meningiomas.[125] In the study, 23 patients with all grades of meningioma were treated with single agent imatinib, starting with 600 mg/day. While the treatment was well tolerated and levels of cytoplasmic imatinib were therapeutically sufficient, the treatment regimen was not significantly effective. For benign meningiomas, median PFS was 3 months, with a 6-month PFS of 45%, while atypical and anaplastic meningiomas demonstrated a median PFS of 2 months, with a 0% 6-month PFS. In a study addressing these results, Gupta et al.[23] found that nelfinavir, a protease inhibitor, potentiates imatinib's efficacy in meningioma cells. Compared with treatment by imatinib alone, the combined treatment resulted in a dose-dependent decrease in tumor survival, growth, and colony formation. Other PDGFR inhibitors, such as sunitinib and CHIR 265, block additional kinases and are currently being investigated.[124]

Other studies have investigated EGFR as another growth hormone receptor target for meningiomas. A recent small Phase II trial examined the efficacy of gefitinib and erlotinib, EGFR inhibitors, in 25 patients with recurrent meningiomas.[82] The 6-month PFS was 25% for benign meningiomas and 29% for atypical and anaplastic meningiomas. Eight patients maintained stable disease. While the treatment was well tolerated, gefitinib and erlotinib did not exhibit significant activity against recurrent meningiomas. Although other EGFR inhibitors exist, very few studies have examined their therapeutic potential for treating meningiomas. The various downstream signaling pathways used by growth factors important in meningioma tumorigenesis provide another therapeutic target. Inhibitors of the MAPK signaling pathway (for example, Raf, MEK inhibitors) and the PI3K pathway (for example, PI3K, Akt, mTOR inhibitors) are worthwhile candidates for future studies.

Another treatment approach to controlling meningioma growth has been inhibiting angiogenesis. Angiogenesis inhibitors have been effective in treating several cancers, including renal cell carcinoma and glioblastomas.[17,45] A number of VEGF and VEGFR inhibitors are available, including ZD6474, PTK787, AEE788, Avastin, and IMC-1C11,[79] and these are currently being tested for use against meningiomas. Additionally, some studies have shown that inhibitors of growth factors, signal transduction pathways, and angiogenesis may induce radiation sensitivity in meningiomas.[18,100] Thus, these inhibitors may be used synergistically with radiation therapies.

The involvement of Cox-2 in meningiomas has led investigators to examine the efficacy of various nonsteroidal antiinflammatory drugs as a therapeutic agent. One such agent is celecoxib, a selective Cox-2 inhibitor. Treatment with celecoxib demonstrated a dose-dependent growth inhibition of both the IOMM-lee cell line and benign meningioma cells in culture.[95] Celecoxib decreased tumor microvascularity, increased cell apoptosis, and decreased Cox-2 and VEGF expression in vivo in a mouse xenograph model.[96] In addition to Cox-2, Pfister et al.[92] identified a number of other potential therapeutic targets involved in eicosanoid formation that are highly

expressed in meningiomas, including the enzymes Cox-1, 5-LO, and PTGER4. Future clinical trials are necessary to determine the potential efficacy of Cox-2 inhibitors and other nonsteroidal antiinflammatory drugs in treating meningiomas.

Challenges and Future Directions

One of the largest obstacles in the development of novel targeted therapies for meningiomas is the limited understanding of the molecular pathogenesis underlying their formation, growth, and progression. A lack of adequate cell lines and animal models for meningiomas has made study of this tumor challenging. While a number of studies have identified common chromosomal aberrations, the majority of the specific genes affected remain unknown. Beyond cytogenetic studies, others have begun to unravel the numerous abnormal signaling pathways involved with tumorigenesis including cell cycle dysregulation, aberrant growth, and angiogenesis. Meningiomas exhibit increased activity in a number of growth factors, growth factor receptors, and their downstream signaling pathways. However, it is difficult to identify which pathways are most critical and which targets are most therapeutically promising. Additionally, the lack of data on the natural course of untreated meningiomas has made it challenging to gauge the benefits of novel therapeutics. A number of studies have reported periods of disease stabilization, but it is possible that placebo may have achieved comparable results. This is especially true in benign and slow-growing meningiomas. Lastly, only a limited number of patients with meningiomas require more than surgery and radiotherapy, thus creating enrollment difficulties for a number of studies.

Despite advancements in our understanding of meningioma pathogenesis, the conventional treatments, including surgery, radiotherapy, and stereotactic radiosurgery, have remained largely stagnant. While these options can be curative for the majority of meningiomas, there exists a subset of inoperable and refractory meningiomas with inadequate treatment options. Current chemotherapy regimens and hormonal therapies have shown minimal activity against meningiomas. A number of potential targets have been tested, and several studies are currently being conducted; however, none has proven particularly effective to date. Future therapies will include combinations of targeted molecular agents, and this will most likely be accomplished through continued progress in the understanding of the genetic and biological changes associated with 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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Statistical analysis: Yang. Administrative/technical/material support: Yang. Study supervision: Yang.

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