Histological classification and molecular genetics of meningiomas

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Meningiomas account for up to 30% of all primary intracranial tumours. They are histologically classified according to the World Health Organization (WHO) classification of tumours of the nervous system. Most meningiomas are benign lesions of WHO grade I, whereas some meningioma variants correspond with WHO grades II and III and are associated with a higher risk of recurrence and shorter survival times. Mutations in the *NF2* gene and loss of chromosome 22q are the most common genetic alterations associated with the initiation of meningiomas. With increase in tumour grade, additional progression-associated molecular aberrations can be found; however, most of the relevant genes are yet to be identified. High-throughput techniques of global genome and transcriptome analyses and new meningioma models provide increasing insight into meningioma biology and will help to identify common pathogenic pathways that may be targeted by new therapeutic approaches.

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Introduction

Meningiomas are common tumours of the CNS that originate from the meningeal coverings of the spinal cord and the brain. Although the cell of origin has yet to be proven, meningiomas are probably derived from arachnoidal cap cells. These cells from the outer layer of the arachnoid mater and arachnoid villi show a striking cytological similarity to meningioma tumour cells.

Meningiomas account for about 30% of all primary brain tumours with an adjusted annual incidence of about 4·5 per 100 000 individuals.[1] These tumours are most commonly reported in elderly patients with peak incidence in the seventh decade of life. There is a clear bias towards women with a female to male ratio of about 2:1. Spinal meningiomas have an even greater predilection for females with a ratio of 10:1, particularly with tumours in the thoracic region. Less common subtypes and high-grade meningiomas are overrepresented in children and men.[2]

Most meningiomas are slow-growing benign lesions, and are typically associated with the symptoms of gradually increasing intracranial pressure. Headaches and seizures are common and other symptoms depend on the size and location of the tumour. On MRI, meningiomas are usually isointense to the cerebral cortex and avidly contrast enhancing.[3]

Ionising radiation is a clear cause of meningiomas and there is a potential role of sex hormones, particularly the progesterone receptor, but this remains unproven.[4,5] Another well-defined cause in those with young onset and no sex predilection is neurofibromatosis type 2 (NF2). Meningiomas (besides schwannomas) are hallmark features of this autosomal dominant disorder caused by germline mutations in the *NF2* gene on chromosome 22q12.[6] There are only a few families with an increased susceptibility to meningiomas, but without alterations of the *NF2* locus.[7] This suggests that there may be additional meningioma predisposition genes. Meningiomas have occasionally been reported in other hereditary syndromes, including Cowden, Gorlin, Li-Fraumeni, Turcot, Gardener, von Hippel-Lindau, and multiple endocrine neoplasia type I.[8,9] However, it remains unclear whether these uncommon associations are causal or merely coincidental.

Histological classification

Clinical characteristics and WHO grading

Although most meningiomas are benign, they have a surprisingly broad spectrum of clinical characteristics, and histologically distinct subsets are associated with high risk of recurrence, even after seemingly complete resection. In rare instances, meningiomas are malignant.

The WHO classification aims to better predict the divergent clinical characteristics of meningiomas with a histological grading system based on statistically significant clinicopathological correlations (panels 1 and 2).[10] There are three types of meningiomas according to

***Panel 1:* The different histological meningioma variants grouped by WHO grade[10]**

Meningiomas with low risk of recurrence and aggressive growth (WHO grade I)
Meningothelial meningioma
Fibrous (fibroblastic) meningioma
Transitional (mixed) meningioma
Psammomatous meningioma
Angiomatous meningioma
Microcystic meningioma
Secretory meningioma
Lymphoplasmacyte-rich meningioma
Metaplastic meningioma

Meningiomas with greater likelihood of recurrence and/or aggressive behaviour (WHO grade II)
Atypical meningioma
Clear-cell meningioma
Chordoid meningioma

Meningiomas with greater likelihood of recurrence and/or aggressive behaviour (WHO grade III)
Anaplastic (malignant) meningioma
Rhabdoid meningioma
Papillary meningioma
Meningiomas of any type or grade with high proliferation index and/or brain invasion

malignancy grades: benign (WHO grade I), atypical (WHO grade II), and anaplastic (malignant; WHO grade III) meningiomas.

Benign meningioma: WHO grade I

About 80% of all meningiomas are slow-growing tumours of WHO grade I. Any histological variant is compatible with WHO grade I, except for the chordoid, clear-cell, papillary, and rhabdoid meningiomas, which are consistently associated with more aggressive clinical features. The histological variants most commonly diagnosed in pathology specimens are meningothelial, fibrous, and transitional meningioma (figure 1, panel 1). Meningothelial meningiomas are histologically composed of characteristic uniform tumour cells that form lobules surrounded by thin collagenous septae. Within the lobules, epithelioid tumour cells have fuzzy ill-defined cell borders that resemble a syncytium. Characteristic nuclear changes include clear spaces (that seem empty of karyoplasm) and rounded eosinophilic cytoplasmic protrusions, referred to as pseudoinclusions. Fibrous meningiomas are mainly composed of spindle-shaped cells that resemble fibroblasts and form intersecting fascicles embedded in a collagen-rich and reticulin-rich matrix. Transitional (mixed) meningiomas combine features of both subtypes and usually present with extensive whorl formation, wherein tumour cells wrap around each other forming concentric layers. The latter have a tendency to hyalinise and calcify to form the characteristic concentric calcifications known as psammoma ("sand-like", based on their gritty, gross appearance) bodies. Tumours that are mostly composed of the latter are referred to as psammomatous meningiomas. In addition to these common meningioma subtypes, several other benign variants can be distinguished (figure 1, panel 1).

The neurofibromatosis type 2 protein is the only tumour suppressor that has been firmly implicated in the development of benign meningiomas. However, the frequency of *NF2* gene mutations varies in the different histological variants. Although fibroblastic and transitional meningiomas carry *NF2* mutations in up to 80% of patients, these can be detected in only about 25% of meningothelial meningiomas.[11] *NF2* mutations are also rare in the secretory meningioma variant, which is histologically characterised by glandular metaplasia and pseudopsammoma bodies (figure 1).[12]

Benign meningiomas of WHO grade I can invade the dura, dural sinuses, skull, and even extracranial compartments, such as orbit, soft tissue, and skin. Although these types of invasion make it more difficult to resect the tumour, they are not considered as atypical or malignant. By contrast, brain invasion is associated with recurrence and mortality rates similar to atypical meningiomas in general, even if the tumour seems completely benign otherwise.[13] Although more common in advanced meningioma variants, brain invasion has not been associated with any particular genetic change yet, but has been reported in tumours without obvious chromosomal imbalance.[14]

Atypical meningioma and other WHO grade II variants

Atypical meningiomas constitute 15–20% of meningiomas. Following gross total resection, benign meningiomas are associated with 5-year recurrence rates of

***Panel 2:* WHO criteria for meningioma grading[10]**

Benign meningioma (WHO grade I)

- Histological variant other than clear-cell, chordoid, papillary, or rhabdoid
- Lacks criteria of atypical and anaplastic meningioma

Atypical meningioma (WHO grade II) (any of three criteria)

- Mitotic index ≥ four mitoses/ten high-power fields (HPF)
- At least three of five parameters:
  - Increased cellularity
  - High nuclear/cytoplasmatic ratio (small cells)
  - Prominent nucleoli
  - Uninterrupted patternless or sheet-like growth
  - Foci of spontaneous necrosis (ie, not induced by embolisation or radiation)
- Brain invasion

Anaplastic (malignant) meningioma (WHO grade III) (either of two criteria)

- Mitotic index ≥20 mitoses/10 HPF
- Anaplasia (sarcoma, carcinoma, or melanoma-like histology)

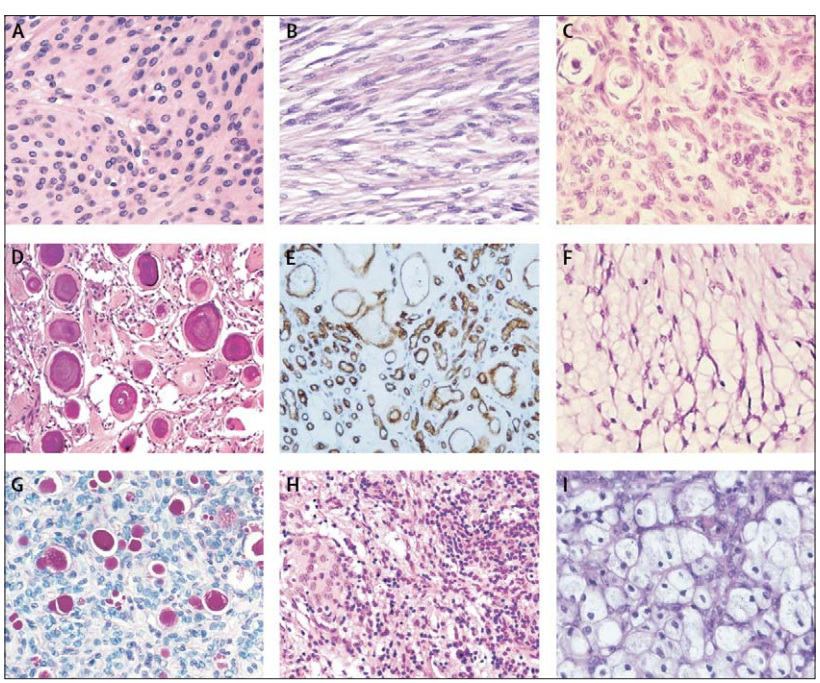

***Figure 1:* Histology of the different benign meningioma variants (WHO grade I)**
Variants of WHO grade I: meningothelial (A), fibrous (B), transitional (C), psammomatous (D), angiomatous (E), microcystic (F), secretory (G), lymphoplasmacyte-rich (H), and metaplastic (I). (A–D, F, H, I: hematoxylin-eosin; E: immunostaining for endothelial cells with anti-CD34 antibody; G: periodic acid Schiff stain). Note syncytial growth of meningothelial cells (A), fascicular growth of fibroblast-like spindle cells (B), formation of multiple meningeal whorls (C), presence of numerous calcified psammoma bodies (D), numerous densely packed blood vessels (E), prominent microcystic degeneration (F), production of periodic acid Schiff positive pseudopsammoma bodies (G), extensive chronic inflammatory infiltrates (H), and xanthomatous changes of tumour cells (I).

only 5%. In contrast, the estimated recurrence rate for totally resected atypical meningiomas is about 40% at 5 years and this continues to increase with additional follow-up time.[13,15] Thus, the diagnosis of atypical meningioma should shorten the intervals of postsurgical clinical follow-up.

The most reliable histological correlate of recurrence risk is increased mitotic activity—ie, four or more mitoses per ten high-power fields (figure 2). Nevertheless, in the absence of increased mitotic activity, other histological features are associated with the likelihood of recurrence and therefore have grading implications. With the WHO 2000 definitions, presence of three of the five following criteria may lead to the diagnosis of atypical meningioma: increased cellularity, high nuclear to cytoplasmic ratio (small cells), prominent nucleoli, uninterrupted patternless or sheet-like growth, and foci of spontaneous (not induced by embolism) necrosis. The issue of brain invasion was less clearly specified in the WHO scheme, although the similar clinical implications suggest that this could be used as another criterion for atypical meningioma (panel 2).

Clear-cell and chordoid variants of meningioma are associated with higher recurrence rates even in the absence of the above criteria.[16,17] Thus, these meningiomas are graded as WHO grade II by definition (panel 1, figure 2). Clear-cell meningioma is composed of sheets of polygonal cells with clear, glycogen-rich cytoplasm positive for periodic acid Schiff, and dense perivascular and interstitial collagenisation (figure 2). Chordoid meningiomas have regions that are histologically similar to chordoma, with cords of small epithelioid tumour cells that contain eosinophilic or vacuolated (ie, resembling **physaliferous cells**) cytoplasm embedded in a basophilic, mucin-rich matrix (figure 2). Clear-cell meningiomas are most common in the spinal cord and posterior fossa, whereas chordoid meningiomas are typically supratentorial. Although genetic features associated with clear-cell meningiomas are still unknown, an **unbalanced translocation** der(1)t(1;3)(p12–13;q11) has been proposed as a specific cytogenetic marker for the chordoid variant.[18] However, this finding is still to be validated and the genes targeted by the translocation are unknown.

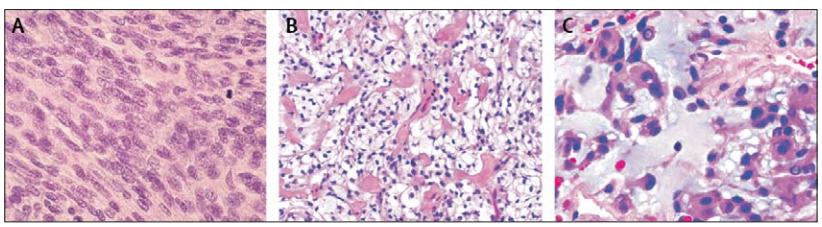

Figure 2: **Histology of WHO grade II meningiomas**
Atypical meningioma with increased mitotic activity (A), clear-cell meningioma with clear, glycogen-rich cytoplasm (B), and chordoid meningioma showing chordoma-like growth of tumour cells in a myxoid matrix (C). All are stained with hematoxylin-eosin.

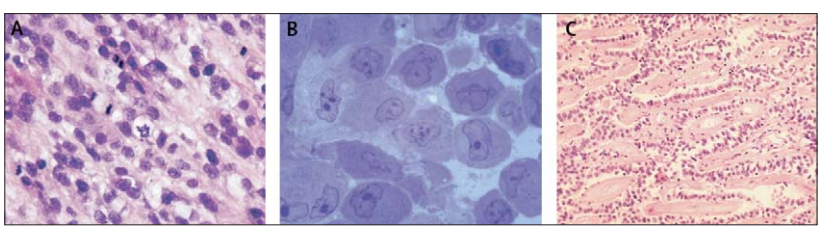

Figure 3: **Histology of WHO grade III meningiomas**
Anaplastic meningioma with cellular anaplasia and numerous mitotic figures (A), rhabdoid meningioma containing large rounded tumour cells with eccentric nuclei (B), and papillary meningioma that shows a pseudopapillary growth pattern (C); A and C are stained with hematoxylin-eosin and B is toluidine blue, semithin section.

Anaplastic (malignant) meningioma and other WHO grade III variants

Anaplastic meningiomas account for 1–3% of all meningioma cases.[10] These tumours have clinical characteristics similar to other malignant neoplasms, which can widely infiltrate neighbouring tissues and form metastatic deposits. Anaplastic meningiomas are associated with recurrence rates of up to 50–80% after surgical resection and median survival is less than 2 years.[13]

Histologically, anaplastic meningiomas have features of malignancy with a mitotic index of 20 or more mitoses per ten microscopic high-power fields being diagnostic (figure 3). Some anaplastic meningiomas are difficult to recognise as meningothelial neoplasms as they can resemble sarcoma, carcinoma, or melanoma. Anaplastic meningiomas usually have large zones of geographic necrosis. However, therapeutic embolisation (iatrogenic) should be excluded as an alternative explanation before grading (panel 2).[19]

Certain meningioma variants are consistently associated with malignant behaviour and therefore invariably correspond to WHO grade III (panel 1, figure 3). Papillary meningiomas, which usually occur in children, show invasion of the brain and other local structures in 75% of patients, recurrence in 55%, and metastasis in 20%.[20,21] These meningiomas are histologically defined by a discohesive growth, which results in a perivascular pseudopapillary pattern and even pseudorosette-like structures similar to those in ependymoma (figure 3). Another highly aggressive meningioma variant is the rhabdoid meningioma, which contains rhabdoid cells with abundant eosinophilic cytoplasm, eccentrically placed nuclei, and paranuclear inclusions that ultrastructurally correspond to whorled bundles of intermediate filaments (figure 3). Both papillary and rhabdoid features may be viewed as progression-associated changes, because they usually present for the first time at recurrence and increase in prominence over time.[20–23]

Physaliferous cells
Tumour cells with an eosinophilic, vacuolated cytoplasm embedded in a basophilic, mucin-rich matrix. Physaliferous cells are typical for chordoma

Unbalanced translocation
The unequal exchange of material between two chromosomes resulting in loss or gain of genetic material

Immunohistochemistry

Immunohistochemistry may be of particular diagnostic value in cases of anaplastic meningioma, although it should be emphasised that even immunohistochemical evidence of meningothelial differentiation can be lacking in some patients. Moreover, there is also a role for immunohistochemistry in meningioma variants dominated by

unusual features. The most commonly used marker in meningioma diagnostics is epithelial membrane antigen, which yields at least patchy positivity in most meningiomas.[24] Depending on the differential diagnostic context, vimentin staining can also be helpful, as all meningiomas strongly express this intermediate filament.[25] Unfortunately, epithelial membrane antigen or vimentin are not specific and therefore additional markers of meningothelial differentiation are sorely needed.

Another important role for immunohistochemistry in meningioma diagnostics lies in the assessment of the proliferative index, which in clinical pathology is usually measured with the antibody MIB-1, the clone that targets the proliferation marker Ki-67 in paraffin-embedded tissue. Raised MIB-1 labelling indices are associated with increased risk of recurrence. Although the specific counting techniques and cut-off levels are not exactly defined and bear a considerable degree of interlaboratory variability, MIB-1 labelling indices above 5% suggest a greater likelihood of recurrence and can be especially helpful as an adjunct to grading in borderline atypical cases.[26,27] Expression of the progesterone receptor is inversely associated with meningioma grade and therefore may also be useful in borderline cases. However, the role of this receptor in routine meningioma diagnostics is not well established.[28–30]

A new immunohistochemical method that can help meningioma grading is the mitosis-specific antibody against phosphohistone-H3. Immunostaining with this antibody highlights mitotic figures and helps to focus the pathologist's attention on the most mitotically active areas within the specimen, also allowing for easy and objective differentiation of mitotic figures from apoptotic nuclei.[31]

Molecular genetics of meningiomas

Chromosome 22q, the *NF2* gene, and its gene product merlin

Monosomy of chromosome 22 is the most common genetic alteration in meningiomas and was one of the first cytogenetic alterations described in solid tumours.[32] About half of meningiomas have genetic losses that involve the chromosomal band 22q12.2.[33,34] *NF2* was identified as the major gene in this region, with mutations identified in virtually all NF2-associated meningiomas and about 50% of sporadic meningiomas.[35–38] The results of one study[39] of sporadic meningiomas suggested that epigenetic *NF2* alterations are an additional mechanism of *NF2* inactivation.

The *NF2* gene product belongs to the 4.1 family of structural proteins that associate integral membrane proteins to the cytoskeleton.[40] It shows strong similarity to the proteins ezrin, radixin, and moesin (ERM proteins) and therefore was named merlin, for moesin-, ezri n- and radixin-like protein.[41] Merlin is also known as schwannomin for its role in schwannoma formation.[42]

Merlin contains an aminoterminal protein 4.1 cell-surface glycoprotein-binding domain (FERM domain; residues 1–313) followed by a predicted alpha-helical region and a non-conserved carboxyterminal domain. The FERM domain of merlin is composed of three subdomains that probably mediate interactions with critical partners.[43,44] Merlin is localised to the cell membrane at regions that regulate cell–cell contact and motility. Several merlin-interacting proteins have been identified. One class includes cell-surface proteins that bind to FERM-containing proteins, such as CD44 and β1-integrin.[45,46] Another class is represented by molecules involved in cytoskeleton dynamics (βII-spectrin, paxillin, actin, and syntenin).[47–50] Lastly, molecules have been identified that may be important for regulation of ion transport, such as the sodium–hydrogen exchange regulatory factor,[51] and endocytosis, such as the hepatocyte growth factor-regulated, tyrosine kinase substrate.[52]

Although the functions of many of these merlin-binding partners have not been studied in vivo, there is recent evidence that the cell-surface glycoprotein CD44 affects merlin-mediated inhibition of cell proliferation and cell motility. The cytoplasmic tail of CD44 binds to merlin at the cell membrane and this seems to be important for providing a growth-arrest signal.[46,53] This CD44–merlin association is tightly regulated by protein phosphorylation.[53–56] In addition, the association between merlin and the actin cytoskeleton is important for the proper subcellular location of merlin. The merlin–actin cytoskeleton interaction may be mediated by several merlin-binding molecules, including βII-spectrin, paxillin, actin, and syntenin. Data from one study[57] have suggested that the association between merlin and paxillin is particularly critical for proper subcellular localisation.

Other protein 4.1 family members

Besides *NF2* mutations with loss of merlin expression, other protein 4.1 family members are also downregulated in meningioma. Loss of protein 4.1B (DAL-1) expression is a common aberration detected in meningiomas of all WHO grades,[58] although one group found it predominantly in higher grade forms.[59] By contrast to loss of DAL-1 protein expression, mutations in the corresponding gene are rare, with only three mutations detected in 83 sporadic meningiomas.[60] These findings suggest that other inactivating mechanisms—eg, epigenetic changes can account for the common DAL1/4.1B silencing in sporadic meningiomas. Another study[61] similarly found no evidence of DAL-1 inactivation either by mutation or loss of heterozygosity in a cohort of tumours from patients with multiple meningiomas.[61]

Recent data suggest that protein 4.1B acts as a tumour suppressor by activating Rac1-dependent c-Jun-NH_2-kinase signalling.[62] Of note, *TSLC1* (tumour suppressor in lung cancer-1), a gene originally identified as deleted in non-small cell lung cancer and that closely interacts

with 4.1B, also has reduced expression levels in high-grade meningiomas.[63] Moreover, another family member, protein 4.1R is commonly downregulated in meningiomas and inhibits meningioma cell growth in vitro.[64] Thus, meningioma development seems to be closely associated with the inactivation of one or more of the protein 4.1 family members.

Putative alternative tumour suppressor genes on chromosome 22q

Although *NF2* is the most frequently altered gene and probably the most important tumour suppressor on chromosome 22q, the frequency of deletions in this chromosomal location exceeds that of *NF2* mutations in meningioma. Furthermore, reports of **interstitial deletions** on chromosome 22 that do not involve the *NF2* region include a case of multiple meningiomas resected from a single patient; no *NF2* gene mutations were detected in this case despite chromosome 22q loss.[65] As a consequence, a second gene involved in the disease on chromosome 22q may exist. Several candidate genes have been proposed. The *BAM22* (*ADTB1,* β-adaptin) gene maps in closest proximity to *NF2* on 22q12.2.[66] This gene is a member of the human β-adaptin gene family, which is involved in the regulation of the intracellular transport of proteins in the trans-Golgi network. In a series of 71 sporadic meningiomas, the *BAM22* gene was inactivated in about 13% of the cases.[66] However, a mutational analysis did not reveal mutations in 110 meningiomas.[67] Other possible candidates are *MN1,* the *LARGE* gene, and *INI1*. The *MN1* gene is located on 22q12.1 and was reported to be lost in a patient with multiple meningiomas, which bore an intact *NF2* gene.[68] Protein function, however, seems to be that of a transcription factor rather than a tumour suppressor. As the name implies, the *LARGE* gene on 22q12.3 is one of the largest human genes[69] and mutational analysis is still needed. Lastly, the *INI1* gene on 22q11.23 was analysed for mutations in 126 meningiomas with 3% of cases bearing an identical mutation in exon 9.[70]

Genes and chromosomes involved in meningioma progression

There is strong evidence that most gene alterations discussed above are early events in meningioma tumorigenesis. For instance, in atypical and anaplastic meningiomas, *NF2* gene mutations occur in about the same number of cases as in benign WHO grade I meningiomas, which suggests that *NF2* is involved in meningioma initiation rather than progression. However, the genetic alterations associated with atypical and anaplastic meningioma are complex, with numerous genomic losses, gains, and amplifications implicated (figure 4). Losses on chromosomes 1p, 6q, 10, 14q, and 18q and gains (DNA copy number increases) on 1q, 9q, 12q, 15q, 17q, and 20q are common in atypical meningiomas. Anaplastic meningiomas share these chromosomal aberrations, but show more frequent losses on 6q, 10, and 14q, additional losses on 9p, and gains or amplifications on 17q23.[14,71–73]

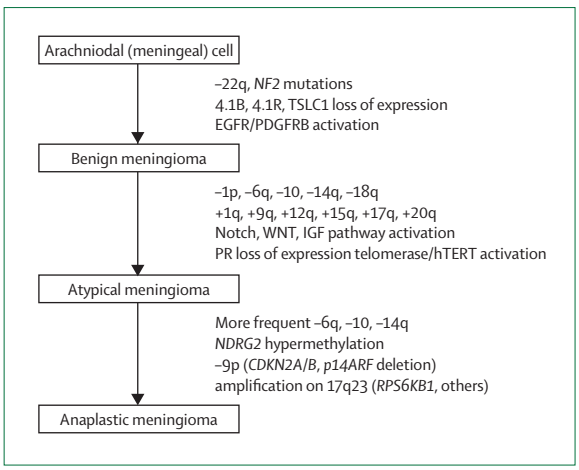

Figure 4: **Genetic alterations associated with meningioma initiation and progression**
Meningiomas probably arise from arachnoidal cap cells or meningothelial progenitor cells. The most common genetic events that lead to meningioma initiation comprise losses on chromosome 22q, *NF2* mutations, and alterations of other members of the protein 4.1 superfamily. In addition, a plethora of molecular changes and genetic pathways that conveys the progression from benign to atypical and anaplastic meningioma have been identified.

Interstitial deletion
Loss of a circumscribed genomic region from within a chromosome

Loss of 1p is the second most common chromosomal abnormality in meningiomas. Two main target regions on 1p33–34 and 1p36 are involved.[74] Recent fine-mapping narrowed the smallest region of overlapping deletion on 1p33–p34 to 2·8 megabases.[75] The region on 1p36 spans about 8·21 megabases of chromosomal sequence.[76] Several candidate genes on 1p have been screened, including *TP73*, *CDKN2C* (encoding $p18^{INK4c}$), *RAD54L*, and *ALPL*.[77–80] However, none of these genes showed any consistent structural alterations; thus, evidence for a major role is lacking. One possible explanation is that the methylation status of most of these genes has not yet been assessed. Of note, a recent study on DNA methylation of multiple promoter-associated **CpG islands** in meningiomas found increased levels of aberrant promoter methylation in tumours with 1p loss.[81] The possibility of gene methylation as a relevant molecular mechanism in meningioma progression is also corroborated by the fact that promoter methylation of the *TP73* gene was detected in 13 of 30 cases with 1p deletions.[82]

CpG island
A region of DNA rich in CpG dinucleotide sites, often located in the 5′-end of genes and promoter regions. Methylation of CpG sites may reduce the transcriptional gene activity and is thus critical to the regulation of gene expression

Several putative target genes located on other chromosomal regions deleted or gained in advanced meningioma have been studied for abnormalities in these tumours. Loss on chromosome 9p represents one of few examples clearly associated with specific genes; namely, the genes *CDKN2A* (encoding $p16^{INK4a}$), *ARF,* and *CDKN2B* (encoding $p15^{INK4b}$) (all located at 9p21). Although *INK4a* and *INK4b* inhibit cell-cycle progression at the G_1/S-phase checkpoint through negative effects on the

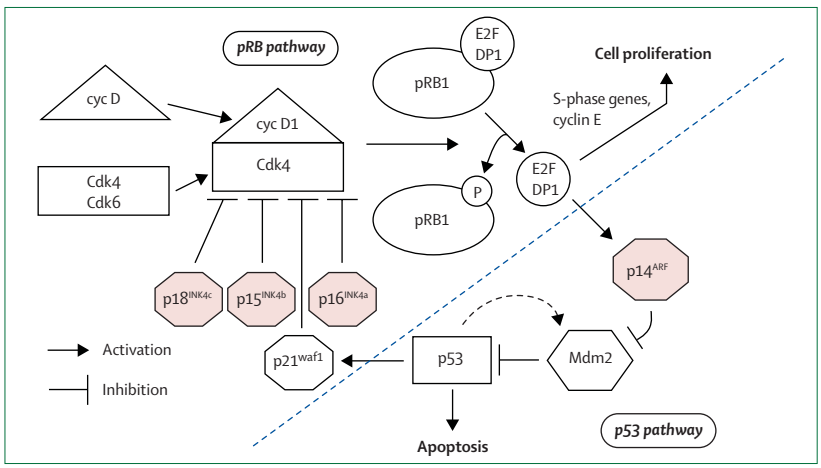

Figure 5: **The interrelationships of pRB and p53 cell cycle regulation pathways**
Anaplastic meningiomas exhibit frequent *CDKN2A* (encoding $p16^{INK4a}$), *ARF*, and *CDKN2B* (encoding $p15^{INK4b}$) homozygous deletions and mutations, indicating that inactivation of the G1/S-phase cell-cycle checkpoint is essential for malignancy. *CDKN2C* (encoding $p18^{INK4c}$) is only rarely mutated in cases of atypical or anaplastic meningioma. While *INK4a*, *INK4b*, and *INK4c* inhibit cell-cycle progression at the G1/S-phase checkpoint through negative effects on the cyclin-dependent kinases Cdk4 and Cdk6, ARF functions as a negative regulator of the Mdm2 oncoprotein, thereby inhibiting p53 degradation. Molecules found to be involved in meningioma progression are shaded in grey. More detailed information is provided in the text.

For information on **telomerase** see page 999

cyclin-dependent kinases Cdk4 and Cdk6,[83] $p14^{ARF}$ functions as a negative regulator of the Mdm2 oncoprotein, thereby inhibiting p53 degradation (figure 5).[84] Homozygous deletions or mutations of *CDKN2A*, *ARF*, and *CDKN2B* are found in most anaplastic meningiomas, which indicates that inactivation of the cell-cycle checkpoint is essential for malignancy.[78] About 70% of patients whose anaplastic meningiomas have 9p21 deletion have a substantially shorter survival than patients whose anaplastic meningiomas lack this alteration.[85] *CDKN2C* (encoding $p18^{INK4c}$) on 1p32 is mutated in only rare cases of atypical or anaplastic meningioma.[78]

Because deletions on chromosome 10 are a common finding in high-grade meningiomas, the *PTEN* gene on 10q23 has been studied extensively. However, mutations were only detected in rare cases and no homozygous deletions were found.[86] Alterations of the *TP53* gene are also uncommon, although there have been some allelic losses of 17p in high-grade meningiomas.[78,87] By contrast, the long arm of chromosome 17 has recently attracted interest since gains are reported in atypical meningioma and 17q21 amplifications are common in anaplastic meningiomas.[14] Two independent studies addressed the role *RPS6KB1*, which encodes for the ribosomal S6 kinase, as a candidate oncogene within this region.[71,88] Gains and amplifications could be detected in a few anaplastic meningiomas. However, *RPS6KB1* may not be the major target of this **amplicon**, which suggests that other genes on 17q are more important.

Amplicon
Most often used to describe a piece of DNA that has been synthesised using amplification techniques such as PCR. In the context of tumour-associated genetic alterations the term refers to a DNA segment with increased copy number that contains one or more amplified and overexpressed genes.

Nowadays, advances in high-throughput expression profiling techniques allow simultaneous screening of thousands of genes within a single tumour. In other brain tumour entities, such as malignant gliomas, gene expression-based classification may correlate better with prognosis than histological classification.[89] High-throughput techniques therefore have the potential to yield relevant gene expression signatures for diagnostic use or may even pave the way for a combined histological and molecular meningioma classification. Of note, hierarchical clustering based on distinct sets of genes (many of them associated with cell-cycle regulation and cellular proliferation) accurately distinguished higher-grade meningiomas from benign meningiomas.[90,91] Also, in line with cytogenetics, genes on chromosome 1p and 14q were commonly downregulated in anaplastic meningiomas.[92] Moreover, expression profiling has identified new candidate genes involved in meningioma progression. For example, the *NDRG2* gene localised to 14q11.2 is consistently downregulated in anaplastic meningiomas and clinically aggressive atypical meningiomas at both the transcript and protein levels. Furthermore, loss of NDRG2 expression was significantly associated with promoter hypermethylation. *NDRG2* therefore serves as a putative meningioma progression associated tumour suppressor gene.[92]

Telomerase activation is implicated as a further mechanism in promotion of tumour progression. Telomerase is an enzymatic complex, which is specifically involved in maintenance of telomeres, the very ends of chromosomes. Activation of the reverse transcription subunit hTERT (reverse telomerase transcriptase) has been extensively studied in meningiomas.[93,94] Although activation of hTERT was only found in up to 37% of benign meningiomas, it was significantly increased in WHO grade II and III tumours (up to 95%), which correlates with higher risk of recurrence and malignancy.[95] Moreover, hTERT may serve as a potential predictor of recurrence because its activation was significantly correlated with a shorter progression-free survival.[96]

Pathway-associated alterations

Understanding signalling pathways that are disturbed in meningiomas is crucial for the development of new targeted therapies. This applies especially to those patients with high-grade lesions for which surgical resection might not be curative. Although in other CNS tumours, such as gliomas, these pathways have been extensively studied, our knowledge of signalling pathways in meningioma tumorigenesis is still limited. High-grade meningiomas commonly have alterations in the retinoblasmtoma protein (pRB)-dependent and p53-dependent pathways via dysregulation of $p16^{INK4a}$, $p15^{INK4b}$, and $p14^{ARF}$ (figure 5).[78] The pRB pathway has a central role in the regulation of G_1 to S-phase transition. Under mitogenic stimuli cyclin D expression is upregulated and the cyclin Ds bind either to Cdk4 or Cdk6, thereby phosphorylating pRB and releasing E2F transcription factors, resulting in the activation of S-phase genes like cyclin E.[97] In anaplastic meningiomas, inhibition of the Cdk4/cyclinD complex is

usually impaired by alterations in the genes *CDKN2A* and *CDKN2B*.[78] The pRB pathway is connected to the p53 cell-cycle regulatory pathway via $p14^{ARF}$.[98] Physiologically, the release of E2F transcription factors on phosphorylation of pRB induces the transcription of $p14^{ARF}$, thereby inhibiting murine double minute 2 protein (MDM2)-mediated degradation of p53. Thus, the p53 pathway is a type of "emergency brake" on the cell cycle in case of aberrant pRB pathway activation. However, in anaplastic meningiomas, this regulatory mechanism is distributed by frequent homozygous deletions of $P14^{ARF}$.[78]

Recently, gene expression profiling data indicated that the Notch signalling pathway might also be dysregulated in meningiomas. HES1 (hairy/enhancer of split), the Notch2 and Notch1 receptors, and the Jagged1 ligand transcripts and proteins were expressed in meningiomas of all grades, whereas TLE2 (transducin-like enhancer of split) and TLE3 were only expressed in higher-grade meningiomas.[99] Another study implicated several members of the WNT (wingless) and insulin-like growth factor (IGF) signalling cascades, associated with losses on chromosomes 10 and 14 in higher grade meningiomas.[90] Both IGF-II and IGFBP2 are expressed in meningiomas, with increased concentrations of IGF-II associated with invasiveness and malignant progression (figure 4).[91,100,101]

Numerous studies have shown increased expression of other growth factors, their receptors, and respective kinases. For instance, the epidermal growth factor receptor is widely expressed on meningioma cells and tumours.[102–105] Moreover, meningioma cells are capable of autocrine expression of the ligands of the epidermal growth factor receptor, such as TGF-α and EGF, associated with aggressive growth.[106,107] Also, the platelet-derived growth factor receptor β is activated in meningiomas[108] and aberrantly stimulated by an autocrine loop as described for the epidermal growth factor receptor.[109,110]

Model systems

A major obstacle in understanding the molecular changes that lead to meningioma formation and progression is the lack of appropriate in vitro and in vivo model systems. Stable in vitro growth of cell lines derived from benign meningiomas could not be achieved because of senescence of these cells in vitro. Thus, until recently most in vitro studies on meningiomas relied on primary early passage cell lines or a few established cell lines derived from aggressive variants of meningiomas.[111,112] This problem was overcome by retroviral transduction of primary human meningioma cells with the hTERT gene, thereby bypassing cellular senescence.[113,114] Meningioma cell lines that were immortalised in this manner maintained classic meningothelial morphological features, including whorl formation, **desmosomes**, and interdigitating cell processes as well as expression of epithelial membrane antigen, vimentin, and desmoplakin. Karyotyping by array comparative genomic hybridisation (array-CGH) revealed similar genetic changes to primary meningiomas—eg, chromosome 22q loss. Moreover, when transplanted subdurally into nude mice, tumours were generated with typical histological features of meningothelial meningiomas. Thus, these cell lines represent promising new tools for further understanding the molecular pathogenesis of meningiomas and providing preclinical screening of new therapeutic approaches.

Although *NF2* knockout mice have provided insight into the role of the *NF2* gene during development, they have been less instructive in elucidating the role of *NF2* in the formation of meningiomas. Recently, a promising conditional knockout mouse was reported, using Cre recombinase technology to specifically inactivate the *NF2* tumour suppressor in arachnoidal cells. These mice developed a range of meningioma subtypes fairly similar to those in human beings.[115] These conditional *NF2* knockouts, when crossed with other strains of knockout or transgenic mice may provide crucial insights into the synergistic or additive effects of *NF2* gene inactivation. Thus, these new in vitro and in vivo models may help to gain mechanistic insights into *NF2*-associated tumorigenic pathways and may contribute to a better understanding of the complex processes that drive meningioma initiation and progression.[116]

Summary and outlook

The WHO classification of meningiomas is still primarily based on morphological parameters. As delineated in this review, however, there has been tremendous progress in our understanding of the molecular mechanisms that underlie meningioma tumourigenesis and progression. Several chromosomal regions and new candidate genes have been identified; these must be further validated as diagnostically and prognostically relevant markers, and may eventually facilitate combined histological and molecular meningioma classification. The use of array-based approaches of global genomic and gene expression analyses and new in vitro and in vivo models will no doubt accelerate progress and contribute to the development of new targeted therapies, particularly for patients for whom surgical and radiation options have been exhausted.

Search strategy and selection criteria

References for this review were identified by searches of PubMed from 1958 until July 2006. Main search terms were "meningioma", "meningioma pathology", "meningioma genetics", "NF2", "merlin", and "meningioma progression". The new "related link function" in PubMed was also used to detect relevant literature crosslinks. Articles were also identified through searches of the authors' own files and the WHO Classification of Tumours of the Nervous System. Only literature published in English was reviewed.

Desmosome
Specialised cell–cell junction, formed between two epithelial (or meningothelial) cells, characterised by dense plaques of protein into which intermediate filaments in the two adjoining cells insert

Contributors

MJR wrote the Review, composed the figures and tables, and did the literature search. AP edited the Review and contributed to the figures and literature selection. GR coordinated authors' activities, edited the Review, and contributed to the literature search and composition of figures and tables.

Conflicts of interest

We have no conflicts of interest.

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