Genetic and molecular alterations in meningiomas

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1. Introduction

Meningiomas are the most common benign intracranial tumors in adults arising from the dura matter. The etiology of meningiomas is mostly unknown, although several risk factors have been described, such as ionizing radiation, head injury, hormones and genetic factors. According to WHO they are classified into 3 grades, grade I, grade II and grade III. Meningiomas express various hormonal and growth factor receptors, such as progesterone, estrogen, somatostatin, transforming growth factor alpha (TGF-alpha) and epidermal growth factor (EGF) receptors, which may be related to their biological behavior and response to treatment. Chromosomal abnormalities linked to meningiomas involve chromosomes 22, 1p, 9p, 10p, 11, 14q, 15, 17, and 18q. In addition, genes that may be involved in the formation of meningiomas include NF2, DAL-1, p14 (ARF), p53, MDM2, Rb, p16 and c-myc. It is likely that detailed molecular information will aid in establishing a molecular grading of these tumors and predict response to treatment and survival.

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1. Introduction

Meningiomas are the most common benign intracranial tumors, accounting for 13–26% of all primary intracranial tumors [1,2]. Intracranial meningiomas affect women more commonly than men, having a 2:1 female: male ratio and usually occur in patients between 50 and 60 years old [1]. The annual incidence per 100,000 people ranges from 2 to 7 for females and 1 to 5 for males [2]. An estimated 2–3% of the population have an incidental asymptomatic meningioma [3]. Meningiomas are by far the most common tumor arising from the dural coverings of the brain at any site, most commonly the skull vault and the skull base [4]. Although many meningiomas have an unknown etiology, several risk factors have been identified, among which are ionizing radiation, head injury, hormones and other receptors binding sites and genetic factors [1,5]. Head trauma has emerged as the most probable etiologic factor that can cause meningioma [2], especially if it occurred 10–19 years before reference date [6].
Although meningioma typically exhibit benign histological features and total resection is associated with favourable prognosis, atypical or anaplastic tumors can be found in 6% of cases and are associated with increased risk of recurrence [7–9]. Among 294 meningiomas that were operated, 92% were found to be benign, 6.26% atypical and 1.7% malignant [7]. Atypical and anaplastic meningiomas are slightly more common in men. Moreover, atypical and malignant meningiomas are more common in the seventh and in the sixth decades, respectively [7].

Spinal meningiomas although rare, are one of the most common spinal tumors and occur more frequently in women than men. They are usually localized lateral to the spinal cord and in the thoracic region [10]. When they are located in the anterior spinal canal they constitute a surgical challenge. With the advent of microsurgical techniques, magnetic resonance imaging and neurophysiological monitoring, complete tumor excision is feasible in the majority of cases with satisfactory clinical outcomes [11,12].

2. Histological grading

Macroscopically meningiomas commonly form well-circumscribed masses that have lobular architecture. Most studies use a modified histological grading system, based primary in the WHO criteria of malignancy such as regions of hypercellularity, loss of architecture, nuclear pleomorphism, mitotic index, sheet-like growth, high nuclear-cytoplasmic ratio, prominent nuclei, tumor necrosis and brain invasion, in order to define atypical and malignant meningiomas. Each of these criteria is given a score from 0 to 3 and then partial scores are added to obtain cumulative scores [13]. Based on WHO classification, meningiomas are graded as grade I, grade II and grade III [13].

Grade I meningiomas, generally follow a benign clinical course and have only occasional mitotic figures, although pleomorphic nuclei do occur. The three commonest architectural patterns within this group are meningothelial, fibrous, and transitional [3]. Two subtypes of WHO grade II meningiomas are recognised, the clear cell and chordoid meningiomas. The term atypical can be used for any architectural pattern, but specific histological features are required, such as mitotic rate of at least four mitotic figures per ten high-power fields and hypercellularity, architectural sheeting, and small cell formation. Anaplastic, papillary and rhabdoid meningiomas are categorized as grade III meningiomas and behave in a very aggressive fashion [14]. These tumors show a high frequency of local and brain invasion, recurrence and metastases [15].

3. Proliferation index

MIB-1/Ki-67 index is the most widely applied proliferation-associated marker. The MIB-1 antibody reacts to the Ki-67 nuclear antigen in formalin-fixed tissue. The MIB-1/Ki-67 assay can be applied easily, performed routinely and is considered as a reliable method, because Ki-67 is expressed in nearly all parts of the cell cycle, with the exception of phase G0 [16]. MIB-1 index correlates with the histopathological grade of meningiomas and can be valuable in histological borderline cases [17]. Furthermore, high MIB-1 index is associated with aggressive behavior and recurrence [18].

4. Molecular markers

Most meningiomas gain several receptors during tumorigenesis (Table 1). Hormone receptor studies were performed mainly by using immunohistochemistry. Almost 61% of meningiomas possess progesterone receptors (PR) that play an important role in meningioma growth [19,20]. Expression of PR is associated with benign histological grade, lower frequency of recurrence and over-all favourable prognosis [21]. In grade II and III tumors there is usually absence of PR. Furthermore, PR negative meningiomas tend to be larger than PR positive [19]. The expression of estrogen receptors (ER) is less consistent [22]. Gonadotropin-releasing hormone (GnRH) receptor, which is related to PR and ER, has been reported in over half of meningiomas [23]. Nevertheless, their clinical significance remains to be elucidated.

Somatostatin receptors are expressed in 70–100% of meningiomas [24,25]. The biological function of these receptors, predominantly type 2a, is unknown, but their activation may be associated with an antiproliferative effect [26,27] [Fig. 1]. Barresi et al. using immunochemistry reported that somatostatin receptors were frequently expressed in high grade meningiomas in accordance with higher microvessel density. Somatostatin analogues may have a potential role in meningioma treatment [28]. Long acting somatostatin was administered in patients with recurrent meningiomas and resulted in partial radiographic response in 31% of patients. Furthermore, 44% of patients achieved progression-free survival at 6 months [27]. Besides that, somatostatin receptors can be used to image the radiolabelled tumors for maximizing resection, detection of residual or early recurrent tumors or for therapeutic purposes [29].

Meningiomas express epidermal growth factor receptor (EGFR) and its ligands, transforming growth factor alpha (TGF-alpha) [30] and EGF [31,32]. Activation of these receptors may promote tumor growth. EGFR monoclonal antibodies have been found effective for the treatment of certain malignancies, thus their plausible therapeutic potential in meningiomas was also evaluated. Norden et al. studied the EGFR inhibitors, gefitinib and erlotinib, in recurrent meningiomas. Although treatment was well-tolerated, neither gefitinib nor erlotinib appeared to have significant activity against recurrent meningiomas [33].

Platelet-derived growth factor (PDGF) has been implicated in meningiomas tumorigenesis. However, its role is still unclear [34]. Expression levels were found higher in atypical and malignant meningiomas than benign [34]. Wen et al. evaluated the therapeutic potential of imatinib mesylate, a PDGFR inhibitor, in patients with recurrent meningiomas but no significant benefit was found [32].

A possible relationship between cyclooxygenase-2 (COX-2) expression and tumoral progression and angiogenesis has been suggested [35,36]. Increased COX-2 expression was found in recurrent meningiomas. COX-2 is an inducible form of the enzyme involved in the first steps of the prostaglandins and thromboxane synthesis. This association may represent a potential area for therapeutic invasion with selective COX-2 inhibitors, namely non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and celecoxib, either as adjunct or in combination to radiotherapy [37]. Treatment with NSAIDs has been shown to curb the tumorigenic properties of prostaglandins in several cancer models via both COX-
Fig. 1. SHP-1, a cytoplasmic protein tyrosine phosphatase (PTP), is activated by somatostatin. Activated SHP-1 dephosphorylates nNOS leading to nitric oxide (NO) production. NO activates NO-sensitive guanylyl cyclase and leads to increased synthesis of cGMP. Increased cGMP inhibits cell growth. SHP-1 impacts upon PI3K activation. SHP-1 and PI3K are required upstream of Ras and Rap1. B-Raf is then activated and in turn activates MEK1/2. MEK1/2 subsequently phosphorylates the ERK-1, which transmits the signal to the nucleus and binds to different transcription factors.

2-dependent and -independent mechanisms [35,36,38,39]. Ragel et al. evaluated the effects of celecoxib on meningioma growth in a mouse xenograft model and found that celecoxib inhibited tumor growth. Celecoxib-treated tumors were less vascular with increased apoptosis [39].

Multidrug resistance is an obstacle to efficient chemotherapy. Various genes have been implicated; among them MDR1, which encodes P-glycoprotein (P-gp) is the most widely studied. P-gp mediates resistance to a range of drugs, including the vinca alkaloids, anthracyclines, etoposide, adriamycin and vinblastine. Meningiomas frequently express P-gp [40,41]. Demeule et al. studied by western blot analysis 60 brain tumors and found P-gp expression to be 10 fold higher in 7/10 meningiomas, suggesting that P-gp might be a meningioma marker [42]. Anderson et al. using immunohistochemical analysis found a significantly greater expression of P-gp in meningiomas compared to normal brain tissue. Nine MRPs show capacity for drug transport [44]. MRP1 is the most widely studied and is expressed in meningiomas. We recently evaluated the immunohistochemical expression of MRPs in brain tumors. In meningiomas, there was expression of the MRP5 only in anaplastic meningiomas, particularly in tumor cells and in some cases in endothelial cells within the tumor [unpublished data]. Lung resistance protein (LRP) has also been associated with multidrug resistance phenotype. Berger et al. using RT-PCR and western blot analysis reported a high expression in meningiomas [45]. A subsequent immunohistochemical study demonstrated an heterogenous expression of LRP in meningiomas [43].

Recent in vitro studies have shown that 60% of meningioma cell cultures underwent apoptosis in response to both radiation and cisplatin, associated with increased expression of Bax/Bcl-2 ratio especially in cells with low levels of p16 (INK4A), Cdk6 and pRB proteins, indicating a possible involvement of the RB pathway [46]. Expression of G-CSF, GM-CSF and their receptors in meningiomas has been correlated with enhanced cell proliferation and higher malignancy suggesting a contribution of these factors to tumor progression [47].

5. Radiation induced meningiomas

Irradiation either alone or in association with other factors is considered to have a role in the formation of intracranial meningioma [48,49]. Doses as low as 1–2 Gy have been associated with increased risk [50]. The latency of meningioma formation is influenced by both the radiation dose and the patient's age [49]. A report of 20 patients with radiation-induced meningiomas showed that the interval from first radiotherapy to meningioma diagnosis varied from 11 to 63 years. However, occasional case reports have indicated appearance of meningioma even 14 months after cranial radiotherapy [51]. Usually patients presenting with meningiomas have received high dosage of radiation at a young age [52]. Meningiomas have been noted after childhood treatment for primary brain tumor or tinea capitis, exposure to dental X-rays, and after exposure to atomic explosions in Hiroshima and Nagasaki [53].

Ionizing radiation has been shown to enhance invasiveness of surviving tumor cells, and several proteolytic enzyme molecules, including urokinase plasminogen activator (uPA), seemed to be upregulated after radiation. uPA and its receptor (uPAR) have been strongly implicated in tumor invasion, angiogenesis and progression. Thus, the specific targeting of proteases could augment the therapeutic effects of radiation and prevent the adverse effects resulting from tumor cells that receive sublethal doses of radiation within the tumor mass [54]. Furthermore, after radiation treatment, meningioma cells overexpress matrix metalloproteinase-9 (MMP-9) at both the mRNA and protein levels. However, the increased MMP-9 expression can be reversed with siRNA-mediated down-regulation of MMP-9, followed by ERK and Akt-mediated apoptosis [55].
6. Cytogenetic alterations

6.1. Early genetic events

Meningiomas are one of the solid tumors in humans that have been shown to have consistent chromosomal abnormalities (Table 2) [56]. One common early phenomenon that has been shown to have consistent chromosomal abnormalities 6.1. Early genetic events is the loss of the one copy of chromosome 22 [1,57]. In benign tumors a normal karyotype or monosomy 22 is usually observed [58,59]. In general these abnormalities are more frequent in transitional and fibrous meningiomas than in the meningothelial variant. Trisomy and tetrasomy 22 are related to younger patient, aggressive histopathological features, a greater incidence of DNA aneuploidy and higher proportion of S-phase tumor cells [60]. Furthermore, trisomy 22 predicts worse outcome [60]. Pfisterer et al. analyzed by fluorescent in situ hybridization (FISH) the frequency and regional distribution of cells with genetic abnormalities of chromosomes 1, 14, and 22 and found that deletion of 22q was more frequent in patients who had recurrence [61]. The number of aberrations of chromosomes 1p, 14q, and 22q correlate significantly to MIB-1 index, with signs of grossly invasive tumor growth.

6.2. Chromosome 22

Up to 60% of meningiomas show a somatic mutation of the neurofibromatosis type 2 (NF2) tumor suppressor gene, located on chromosome 22q12.2. The NF2 protein Merlin belongs to the band 4.1 family of proteins that regulates cell growth and motility by linking the cytoskeleton to cell membrane proteins [63]. Kros et al. determined the NF2 status by loss of homozygosity (LOH) analysis, karyotyping and FISH and found that abnormalities of chromosome 22q were more frequent in transitional and fibrous meningiomas than in the meningothelial variant [64]. A subsequent study also reported an association between the histological variant and the frequency of NF2 mutations. Nearly 70–80% of fibrous and transitional meningiomas carrying NF2 mutations, compared with only 25% of meningothelial meningiomas, suggesting the presence of different molecular subgroups of meningiomas [63].

Besides NF2 mutations, another protein of the 4.1 superfamily the DAL-1 or protein 4.1B has been implicated in familial meningiomas and meningioma evolution. Loss of expression of this protein is an early event in tumorigenesis and has been found at the DNA level by FISH and PCR-based loss of heterozygosity, at RNA level by RT-PCR and at protein level by western blot and immuno-histochemistry [65,66].

6.3. Chromosome 10

LOH on chromosome 10 has been found in 73.4% in benign, 80% in atypical and 86.75% in malignant meningiomas. This high incidence of heterozygosity may serve as a marker of clinical relevant alterations useful for the diagnosis and patients treatment [67]. Also, there is a significant correlation between tumor location, grade, time to recurrence, patient’s survival and LOH of chromosome 10 [68]. Mihaila et al. suggested that genetic differences exist among different meningiomas subtypes. Meningiomas with LOH for D10S1587 or D10S209 were more likely to be meningothelial or transitional. Furthermore, LOH at D10S179 and D10S169 were more likely to be present in atypical or malignant meningioma and LOH at D10S169 could also be predictive of tumor recurrences [67].

6.4. Progression-associated genetic aberrations

A number of cytogenetic alterations are associated with meningioma progression. The value of numerical abnormalities, in various chromosomes, has been explored in order to help the prediction of relapse-free-survival. Adverse prognostic factors and shorter relapse-free survival were associated with abnormalities in 9, 11, 15, and 17 and the sex chromosomes [69]. The genetic alterations in atypical and anaplastic meningiomas are complex and involve losses on 1p, 6q, 10, 14q and 18q, as well as gains on multiple chromosomes. The relevant genes are still unknown.

Deletions on 1p are a frequent chromosomal abnormality in meningiomas [69]. The frequency increases with higher histologic grade and may hold a role in meningioma progression. Using double-target FISH, Ishino et al. supported the existence of tumor suppressor genes on 1p associated with malignant progression of meningiomas [70]. Tumors with 1p-deletions recur more frequently [71]. Nagasaka et al. analyzed the chromosome 1p/19q state by FISH and found that genomic abnormalities of 1p/19q were significant in atypical and in low grade meningiomas. All atypical meningiomas had chromosome 1 deletions, whereas only 1/16 low grade meningiomas had 1p deletion. A 19q instability was detected in 6 out of 7 atypical meningiomas and only in 2/16 low grade meningiomas. Thus, identification of these genetic aberrations may prove useful for meningiomas grading and possible identification of tumors with high risk for recurrence [72].

Furthermore, 14q status has been suggested to predict the risk of recurrence in benign meningiomas [73]. Patients with monosomy 14/14q – more often are male and have greater incidence of recurrence and shorter relapse-free survival time [74,75]. Loss of chromosome 14 was also found in anaplastic meningiomas and associated with malignant progression [58].

Deletions on chromosome 10 has been also implicated in meningiomas progression. Phosphatase and tensin homologue deleted on chromosome 10 (10q23.3) (PTEN), was identified in 1997 as a tumor suppressor gene [76,77]. Mutations of the PTEN have been associated with malignant progression in meningiomas [78]. The phosphatidylinositol 3-kinase (PI3K) pathway is a likely mediator of meningioma cell proliferation and PTEN is the sole central negative regulator of PI3K signaling because no other protein compensates if there is a loss of its function. PTEN disruption leads to the serine/threonine kinases AKT/protein kinase B, S6 kinase, and mTOR [79,80]. mTORC1 regulates cell growth and mTORC2 directly phosphorylates AKT at S473 which is necessary for the full activation of AKT and facilitates the Thr308 phosphorylation by PDK1 [83] [Fig. 2]. Both complexes can serve as drug targets in cancers lacking PTEN expression [84].

Alterations on chromosome 9 are also frequent detected abnormalities mainly in anaplastic meningiomas. Alterations of the CDKN2A, p14ARF, and CDKN2B tumor suppressor genes at 9p21.
Fig. 2. PTEN/PI3K/AKT signaling pathway and targets for drug therapy. Activated PI3K induces the production of PIP3 which in turns activates AKT. AKT regulates cell growth via activation of mTOR. mTOR is the catalytic subunit of 2 complexes (mTORC1 and mTORC2). mTORC1 regulates cell growth and mTORC2 directly phosphorylates AKT at S473 which is necessary for the full activation of AKT.

are common[85,86]. The methylation of the p14 (ARF) gene, located on 9p21, was found in 8.6% of benign, 20% of atypical and in 50% of anaplastic meningiomas. P14 binds to MDM2/p53 complex and inhibits MDM2 mediated degradation of p53. Loss of the immunohistochemical expression of MDM2 protein was found in high grade meningiomas. These alterations in the p14MDM2-p53 pathway may contribute to meningioma’s malignant progression[87]. Several candidate growth regulatory genes have also been identified including the Tumor Suppressor in Lung Cancer-1 (TSLC-1) and the S6-Kinase genes, which may hold a role in formation and progression of meningiomas[57,88].

Furthermore, epigenetic change may be involved in malignant progression of meningiomas. One such mechanism is the CpG island aberrant promoter methylation that contributes to the silencing of NF2 [89]. Jun et al. also found that epigenetic mechanisms are common across meningiomas of all grades. Among them, WNK2 was found to be grade specific, because it was methylated in 83% and 71% of grade II and III meningiomas respectively [90]. WNK2 negatively regulates the EGF-induced activation of the ERK/MAPK-pathway and the downstream cell cycle progression [91].

6.5. Telomerase

Telomerase activation has been reported to be a prominent finding in meningioma progression. Telomerase is a reverse transcriptase that stabilizes chromosomal length allowing them to divide virtually forever. The main components of this telomerase complex are a reverse transcriptase (hTERT) and an integral RNA component (hTR). Atypical and anaplastic meningiomas had increased telomerase activity or elongated telomeres compared to benign tumors [92]. Telomerase activity has also been associated with poor outcome [93]. Maes et al. reported that hTERT expression might be a more sensitive marker than telomerase activity and constitute an early event in carcinogenesis [94].

7. Conclusions

In this paper we reviewed the various molecular markers and genetic alterations that contribute to meningioma formation and grading. Extensive knowledge obtained by these markers may be used not only for effective treatment strategies but also for predicting recurrences and determining prognosis.

References


