Rapid intraoperative molecular characterization of glioma

There has been a major change in the conceptual understanding of the molecular pathogenesis of glioma by the introduction of a series of diagnostic, prognostic, and predictive biomarkers. Molecular markers of relevance for diagnosis (D), prognosis (P), or therapy prediction (T) are mutations of the isocitrate dehydrogenase (IDH) (D,P), co-deletion of chromosomes 1p/19q (D, P, T), promoter mutation of telomerase reverse transcriptase (TERT) (D,P), loss of alpha thalassemia/mental retardation syndrome X-linked (ATRX) (D), and methyl-guanine DNA-methyltransferase (MGMT) promoter methylation status (T). As long as the molecular status is only relevant for postsurgical treatment decisions and not to tailor presurgical decisions or approaches, the current molecular workup seems sufficient.

While the surgical strategy is a matter of little debate in patients with little risk for complications of anesthesia and the tumor sitting non-eloquent, the decision on the surgical management is less straightforward in a higher-risk patient. Molecular stratification could drive treatment decision making. Patients with potential high-grade gliomas should undergo a maximal safe resection of the contrast-enhancing part of the tumor with preservation of neurologic function. In a retrospective series from the Massachusetts General Hospital it has been proposed that mainly patients with IDH mutant gliomas may benefit from maximal resection of the T1 contrast enhancing plus T2 tumor volume.

If we take that seriously, there is a need for more rapid, intrasurgical molecular testing in order to allow for a course definition based on molecular data becoming available. The additional advantage of such an approach would be a more rapid decision on the potential tumor diagnosis and prevention of further biopsies, eg with the detection of an IDH mutation, in cases of uncertainty between a glial tumor and another lesion biology.

This challenge has been taken on in the recent work by Shankar et al. They propose an assay to test IDH1 R132H/C, TERT C228 T or TERT C250 T promoter mutations using a quantitative polymerase chain reaction (PCR)-based method to detect cancer-specific mutations in specimens with low tumor density through (1) the inclusion of peptide nucleic acid (PNA) oligonucleotides that block amplification of wild-type alleles and (2) the incorporation of locked nucleic acid (LNA) into the detection probes to increase specific binding to the mutant allele. The assay, coined OperaGen for “operative genotyping”, was carried out during glioma resection in two cases, ruling out the presence of 0.1% mutant allele fraction within 60 minutes. In these, a TERT C228 T mutation in a glioblastoma tissue and an IDH1 R132H mutation and no TERT promoter mutation, which raise the possibility of a World Health Organization (WHO) grade II or III glioma, were detected and confirmed by routine pathological and molecular diagnostics.

The OperaGen assay represents a further stretch in taking on comprehensive and decisive molecular diagnostics as early as possible in the management of patients with glioma. The yet incomplete development of 2-hydroxyxylutarate MR spectroscopy is aimed at presurgically defining the pivotal IDH mutation by detecting the oncometabolite of the neomorphic function of mutated IDH. The same will be true for detecting glioma-specific mutations in peripheral blood or cerebrospinal fluid.

The technical advantage of OperaGen is the rapid detection of certain mutations, which would not be possible using techniques like next-generation sequencing. In addition to the yet to be prospectively demonstrated IDH mutation-dependent benefit from more radical surgery, the intraoperative molecular diagnosis might enhance the likelihood of demonstrating a definite diagnosis and limit the potential hazards associated with multiple sampling or reduce the need for staged craniotomies.

The neurosurgical/pathological community will have to further expand on the topic with prospective, larger series to demonstrate feasibility and practical use.

References

Magnetic resonance image features identify glioblastoma phenotypic subtypes with distinct molecular pathway activities

Imaging genomics is a relatively new field of study that examines the relationship between gene expression and imaging features. The goal is to exploit the non-invasive and global surveillance advantages of MRI in combination with the detailed molecular data provided by tissue sampling in order to develop a more comprehensive understanding of tumor biology. To date most studies have examined differences in gene expression between various predetermined tumor groups (for instance, tumors with little versus abundant edema).

A modified strategy has been employed by Itakura et al in which tumors were clustered based on similarities and differences in quantitative imaging features extracted from contrast-enhanced MRI without the use of predefined categories. Gene expression from these clusters was then analyzed. The authors used two cohorts of unilateral, solitary glioblastoma - one for development and one for validation (n = 121 and 144), respectively. Multifocal tumors were excluded. Quantitative values using histogram statistics including texture and roughness were extracted from post-contrast T1-weighted images. Consensus clustering with 10,000 iterations was used to achieve robust results and to minimize cluster selection bias. Three clusters were developed. Cluster 1 was typified by highly irregular tumors with many concavities. The appearance was similar to tumors with multicentric disease and thus designated “pre-multifocal.” Cluster 2 (“spherical”) was made up of spherical tumors with regular, well-circumscribed edges. Cluster 3 (“rim-enhancing”) tumors exhibited central hypointensity with a T1 hyperintense (enhancing) rim. The authors found that the clusters had different survival rates: patients with cluster 3 tumors lived the longest, followed by cluster 2, with cluster 1 having the shortest survival. This appeared to be independent of age, sex, KPS, MGMT hypermethylation and EGFR amplification. Signaling pathways based on gene expression also varied between the clusters. For instance, cluster 1 showed evidenced of signaling events mediated by stem factor receptor (c-Kit). Cluster 2 showed a decrease in multiple pathways including c-KIT, VEGFR1, Tie-2, and c-MET. For cluster 3, up-regulated pathways included VEGFR1, Tie2, and PDGFR-B.

The potential impact of this approach is based on the ability to monitor signaling pathways through non-invasive imaging. This could help select and monitor targeted therapies. For instance, bevacizumab might be more effective for cluster 3 than cluster 2 tumors, a hypothesis the authors plan to test in the near future.

Reference

DNA methylation and somatic mutation converge on the cell cycle and define similar evolutionary histories in brain tumors

Analyses of mutation patterns in different parts of tumors and comparison with patterns after recurrence have provided insights into the genetic evolution of the tumors and allowed construction of phylogeny. This is of particular interest as it informs on mechanisms of tumor heterogeneity and acquisition of treatment resistance, which are important for the design of treatment strategies.

Mazor et al have taken the question to a next level and asked whether epigenetic alterations, in particular DNA methylation, also allow deconstruction of tumor evolution and how it compares to the phylogeny of the tumors. To this end they have determined the DNA methylation profiles of a set of IDH mutant 1p/19q intact low-grade gliomas WHO grade II for which they had multiple parts of tumors, including the corresponding recurrences progressed to grade III or IV. Given the IDH mutant status the tumors all exhibited the CpG island methylator phenotype that was also preserved throughout progression in all cases. The patient-specific methylation profile was quite conserved. Nevertheless, they were able to identify a methylation profile specific for the evolution to glioblastoma (GBM) that was independent of adjuvant therapy. In integrating transcriptome information, they searched for changes in the methylome that had an effect on gene expression. Tumor grade-dependent methylation changes were observed, but most prominent was hypomethylation upon progression to GBM. Strikingly, hypomethylation was enriched at genes that are associated with age-related (fetal brain to adult brain) increases in methylation. Affected genes were enriched for association with cell cycle function, from which the authors concluded that the epigenetic alterations contributed to an increase in proliferation similar to the genetic alterations associated with malignant progression. Finally, they constructed phyloepigenetic trees and compared them with the phylogenetic trees based on somatic mutation analysis. The spatial and temporary evolution of the tumors based on the construction of phylogenetic and phyloepigenetic trees displayed high similarity.

Taken together, this work elegantly shows the interdependence of genetic and epigenetic alterations in malignant progression of tumors. This interdependence needs to be taken into account for the understanding of tumor biology in order to design efficacious strategies for therapy. This is just the beginning of the integration of tumor epigenetics. It will be of
interest to confirm the findings in larger studies, in particular in the context of clinical trials, to see the impact of defined therapies on phylogeny and phyloepigeny of the tumors. It also raises the question of how oligodenrogliomas that are IDH mutant- and 1p/19q-codeleted are different, as they do not progress to GBM.

Reference

The value of 5-ALA in low-grade gliomas and high-grade gliomas lacking glioblastoma imaging features: an analysis based on fluorescence, MRI, 18F-FET-PET, and tumor molecular factors
To date, the use of 5-aminolevulinic acid (5-ALA) for fluorescence-guided resections is well established for malignant gliomas, mainly glioblastomas.1 The application of 5-ALA for lesions regarded as low-grade gliomas is less clear, with only 10% to 20% of these tumors showing visible porphyrin accumulation. In up to 30–40% of non-enhancing tumors on MRI, anaplastic foci are detected at biopsy, and it has been suggested that they could be identified by amino acid PET and intraoperative fluorescence.2

The aim of the study of Jaber and colleagues, published online in the September 2015 issue of Neurosurgery,3 was to determine which patients with suspected gliomas without unambiguous features of glioblastoma benefit from preoperative 5-ALA administration by disclosing tumor fluorescence useful for the detection of high-grade tumoral areas.

Patients harboring gliomas without necrosis and rim enhancement on MRI were given 5-ALA. Fluorescence was recorded intraoperatively, and biopsy specimens were collected from fluorescing tissue. World Health Organization (WHO) grade, Ki-67/MIB-1 index, IDH1 (R132H) mutation status, O-methylguanine DNA methyltransferase (MGMT) promoter methylation status, and 1p/19q co-deletion status were assessed. Predictive factors for fluorescence were derived from preoperative MRI and 18F-FET PET. Classification and regression tree analysis and receiver-operating characteristic curves were generated for defining predictors.

Of 166 tumors, 82 were diagnosed as WHO grade II, 76 as grade III, and 8 as glioblastomas grade IV. Contrast enhancement, tumor volume, and 18F-FET PET uptake ratio >1.85 predicted fluorescence. Fluorescence correlated with WHO grade (P < .001) and Ki-67/MIB-1 index (P < .001), but not with MGMT promoter methylation status, IDH1 mutation status, or 1p19q co-deletion status. The Ki-67/MIB-1 index in fluorescing grade III gliomas was higher than in non-fluorescing tumors, whereas in fluorescing and non-fluorescing grade II tumors no differences were noted.

Overall, this is the first study of a large cohort of grade II and III gliomas that analyzed the propensity of 5-ALA for intraoperative marking of tumor tissue. The most important data are that any form of contrast enhancement on MRI resulted in a high likelihood of finding useful fluorescence (78%), and that in approximately 20% of non-enhancing tumor areas with clear fluorescence were seen.

The major limitation of this study is that the authors could not provide information on whether intraoperatively observed areas of fluorescence corresponded to preoperative areas of contrast enhancement on MRI or areas of hypermetabolism on the 18F-FET PET.

Future studies should analyze whether fluorescence in lower grade gliomas could identify subtypes with worse prognosis and guide effective tumor resections.

References