Transformation of quiescent adult oligodendrocyte precursor cells into malignant glioma through a multistep reactivation process

Much debate has gone into the cell of origin of adult gliomas, with recent studies pointing to both neural stem cells (NSCs) as well as oligodendrocyte progenitor cells (OPCs). In adults, OPCs rarely proliferate, and whether adult OPCs can be transformed and give rise to high-grade gliomas is unknown. Using a temporally regulated Cre transgene mouse model, Galvao et al show that deletion of tumor suppressor genes p53 and NF1 in adult OPCs (termed conditional knockout [CKO] mice) gives rise to gliomas with 100% penetrance. They demonstrate using a tamoxifen-driven ROSA26-LSL-tdTomato (tdT) reporter transgene that the NG2-Cre-ER does not label adult NSCs but only platelet-derived growth factor (PDGF) receptor α (PDGFRα)+ OPCs, proving the specificity of this model to OPCs. The gliomas arising in these mice were found to be most similar to the proneural (PN) subtype of glioblastoma (GBM) and maintain signatures resembling OPCs despite transformation. Interestingly, they found that these gliomas did not simply exhibit linear expansion of a cell population but rather underwent a multistep transformation with an early burst of proliferation followed by a dormant period with a final reactivation of proliferation. The tumor growth could be attributed not only to increased proliferation, but also to defective differentiation of transformed OPCs to oligodendrocytes.

Given the previously attributed role of mTOR signaling in similar reactivation processes in other stem cell types, the authors tested the effect of mTOR inhibitor temsirolimus by administering it to CKO mice. mTOR inhibition, as evidenced by reduction of its downstream target phosphorylated S6 ribosomal protein (pS6), caused reduction of OPC cell numbers due to G1 to S cell cycle transition arrest. Treatment of transformed primary OPC-derived glioma cells with temsirolimus in vitro also caused proliferation arrest, demonstrating the utility of mTOR inhibition in growth suppression of gliomas.

This study convincingly demonstrates that transforming OPCs give rise to gliomas and that mTOR signaling plays a vital role in this process. While conventional mTOR inhibition is met with resistance in GBM patients, it remains to be seen if the newer rapamycin derivatives such as temsirolimus could be effective in these patients, in particular those exhibiting the PN subtype.

Specific targeting of pediatric brainstem gliomas with histone demethylase inhibitors

Over recent years, the identification of novel brain tumor driver mutations by next-generation sequencing has led to the remarkably rapid development of new and promising therapeutic strategies. For example, a few short years after the identification of recurrent IDH1 mutations in malignant glioma, novel mutant IDH1-specific small molecule inhibitors have been developed and the first clinical trials are now underway. Similarly, the recent identification of H3F3A (Histone 3.3) mutations in diffuse intrinsic pontine glioma (DIPG) and in pediatric malignant glioma has created unique therapeutic opportunities. A study recently published by Hashizume et al in Nature Medicine4 has shown that these mutations confer unique drug sensitivities in preclinical cellular and animal models of brainstem glioma that may be of great clinical significance in these notoriously challenging tumors.

Previously published genomic sequencing studies identified the presence of recurrent monoallelic mutations of H3F3A in the majority of pediatric brainstem gliomas.1 The most common mutation observed is K27M, which removes a key post-translational methylation site that is vital for regulation of the H3F3A function and chromatin remodeling. The K27M mutation has been shown to have a dominant effect, as a result of sequestration of the histone lysine methyltransferase EZH2, which ultimately leads to a decrease in total cellular H3K27 methylation. Therefore, it is thought that the H3F3A K27M mutation suppresses histone methylation and promotes gliomagenesis by altering chromatin structure and gene expression programs favoring tumor growth. The authors of this study hypothesized that this reduction in methylation could be prevented by inhibition of demethylase activity to compensate for the
effects of the dominant driver K27M mutation in these tumors. The authors chose to use a small molecule inhibitor (GSKJ4) of the demethylases JMJD3 and UTX that are known to demethylate H3K27. GSKJ4 selectively inhibited growth of cells carrying the K27M mutation compared with malignant glioma cells not driven by this mutation. Demethylase inhibition by GSKJ4 induced apoptosis, reduced the numbers of cells in S phase, and completely blocked clonal growth of H3F3A K27M-expressing cells. siRNA studies showed that JMJD3 knockdown inhibited the growth of K27M cells, and GSKJ4 had no additional effect on these cells. In contrast, siRNA depletion of UTX had little effect. This suggests that inhibition of JMJD3 by GSKJ4 is responsible for its effects on K27M mutant cell lines.

In vivo studies showed that GSKJ4 treatment of nude mice bearing subcutaneous and brain stem xenografts of H3F3A K27M glioma cells reduced tumor growth and prolonged animal survival, and importantly, GSKJ4 was able to enter the brain of treated animals. This study is the first demonstration that targeting demethylase enzymes in H3F3A K27M expressing brainstem gliomas is a promising therapeutic strategy that targets the specific vulnerability created as a result of this novel driver mutation. This will hasten the development of a new generation of drugs targeting demethylases that have pharmacokinetic and toxicity profiles suitable for use in patients with this incurable and fatal disease.

Pharmacodynamic and therapeutic investigation of focused ultrasound-induced blood-brain barrier opening for enhanced temozolomide delivery in glioma treatment

Focused ultrasound (FUS) can be used to deliver concentrated ultrasound energy to tissue. In the brain, the combination of focused ultrasound and microbubbles has been shown to disrupt the blood-brain barrier (BBB) and promote the delivery of intravascular constituents into the brain parenchyma. Examples include chemotherapeutics, biologics such as trastuzumab (Herceptin) and antibodies to beta-amyloid, small interfering RNA, and even stem cells. FUS has been extensively explored as a way to improve drug delivery to gliomas in animal models.

Following up a previous study from 2013, Liu and colleagues recently examined the impact of FUS on delivery of temozolomide (TMZ) to a U87 mouse glioma model. Unlike their previous work, the authors were able to directly measure TMZ concentrations in tumor and normal brain, providing insight into the effects of FUS on TMZ pharmacodynamics. To do this, the authors implanted U87 cells into the striatum of mouse brains. The presence of tumor was confirmed by MRI and the animals were orally dosed with TMZ. Tumor and contralateral normal brain were exposed to focused ultrasound for 60 seconds following intravenous administration of microbubbles. Plasma and tissue concentrations of TMZ were monitored with liquid chromatography/mass spectrometry.

The authors found that FUS resulted in a 2.7-fold increase in the concentration of TMZ in brain tissue. FUS caused only a slight increase in the concentration of TMZ in tumors, but this was not statistically significant (P = 0.12). However, the degradation rate of TMZ was reduced in tumors following FUS treatment. Therefore, the average TMZ concentration over time was higher in tumors exposed to FUS. The authors also assessed the impact of FUS on tumor growth and mouse survival. Tumor growth was suppressed with TMZ + FUS in comparison with TMZ alone, particularly for lower TMZ (2.5 mg/kg) doses. The addition of FUS to oral TMZ (2.5 mg/kg) increased median survival from 40 to 45 days. Furthermore, all 6 animals treated with 25 mg/kg TMZ and FUS survived longer than 70 days, whereas only 50% of animals that received 25 mg/kg TMZ alone survived for a similar period. Thus, the authors demonstrated that FUS alters TMZ pharmacodynamics in normal brain as well as in tumor tissue and that FUS prolongs survival in mice treated with varying concentrations of TMZ, confirming their earlier findings.

One potential caveat for the translation of these studies to human patients is that the “leakiness” of glioblastoma vessels may be greater than that of implanted U87 tumor vessels, which could diminish the beneficial effect of FUS-induced opening of the BBB. However, as the authors point out, the BBB tends to be more intact at the margins of the tumor where infiltration of the adjacent brain parenchyma occurs; targeting these areas may help to diminish the potential for tumor recurrence and spread. Thus, the current work adds to a growing body of literature showing that FUS can increase the effectiveness of TMZ and other clinically used anti-glioma drugs in preclinical models of gliomas, suggesting that human clinical trials may be not far off.

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Streamlining diagnostic classification of diffuse infiltrating gliomas

Among the limitations of the current WHO classification for grade II and III diffuse gliomas are high interobserver variability among neuropathologists and the failure to incorporate molecular characteristics that convey ontogenic and prognostic significance. To address this, the Haarlem consensus guidelines...
proposed an integrated neuropathologic diagnosis that layers histologic features, tumor grade, and pertinent molecular data as a basis for the upcoming WHO revision.\(^1\)

In a recent article, Reuss and colleagues\(^2\) highlight the value of the molecular classification of currently defined low- and intermediate-grade gliomas. A series of 405 archived diffuse gliomas, all graded II/III by WHO 2007 criteria, underwent thorough molecular analysis including immunohistochemistry for the common IDH1 R132H mutation, 1p/19q codeletion status, ATRX nuclear immunostaining, and 450 K Illumina chip for copy number data. Tumors lacking staining for mutant IDH protein underwent sequencing of IDH1 and IDH2; 7p gain, 10q loss TERT promoter, EGFR amplification status, and H3F3A status were also assessed when pertinent. All tumors were then assigned a new integrated diagnosis adhering to the Haarlem guidelines. The diagnoses of mixed glioma and glioblastoma with oligodendrogial component were excluded by defining 1p/19q codeletion as necessary and sufficient for oligodendroglioma diagnosis. Anaplastic astrocytomas with wild-type IDH, 7p gain, and 10q loss were reclassified as glioblastoma. These analyses confirmed the value of immunohistochemistry for ATRX nuclear staining.\(^3\) Fully 97% of tumors with their integrated diagnosis classified as astrocytomas had loss of ATRX staining, compared with 70% of astrocytomas defined according to the 2007 WHO classification. Similarly, only 3% of oligodendrogliomas by integrated diagnosis had loss of ATRX expression. 1p/19q codeletion and loss of nuclear ATRX expression were mutually exclusive, and the latter was a highly sensitive and specific marker for grade II-III astrocytomas. This further supports the contention that from the molecular standpoint the diagnosis of mixed glioma does not exist;\(^4\) in these investigators’ hands one third of mixed gliomas are oligodendrogliomas and two thirds are astrocytomas. Applying the integrated diagnosis to a subset of 100 anaplastic gliomas from the NOA-04 clinical trial\(^5\) predicted time to tumor progression and overall survival significantly better than did the purely histology-based WHO 2007 classification.

Based upon the value of ATRX immunohistochemistry for astrocytic tumors and the complementarity of 1p/19q codeletion and loss of nuclear ATRX expression, the authors proposed an algorithm for classifying grade II/III gliomas with the first step being immunohistochemistry for ATRX and IDH1 R132H. Tumors without ATRX loss undergo 1p/19q assessment. Codeleted tumors are oligodendrogliomas, whereas non-codeleted tumors undergo IDH sequencing; those that are IDHwt may well be glioblastomas and should be considered for further molecular testing. This approach has substantial potential value both in terms of cost (allowing many tumors to avoid molecular testing for 1p/19q codeletion) and rapidity of diagnosis (since many astrocytic tumors will be diagnosed with immunohistochemistry alone). These findings will require validation in other patient cohorts. Moreover, their algorithm places a heavy emphasis on ATRX immunohistochemistry; whether this antibody proves to be as easy to use as the H09 antibody for the IDH1 R132H antibody, and whether the results as reliably interpretable and reproducible in other neuropathology laboratories needs to be confirmed.

**References**