Research Highlights

Highlights from the latest articles on biomarkers for brain tumors

CD44 as a therapeutic target for glioblastoma

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Evaluation of: Xu Y, Stamenkovic I, Yu Q: CD44 attenuates activation of the Hippo signaling pathway and is a prime therapeutic target for glioblastoma. *Cancer Res.* 70(6), 2455–2464 (2010).

Glioblastoma multiforme (GBM) is the most common and aggressive form of primary human brain tumor. Despite major advances in the management and treatment of these tumors, the median survival remains dismal. Approximately 14,000 new cases of malignant glioma are diagnosed each year in the USA, with 60–70% of those tumors being GBM. Resistance of GBM to conventional chemotherapy and radiation therapy has necessitated a search for more effective therapies. Similar to other solid malignancies, the surrounding microenvironment provides a niche for GBM tumor progression and modulates the response of cancer cells to therapy.

The present study has analyzed the role of glycosaminoglycan hyaluronan receptor, CD44 in GBM progression and the response of GBM cells to chemotherapy. Mining of four independent gene expression data sets demonstrated consistent upregulation of CD44 transcripts in human GBM compared with normal brain. In addition, CD44 was overexpressed in the majority of GBM cell lines and all the GBM tumor tissues included in this study. Downregulation of CD44 expression inhibited subcutaneous growth of human glioma cells by inhibiting proliferation and promoting apoptosis. Knockdown of CD44 expression also inhibited intracranial (IC) growth of human gliomas and sensitized them to cytotoxic drugs. A combination of CD44 knockdown and treatment with the cytotoxic drugs temozolomide and carmustine, resulted in a synergistic inhibition of IC glioma growth with markedly prolonged median survival of the experimental animals.

The authors further studied the molecular mechanisms behind the chemosensitizing effects of CD44 downregulation, by analyzing GBM cell response to oxidative/cytotoxic stress. Downregulation of CD44 attenuated activation of the Hippo signaling pathway that plays an important role in restraining cell proliferation and promoting apoptosis. The cascade of events included sustained activation of JNK/p38 stress kinases, and upregulation of p53, p21 and puma. CD44 also modulated proliferation induced by EGF and HGF through activation of ERK phosphorylation. Finally, development of CD44 antagonist fusion proteins markedly inhibited growth of GBM and significantly extended survival of both immunocompromised and immunocompetent mice bearing the IC tumors. One particular antagonist showed widespread anti-GBM effects, inhibiting both hyaluronan-dependent and -independent CD44-mediated activities. On systemic delivery, it accumulated specifically into IC tumors with little accumulation in the surrounding normal brain.

CD44 is a major cell surface cancer stem cell marker in a number of tumors with its role in cancer stem cell physiology not well understood. It is a versatile molecule and participates in multiple functions, including modulation of diverse signaling pathways and regulation of interactions between tumor cells and the host tissue microenvironment and tumor cell responses to various forms of stress. Thus, CD44 may serve as an ideal therapeutic target to sensitize malignant gliomas and other cancers to radiation and chemotherapy.

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future medicine part of fsg

STAT3 phosphorylation: marker of aggressive glioblastomas

Evaluation of: Birner P, Toumangelova-Uzeir K, Natchev S, Guentchev M: STAT3 tyrosine phosphorylation influences survival in glioblastoma. *J. Neurooncol.* (2010) (Epub ahead of print).

The WHO divides astrocytomas into four grades. Of these, WHO Grade III (anaplastic astrocytoma) and WHO Grade IV (glioblastoma multiforme [GBM]) are the most malignant subtypes. Gliomas, including GBM show aberrant expression of a number of signaling pathways. One such pathway is JAK/STAT, which drives the expression of a large number of genes, such as VEGF, IL-10 and Bcl-2. JAK/STAT signaling also induces an important family of negative regulators. It is critical for the growth and survival of a variety of human malignancies, making it a focal point of cancer research. In GBMs, one member of the STAT family, STAT3, was shown to be constitutively activated by tyrosine phosphorylation at a single site (Y705) close to the carboxy terminus. In addition, it is one of the master regulators of mesenchymal transformation, leading to the mesenchymal phenotype that is a hallmark of tumor aggressiveness in GBM.

The current study investigated the prognostic relevance of activated STAT3 in 111 patients with supratentorial glioblastomas and 25 patients with grade III gliomas. The authors determined cellular phosphorylated STAT3 (pSTAT3) status by immunohistochemistry and correlated it with an overall survival. Overall survival was defined as the period from primary surgery until death of the patient. They observed pSTAT3-positive staining in 76.6% of GBM samples and 72% of glioma cases. In most GBM samples, strong nuclear staining was observed. The number of cells with positive pSTAT3 staining correlated significantly with patient survival. On multivariate analysis the patient's age and the number of pSTAT3-positive tumor cells were found to be independent prognostic factors for worse outcome in GBM.

Previous studies have demonstrated conflicting roles for STAT3, sometimes acting as a tumor suppressor and sometimes acting as an oncogene. The current study demonstrated that in GBM the pSTAT3 activation correlated with shorter overall survival, thus indicating an oncogenic function of STAT3. How activation of STAT3 influences patient survival in GBM remains unclear. There are ongoing Phase 0 and Phase I clinical trials for STAT3 inhibitors; however, the role of STAT3 in human cancers needs to be fully understood. GBMs presents a much more complex picture, with major challenges in both delivery and pathway targeting. If proven, selection of a target like STAT3 could be of interest to intervene against both the traditional tumor cell as well as the tumor stem cell as a GBM therapeutic modality.

MGMT promoter methylation as a predictive biomarker of radiation response

Evaluation of: Rivera AL, Pelloski CE, Gilbert MR *et al.*: MGMT promoter methylation is predictive of response to radiotherapy and prognostic in the absence of adjuvant alkylating chemotherapy for glioblastoma. *Neuro Oncol.* 12(2), 116–121 (2010).

Glioblastoma (GBM), the most common primary brain tumor in adults, is usually both aggressive and fatal. The current standard treatment for newly diagnosed GBM is based on a Phase III trial conducted by the European Organization for Research and Treatment of Cancer (EORTC) and includes surgical resection followed by adjuvant radiotherapy plus an alkylating agent temozolomide (TMZ). The improved overall survival in GBM patients treated with radiation plus TMZ is due, in part, to decreased levels of *O*6-methylguanine-DNA methyltransferase (MGMT) protein attributed to epigenetic silencing mediated by *MGMT* gene promoter methylation. *MGMT* is a ubiquitous DNA repair enzyme and its status can be correlated to tumor cell sensitivity to TMZ therapy. Although, its role in predicting TMZ response has been established, its predictive value for response to radiation has not been well investigated.

The current study examined *MGMT* promoter methylation as a predictive marker of response to radiotherapy in 225 GBM specimens collected from patients treated with radiation alone and analyzed its association with clinical outcomes. Out of 225 cases, 183 were evaluable to assess the response to radiotherapy. In this cohort, the group of GBM patients with an unmethylated *MGMT* promoter had a tumor progression rate that was twice that seen in methylated cases (58 vs 29%, respectively). When the median overall survival time was examined it was found that patients with methylated *MGMT* had better survival (63 weeks) compared with patients with unmethylated tumors

(51 weeks). The actuarial 2-year overall survival rate in patients with tumors with *MGMT* promoter methylation was nearly double when compared with the *MGMT* unmethylated group (30 vs 16%). Thus, the *MGMT* promoter methylation status was observed to be an independent prognostic factor for tumor progression and overall survival when age, performance status and extent of resection were accounted for. The authors claimed that *MGMT* promoter methylation is a prognostic biomarker in GBM even in the absence of chemotherapy with alkylating agents and may represent a surrogate marker of a more treatment-responsive tumor in general.

Although the mechanism for TMZ sensitivity in methylated tumors is well established, there is no evidence showing that *MGMT* is involved in the repair of radiation-induced DNA damage. It is possible that there is an overlap of other DNA repair pathways that result in altered repair of methylated tumors after radiation. Alternatively, *MGMT* promoter methylation may be a surrogate marker for an unidentified process that contributes to the overall aggressive biology of GBM tumors.

MGMT promoter methylation is on the verge of entering clinical decision-making and is currently used to stratify GBM patients for clinical trials. However, in the Phase III EORTC trial the modest effect of TMZ in patients lacking *MGMT* promoter methylation have raised new questions (i.e., whether TMZ should be withheld from patients with tumors that lack *MGMT* promoter methylation). Therefore, reviewing all the available data, the current study included suggests that more convincing data are needed to establish a link between *MGMT* methylation, radiation and TMZ treatment in GBM.