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Submitted abstracts
Innehåll

The broad reach of BET inhibitors in Myc-induced cancers - opportunities for combination treatments of glioma .................................................................3

Modeling the glioma perivascular niche ........................................................................................................5

Establishment and characterization of experimental in vivo models of pediatric brain tumors ........6

Understanding the dynamic interplay between genetically different cancer cell clones in glioblastoma .................................................................................................................................7

Case study of circulating tumor cells for monitoring of glioblastoma multiforme patients .................8

Update of valganciclovir add-on therapy in glioblastoma. Effect in newly diagnosed and in recurrent patients. ..........................................................................................................................8

Pilot analysis of early treatment response by parametric response maps in malignant glioma. ....9
Abstract

Bromodomain and extraterminal (BET) proteins bind acetylated proteins, including histones, and regulate transcription. Recently, BET inhibitors (BETi) have been developed that show promise as potent anti-cancer drugs against various solid and hematological malignancies, including glioma. We recently showed that that BET inhibition exhibited global transcriptional effects in cancer cells from Myc-transgenic mice (Bhadury et al., PNAS 2014). Contrary to the proposed mechanism of action, Myc transcription was not affected suggesting that Myc suppression is not required for BET inhibitors to exert anti-cancer effects. Moreover, we discovered that the induced genes were similar to those induced by histone deacetylase inhibitors (HDACi); and HDACi synergized with BETi. Follow-up studies resulted in the unexpected discovery of compounds whose mechanism of action point to that BET inhibitors have been tested in several clinical trials over thirty years ago. The identification of these old BET inhibitors can provide important clues on which patients will benefit from this promising class of anti-cancer drugs. We have now shown that one these compounds can halt the proliferation of solid cancer cancer cells as well, including lethal diseases such as glioma and uveal melanoma. Interestingly, combination treatment with an FDA-approved targeted therapy enhanced the effect of BET inhibition, resulting in cell death. We are now developing one of the old compounds for clinical use again by both medicinal chemistry efforts, as well as identification of a biomarker of response to guide which glioma and melanoma patients are sensitive to the combination therapy.
High-throughput screening (HTS) for the identification of compounds capable of interfering with astrocyte-dependent growth of glioblastoma cells

Alessandro Mega

Abstract

Glioblastoma multiforme (GBM) is among the most lethal tumor types. The pathological tissue contains different tumor-associated cell types, including astrocytes, which contribute to cancer biology. Our work aims at exploring the potential role(s) of crosstalk between astrocytes and malignant cells in GBM with regard to growth and drug response of the malignant cells. According to completed studies, GBM cell lines, as well as a panel of primary GBM cultures, have shown increased growth upon co-culture with astrocytes. Ongoing studies are performed with the double intention of identifying the molecular details of the underlying pathogenic paracrine crosstalk and identification of small molecule inhibitors able to interfere with astrocyte-dependent GBM growth. A HTS has been established to screen low molecular weight compounds. After a pilot screening of 1700 compounds (Prestwick + Enzo libraries), 63 molecules have been identified and are currently being validated. Of these molecules, 10 specifically inhibit GBM in co-culture and 53 only have an effect on GBM monoculture. Interestingly, temozolomide (the current standard of care for GBM) fell in the category of drugs that only inhibit GBM monoculture, suggesting a protective role of the astrocytes on the malignant cells. More libraries will be screened in upcoming studies. Future studies will continue efforts to develop in vivo-active inhibitors of astrocyte-dependent growth. Inhibitors will also be used in chemical biology studies to help identifying molecular pathways involved in the growth-supportive effect of astrocytes.
Modeling the glioma perivascular niche

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Abstract

Despite aggressive treatment, essentially all glioblastoma multiforme (GBM) tumors recur as incurable lesions. Tumor cells with stem cell characteristics are thought to be responsible for therapeutic resistance in brain tumors. Such cells reside specifically within a perivascular niche (PVN) thought to maintain their aggressive and stem-like phenotype. The heterotypic cell-cell and matrix-cell interactions that define the niche, as well as the signaling pathways controlling tumor cell stemness, remain poorly understood. Furthermore, most identified drivers of the aggressive stem-like phenotype are essentially non-druggable transcription factors. It is likely that interfering with stem cell signaling pathways is key to sensitize resistant cells to standard DNA damage-inducing therapeutics not only in glioma, but also in other solid tumor types. Using genetically engineered mouse models of GBM, we are mapping the therapy-resistant PVN to (1) identify novel niche components, and (2) understand the signaling pathways underlying therapeutic resistance, with the overall aim of developing novel therapeutic strategies targeting the resistant stem cell character of PVN tumor cells. We recently identified Osteopontin-CD44 signaling as one PVN-specific pathway controlling glioma stemness by signaling through hypoxia-inducible factors, resulting in a pseudo-hypoxic phenotype of PVN glioma cells. Our findings have revealed novel pathways in tuning of the hypoxic response in both well- and poorly oxygenated tumor areas. We are currently developing strategies to exploit these new insights in therapeutic targeting of tumor stemness and pseudo-hypoxic phenotypes in glioma and other solid cancers.
Establishment and characterization of experimental in vivo models of pediatric brain tumors

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Abstract

Established tumor cell lines are extensively used for screening of novel therapeutic compounds. However, during long-term culturing cells adjust to these conditions and may acquire additional phenotypic or genetic aberrations, which may cause them to differ significantly from the original tumor. Predisposed genetic mouse models more closely recapitulate several aspects of human disease but they usually require complex breeding schemes and may suffer from incomplete tumor penetrance and a variable age of tumor onset. In order to establish reproducible transplantable pediatric brain tumor models for therapeutic studies, we have acquired (1) primary tumor cells of a human group 3 medulloblastoma, (2) isolated tumor cells from the retrovirally induced glioma mouse model PDGFB;Trp53-/- and (3) a glioma cell line (KR158b), originally established from the genetic mouse model Nf1-/-;Trp53-/-;cis. Tumor cells were stereotactically injected into the cerebellum of NOD SCID mice (medulloblastoma) or the cerebrum of C57BL/6 mice (gliomas). Mice developed brain tumors with a latency of 17-18 weeks (medulloblastoma) and 30-70 days (gliomas). The medulloblastoma model has to date been serially transplanted for >3 generations and replicates the histology and immunoprofile of the primary tumor. All three models will be further characterized with regards to histology, immunophenotype, stromal compartments, cytokine secretion and methylation profile, and then utilized for studying the treatment efficacy and immune modulation following intratumoral cytostatic deliveries and immunotherapeutic approaches.
Understanding the dynamic interplay between genetically different cancer cell clones in glioblastoma

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Abstract

Glioblastoma is the most frequent and aggressive type of brain tumor in adults. Intratumoral heterogeneity is a hallmark of glioblastoma and has been suggested to be a key-contributing factor for their aggressiveness and difficulty to treat. Gene expression based analyses classify glioblastoma tumors into four subclasses: proneural, neural, classical and mesenchymal. However, recent publications have shown that individual glioblastomas are heterogeneous and contain a mixture of cells that display gene expression patterns representing these four subclasses. Moreover, it has been shown that interclonal communication between genetically distinct glioblastoma tumor subclones can affect the overall tumor growth.

To further investigate the impact of genetically heterogeneous glioma cell populations we have used the U343 cell culture system, which consists of a set of cell clones derived from a single glioblastoma, including U343MG, U343MGa, U343MGa-31L and U343MGa-CI2:6. We found that U343MG display invasive capacity in vitro and express relatively higher mRNA levels of mesenchymal markers, including SNAI2, FN1 and LAMC1. In contrary, the other clones were less invasive and expressed high mRNA levels of the stem cell marker SOX2 and the astrocytic marker GFAP. By genomic copy number analysis a set of common gains and losses indicated a common tumor cell ancestor, while specific alterations illustrated how the different clones have genetically diverged during the tumor evolution.

By combinatorial co-culture and conditioned media based experiments we found that the U343 clones affected each other’s proliferation rate via secreted factors. By Secretome Protein Enrichment with Click Sugars (SPECT) followed by mass spectrometry analysis we have identified proteins that are secreted by the U343 cultures. Following this, functional genomic approaches will be taken to identify what specific proteins are eliciting these inter-clonal signaling effects.

This study shows that subclones from a heterogeneous glioblastoma can display different phenotypic characters including invasive capacity and gene expression patterns, and may affect each other via secreted factors. Knowledge about cell-to-cell communication in glioblastoma may provide novel therapeutic targets.
Case study of circulating tumor cells for monitoring of glioblastoma multiforme patients

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Abstract

Detection of circulating tumor cells (CTCs) is proposed as a prognostic marker of aggressive development of solid tumors. Here we describe use of CTCs for monitoring of a disease status for 4 patients with glioblastoma multiforme. CTCs were recovered from the blood of patients, and cultured in vitro to assess their proliferation, spheroid formation capacities and expression of E-cadherin and vimentin. We observed that the low recovery of CTCs correlated with a stable disease, and the high recovery of CTCs correlated with a progressive disease. Therefore, the reported case study confirms presence of CTCs in GBM patients, and show potential prognostic value of CTCs recovery.

Update of valganciclovir add-on therapy in glioblastoma. Effect in newly diagnosed and in recurrent patients.
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Abstract

Background: Since the overall survival of patients with glioblastoma is still very limited with current combined therapy of surgery and radiochemotherapy, we have studied the potential effect of adding valganciclovir early after diagnosis, or at relapse. Our earlier retrospective results on 50 patients have shown significant prolonged survival ; the median survival increased to 24 months and the 2 years survival was 62%.

Method: This is an updated retrospective analysis of glioblastoma patients treated at a single institution. Valganciclovir tablets 450mg 2x2 were given during the initial 3 weeks as induction therapy, followed by maintenance 1 x 2 continuously, until disease progression and/or late palliative situation. Ten patients receiving valganciclovir during shorter period are not included : early progression (3), hematologic toxicity grade III (1), non compliance (4) patient stopped intake early (2).

Results: Seventy-six patients with newly diagnosed glioblastoma received valganciclovir for 6 months (which was the duration of the first phase II exploratory study: VIGAS) or longer. The follow-up is at least 1 year. The median 2-year survival is 80 %. Of the patients that have died, the median survival is 5 years (9-72 months, n=56), the patients with shorter survival had partial resection. Twenty patients are alive, and the median survival is > 20 months , the longest survival so far is > 8½ years. In twenty-five patients with recurrence, valganciclovir was added to salvage therapy ( 3 underwent reoperation before starting valganciclovir), relapse treatment was heterogeneous (second line chemotherapy-CCNU in general, a few received re-irradiation up to 34 Gy and 3 radiosurgery). The median survival after recurrence was 9 months (1-24 months, n=25).

Conclusion: The addition of valganciclovir prolonged survival in newly diagnosed glioblastoma patients. There was no additional toxicity when valganciclovir was combined to chemotherapy. The effect in recurrent patients is more limited, because patients were treated late. Earlier therapy at recurrencein patients with good function may have better effect. Larger randomized trials of anti CMV therapy are planned.
Pilot analysis of early treatment response by parametric response maps in malignant glioma.

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Purpose: The postoperative treatment for patients newly diagnosed with malignant glioma is currently limited to one standard regime of concomitant radiotherapy with Temozolomide (TZ). The aim of this study was to identify non-responders to standard treatment at an early stage using the parametric response map (PRM) [1], which assesses voxel-wise progression of quantitative MRI parameters.

Methods: A pilot PRM study was performed on five patients newly diagnosed with malignant glioma, operated with either resection or biopsy. The patients received postoperative concomitant radiotherapy according to standard regime with TZ. Eight months postoperatively when standard treatment had been completed, the patients were classified using conventional MRI and clinical methods, as either having stable disease (SD, including partial response) or progressive disease (PD). For the purpose of this study, MRI examinations were performed at two additional time points: before postoperative treatment start (w0) and three weeks into treatment (w3).

The imaging protocol included T1-weighted post gadolinium (T1W-Gd), diffusion weighted imaging (DWI), and dynamic susceptibility-weighted contrast (DSC) MRI. Mean diffusivity (MD) maps were calculated from the DWI data and cerebral blood volume (CBV) maps were calculated from the DSC MRI data. The CBV maps were normalized to create relative CBV (rCBV) maps, using the mean cerebellar value as a reference, defined using FreeSurfer [2]. All MRI images were spatially co-registered to the T1W-Gd image at w0 using Elastix [3,4].

The PRM procedure was performed for MD, and is illustrated for two patients in Figure 1. At both time points the contrast enhancing parts of the tumours were defined and manually delineated as regions of interest (ROI, row 1-2, green). PRM (row 3) were created in the intersection of the w0 and the w3 ROI (row 1-2, red) by voxel-wise subtraction of the w0 image from the w3 image. Voxels with significantly increased or decreased MD at w0 compared to w3 were colored red or blue, respectively, while unchanged voxels were colored green. Scatter plots of voxel MD values at w0 versus w3 are shown in row 4. The white diagonals correspond to the thresholds for determining significant change (±0.55 μm²/ms). The plot shows the percentages of voxels where the MD increased (PRMMD, red), decreased (PRMMD, blue) and was unchanged (PRMMD, green). In this study PRMMD and PRMMD were compared between the PD-group and the SD-group.

Results: Out of the five patients, two were classified as having SD and three were classified as having PD eight months postoperatively. Already at three weeks after postoperative therapy start the SD group exhibited a distinctly higher PRMMD than the PD group: 11 and 8 % versus 3, 3, and 1 %. This is shown in Figure 2 by a box plot of the distribution of PRMMD, and PRMMD for the two groups. Figure 1 shows PRM and scatter plots for two example patients. The SD patient (left) exhibited both increase (red) and decrease (blue) locally, while the PD patient was mostly unchanged (green). The PRM analysis of rCBV is pending due to issues with co-registration.

Discussion and Conclusions: Previous PRM studies of malignant gliomas have shown that, three weeks into postoperative standard treatment, PRMMD predicted treatment response and improved survival [5,6], whereas PRMMD predicted a worse survival [1,7]. Pure ROI-based metrics, such as ROI mean rCBV, however, have been shown less reliable in predicting outcome [1]. Suggested mechanisms for the increase in MD include an increased extracellular space due to treatment-induced tumor cell kill [8]. The preliminary results of this pilot study support PRMMD at three weeks as an early biomarker of treatment response. A limitation of the study was small sample size.

In conclusion, PRM based on diffusion metrics may be a tool for early prediction of treatment response for malignant gliomas. This could open up opportunities for individualized therapy.