at D9, p=0.05 at D12), whereas the other antibodies did not slow down tumor growth significantly. If combined with radiotherapy, anti-VEGF-A showed no variations to the control arm. Unexpectedly, the combination of radiotherapy with anti-Ang-2/VEGF-A (p=0.01 at D15), and even more with anti-Ang-2 (p=0.04 at D9, p= 0.008 at D12, p=0.003 at D15) lead to an increased tumor diameter.

In conclusion, vascular normalization was most effectively achieved by dual targeting of Ang-2 and VEGF-A, which resulted in improved response to chemotherapy, but attenuated effectiveness of radiotherapy. These findings are important for the development of novel antiangiogenic strategies, and planning of future clinical trials in glioblastoma.

OS3.2 DELTA-AMINOLEVULINIC ACID CAN DECREASE THE RADIORESISTANCE OF GLIOMA STEM CELLS WITH MESENCHYMAL PHENOTYPES IN VITRO AND IN VIVO

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INTRODUCTION: High-grade gliomas (HGGs) is divided into 3 major subtypes based on core gene signatures; mesenchymal (MES), proneural (PN) and proliferative. Among them, HGGs profiled in the MES subtype tend to show worse prognosis than others. Recently, also as for glioma stem cells, two mutually exclusive subtypes with distinct dysregulated signaling and metabolic pathways has been identified; MES-GSC and PN-GSC. It is known that MES-GSC displays more aggressive phenotypes and is markedly resistant to radiation as compared with PN-GSC. Delta-aminolevurinic acid (ALA) is the first metabolite in the porphyrin synthesis and the exogenous administration of ALA increased the endogenous production of photosensitizer protoporphyrin-IX (PpIX) in HGG tumor cells. Recently, several reports have revealed that ALA has an ability to enhance the therapeutic efficacy of X-ray radiotherapy (XRT) for various malignant tumors in vitro and in vivo. The aim of the present study is to investigate whether ALA can decrease the radioresistance of GSCs.

MATERIAL & METHODS: We used 3 MES-GSCs and 2 PN-GSCs established from surgical specimens of human malignant gliomas. We defined the above GSCs cultured in an adherent condition with FBS and without glutamine for more than 2 weeks as glioma "differentiated" cells (GDCs). Firstly, we quantified the intracellular PpIX in the GSCs and their corresponding GDCs by FACS and LC. Then, we assessed the in vitro therapeutic effect of ALA-XRT on them; MTT-assay, sphere- or colony- forming assay, and apoptosis assay were performed. We made an intracerebral glioma model using one MES-GSC to evaluate in vivo therapeutic effect of ALA-XRT; MES-GSC transplanted mice were randomly divided into 4 groups, ALA-XRT, XRT only, ALA only and control. Total 30 Gy in 3 Gy fractions of X-ray was irradiated to the mice in 1st and 2nd group. The intraperitoneal injection of 240 mg/kg ALA was done 6 hours prior to each irradiation for the mice in 1st and 3rd group.

RESULTS: 1) All MES-GSCs showed significantly higher PpIX accumulation than their corresponding MES-GDCs. Meanwhile, two PN-GSCs showed lower intracellular PpIX level than their corresponding PN-GDCs. 2) All MES-GSCs showed higher resistance to X-ray than their corresponding MES-GDCs, but the radioresistance of MES-GSCs significantly decreased by ALA in vitro. 3) ALA significantly increased the ROS production during XRT in a dose dependent manner both in MES-GSCs and MES-GDCs in vitro. 4) In the in vivo therapeutic experiment, median survivals of control, ALA-only, X-ray only, ALA-XRT group were 19, 22, 30, 38 days, respectively. (ALA-XRT vs. XRT only: p=0.02, Control vs. XRT only: p<0.001, Control vs. ALA only: p=0.09)

CONCLUSIONS: MES-GSCs had a higher activity of porphyrin synthesis from exogenous ALA than PN-GSCs. In fact, the redioresistance of MES-GSCs can be effectively decreased by the concomitant use of ALA both in vitro and in vivo.

OS3.3 ROLE OF PERICYTES IN ANGIOGENESIS-DEPENDENT GROWTH OF MALIGNANT GLIOMA

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BACKGROUND: Despite advances in multimodal therapy, the prognosis for patients with malignant glioma remains dismal. Aberrant angiogenesis and diffuse glioma cell invasion are major obstacles to effective treatment. We have established two sibling glioma subclones, J3T-1 and J3T-2, which show different invasive and angiogenic phenotypes. One exhibits angiogenesis-dependent cell growth and no single-cell invasion, and the other exhibits massive single-cell invasion without angiogenesis. Pericytes were recently demonstrated to have important roles in angiogenesis and tumor infiltration. In normal brains, pericytes play critical roles, including supporting vascular structures, maintaining the blood-brain barrier, and initiating vessel sprouting. The significance of multi-layered pericytes in malignant glioma was previously unknown. In this study, we investigated the role of pericytes in human glioma specimens and our glioma models.

METHODS: Histopathologic analysis of animal glioma models and human glioma specimens was performed. Quantitative analysis of CD31 (marker of endothelial cells)-positive or α-smooth muscle antigen (α-SMA, marker of pericytes)-positive vessels was performed by immunohistochemical staining. $CD31^+$ or $\alpha\text{-SMA}^+$ microvessel number density (MVND) and the proportion of pericyte-covered vessels were calculated.

RESULTS: In human glioma specimens, $CD31^+$ MVND and $\alpha\text{-SMA}^+$ MVND were significantly increased with increasing glioma grades. The proportion of pericyte-covered vessels also increased as the malignancy upgraded. Pericytes were monolayered in grade II glioma, and no multilayered pericytes were identified. In grade III and IV glioma, the proportion of multi-layered pericyte-covered vessels was 11.0 and 16.9%, respectively. In both of our animal glioma models, approximately half of the vessels were covered with pericytes. Multi-layered pericytes were only observed around neovasculatures with glioma cell cuffing in J3T-1 tumor.

CONCLUSION: In our glioma models, multi-layered pericytes were only identified in the angiogenesis-dependent growth phenotype (J3T-1) and showed a similar vascular structure as human malignant glioma specimens. These results suggest that multi-layered pericytes may act as a scaffold of glioma migration around neovasculature. Our glioma models are suitable for the molecular and pathologic analysis of angiogenesis and the development of novel therapeutic strategy targeted for angiogenesis.

OS3.4 EFFECT OF BEVACIZUMAB ON THE MORPHOLOGY AND FUNCTION OF NEURONS AND GLIAL CELLS

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VEGF (vascular endothelial growth factor) is a dimeric polypeptide which acts primarily through its binding to VEGFR-2. The main effects of VEGF are proliferation of endothelial cells, angiogenesis and increased vascular permeability. VEGF also plays an important role in brain development and function. In glioblastoma multiforme (GBM) it stimulates neoangiogenesis and bevacizumab, a VEGF antibody, is used in GBM treatment. Recently, it has been reported that treatment with bevacizumab may cause a decline in neurocognitive function of GBM patients.

AIMS: The aim of this project is to analyze the effects of VEGF and bevacizumab on the morphology and function of neurons and glial cells.

METHODS: Dissociated cortical and hippocampal cell cultures of juvenile rats were treated with VEGF, bevacizumab and VEGF + bevacizumab. The morphology and cell viability were analyzed via immunocytochemistry. Gene and protein expression were investigated by quantitative PCR and western blot, respectively. Hippocampal slices were used for field excitatory postsynaptic potential recording and patch clamp analyses in the CA1 region to investigate neuronal function.

RESULTS: Bevacizumab significantly decreased the number of cortical neurons and glial cells after long-term incubation. Additionally, an increase of the dendritic length was obvious after 10 days of incubation with bevacizumab, but returned to control level after 30 days. In hippocampal cultures, cell viability was not affected by bevacizumab; however, the dendritic length increased at day 10, but decreased after long-term treatment. Moreover, bevacizumab upregulates LTP-associated genes (long-term potentiation) after 10 days (PKCα and CaMKIIα), but downregulates NMDA receptor gene expression after 30 days. Preliminary results on the protein level showed a decrease in CamKII α in hippocampal cells after 10 days of incubation with bevacizumab. Besides this, LTP, passive and active properties of hippocampal neurons are significantly impaired with bevacizumab.

CONCLUSIONS: Bevacizumab has a cytotoxic effect in cortical cultures, decreases the dendritic length in hippocampal neurons after long-term treatment, and impairs hippocampal function.

OS3.5 GENE EXPRESSION LANDSCAPE INCLUDING MIRNAS OF DELAYED RADIATION NECROSIS IN BRAIN

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IINTRODUCTION: Delayed brain radiation necrosis (DBRN) appears months to several years after radiotherapy for brain diseases