a molecularly diverse panel of GBM patient-derived cancer stem-like cells (CSC) and orthotopic mouse xenografts (PDX). Transforming to a normal distribution (log10), 4/13 of cell lines had a PDGFRA mRNA expression (RPKM) higher than 1.5. Similarly, one PDX line had a staining index of greater than 10, 11 (27.5%) had an index between 5–10. The range of intra-tumoral variance, represented by standard deviation, was 0.09–24.25 highlighting the heterogeneity of PDGFRα expression. PDGFRα phosphorylation (Y754) did not differ between 8 cell lines cultured in NMGF, but deviated in alternate medias without growth factors, supplemented with FBS. In comparison, MYC(N) mRNA expression is only elevated in the context of a known amplification. Furthermore, a a MYC activity signature consisting of 18 target genes was only evident in the 5 amplified CSC lines. Taking advantage of genomic heterogeneity, we have isolated subclones lacking PDGFRA amplification from a PDGFRA amplified GBM CSC. The absence of PDGFRA amplification reduced the self-renewal potential to 37% of the PDGFRA amplified cell population (p=0.001) in clone 1 and 57% in clone 2 (p=0.013). Pertaining to determinants of in vivo survival, MYC was altered in 80% of short survivors (2/5 MYC, 2/5 MYCN amplification) and in 0% of long survivors. Myc signature was highly correlated with in vivo survival (Pearsons' corr. = -0.77) and MYC gene expression was correlated with in vivo TMZ resistance (corr. = 0.7). **CONCLUSION:** These results suggest that PDGFRα expression and activity can occur in the absence of gene amplification, while Myc activity is dependent on gene amplification. Both oncogenes drive oncogenic pathways that should be explored as therapeutic targets.

P11.55 THYMOQUINONE ENHANCES THE SENSITIVITY OF B16-F10 MELANOMA CELL LINE TO GAMMA KNIFE RADIOSURGERY

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BACKGROUND: Melanoma patients with brain metastasis have poor prognosis. Gamma Knife radiosurgery (GKR) is an effective treatment to control brain metastasis from melanoma. Thymoquinone (TQ) has gained attention as a potential therapeutic option due to its anti-tumor effects on various cancers. The aim of the study was to invetsigate the effect of GKR in B16-F10 melanoma cells in vitro and in established intracerebral melanoma model in mice in vivo, and the sensitizing efficacy of TQ towards GKR. **MATERIAL AND METHODS:** Effects of GKR and combination treatment of GKR and TQ were studied on B16-F10 melanoma cells via analyzing cytotoxicty by adenosine triphosphate (ATP) assay; apoptosis induction by acridine orange staining and genotoxicity by comet assay. Expression of STAT3, p-STAT3 (Tyr705) and apoptotic pathway proteins was investigated by western blot analysis. Enzyme Linked Immunosorbent Assay (ELISA) was performed to assess the expression of inflammatory cytokines. Also survival was evaluated in mice with brain tumor after GKR and its combination treatment with TQ. **RESULTS:** The effects of GKR on cytotoxicity, apoptosis and genotxicity were significantly enhanced by TQ on B16-F10 melanoma cells. GKR induced apoptosis through inhibition of p-STAT3 expression, which subsequently regulated pro- and anti-apoptotic proteins such as caspase-3, Bax, Bcl-2, survivin. Combination TQ with GKR irradiation enhanced the apoptotic effect of GKR irradiation. Furthermore, GKR decreased the levels of tumor-related inflammatory cytokines on B16-F10 melanoma cells. This effect was more pronounced when TQ was added to GKR irradiation. GKR with 15 Gy improved the survival of mice with intracerebral melanoma compared to untreated mice. Although, the additive effect of TQ in combination with GKR irradiation on B16-F10 melanoma cells in vitro, TQ did not add any significant survival benefit to GKR treatment in mice with intracerebral melanoma. **CONCLUSION:** Our findings indicate that TQ would be a potential therapeutic and sensitizing agent in addition to GKR to enhance the anti-tumor effect of irradiation. Further studies are required to support our findings.

P11.56 ONCOLYTIC VIRUS AND TUMOR MICROENVIRONMENT MODULATORS AN ALLIANCE TO FIGHT GLIOBLASTOMA

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BACKGROUND: Glioblastoma is the most common and malignant brain tumour in adults with a dismal overall survival. Several works demonstrated that its tumoral microenvironment is enriched of immunosuppressive cell populations resulting in a challenge for its treatment. In this study, we propose to use an oncolytic adenovirus (Delta-24-RGD) with an inhibitor of myeloid derived suppressor cells (MDSCs; pexidartinib). Therefore, we hypothesis that we will act on two arms of the immune response: the efector arm (with the virus) and the inhibitor arm (with the Pexidartinib) resulting in a greater antitumor immune response. **MATERIAL AND METHODS:** We examined the benefit in survival of the combination Delta-24ACT with pexidarnitib (MDSC inhibitor) or a complement inhibitor in C57BL/6 mice bearing the CT-2A or the GLS61-5 murine glioblastoma models orthotopically. Three viral doses ($1x10^8$ pfu/mouse) were administered at 4, 8 and 11 days post cell implantation and the tumor microenvironment immunomodulators were administered following different schedules. Survival in different treatment groups were compared using the log-rank test. **RESULTS:** We observed that the combination of Delta-24-ACT and pexidartinib, led to an increase in the median survival of the treated mice when compared with single treatments (PBS=24 days, pexidartinib=24 days, Delta-24-ACT= 35 days and Delta-24-ACT+Pexidartinib= 36 days). Moreover, the treatment led to 8 long-term survivors; Pexidartinib=1, Delta-24-ACT=3 and Delta-24-ACT+Pexidartinib=4. These data underscore that the combined treatment increase significantly the number of long-term survivors. These long-term survivors were subjected to a rechalleng experiment to assess the generation of immunological memory which is currently on going. We have observed that the combined treatment increase the tumor infiltratrion of CD4, CD8 and led to a less immunosuppressive microenvironment. Immunohistochemistry of macrophages and nanostring of FFPE brain samples in order to evaluate the differences of the cell populations present in the tumoral microenvironment and their functional studies is currently on going. These experiments are being also conducted in GL261-5. **CONCLUSION:** Our data suggest that the combination of the Delta-24-ACT with the capacity of activate the immune system and a drug that downregulates the immunosupression results in a survival benefit in model of gliomas and warrants further investigation.

P11.57 A 3D BRAIN ORGANOID COCULTURE SYSTEM DELINEATES THE INVASIVE CELL COMPONENTS IN GLIOBLASTOMA

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BACKGROUND: Glioblastoma (GBM) cell infiltration into the surrounding normal brain tissue where the blood brain barrier is intact, represents a major problem for clinical management and therapy. There is a vital need to understand the molecular mechanism that drives tumor cell invasion into the surrounding brain. We have previously developed a 3D coculture model where mature brain organoids are confronted with patient-derived glioblastoma stem-like cells (GSCs). In such a coculture system, single cell invasion into the normal brain tissue can be studied in detail. Here, we first describe in detail, by RNA-seq and proteomics, the differentiation of various neural cell lineages into mature brain organoids as well as their cellular organization. By real-time confocal microscopy and imaging analyses we also determine the speed of tumor cell invasion into the brain. Finally, we used this coculture system to delineate in detail the cellular heterogeneity within the invasive compartment and their gene expression. **MATERIAL AND METHODS:** Immunohistochemistry and immunofluorescence were used to determine the expression and distribution of mature neurons, astrocytes, oligodendrocytes, and microglia within the brain organoids. Proteomics and RNA-seq were used to determine brain development ex-vivo. To assess the clonal composition of the GBM-invasive compartment, we used cellular (RGB) barcoding technology. By advanced imaging, we tracked in real time the invasion of barcoded cells into the brain organoids. Finally, we isolated invasive cells and non-invasive cells from our coculture system and used single cell sequencing to analyze their gene expression profiles and molecular phenotypes. **RESULTS:** Immunohistochemistry and immunofluorescence showed that brain organoids, after 21 days of differentiation, display a highly cellular and structural organization. RNA-seq and proteomics, performed at different time points of organoid differentiation, revealed that the brain organoids develop into mature brain structures after 21 days as verified by a comparative analysis to normal rat brain development *in vivo*. Imaging analyses showed that multiple clones within the GBMs have the capacity to invade into the brain tissue with an average speed of ~ 20 μm/h. RNA-sec analysis of the invasive compartment revealed a strong up-regulation of genes and pathways associated with anaerobic respiration (glycolysis). **CONCLUSION:** We describe a highly standardized brain organoid coculture system that can be used to delineate GBM inva-