are able to specifically block glioblastoma invasion in vitro and in vivo, where they also prolong survival in glioblastoma animal models. To better understand the functions of GSK-3 in glioblastoma we used proteomics which revealed major changes in cytoskeletal proteins, with downregulation of the EMT marker vimentin being the most significant alteration. Vimentin is an intermediate filament protein that functions as an organizer of a number of critical proteins involved in attachment, migration, and cell signaling. The downregulation of vimentin was rapid and due to alterations in its dynamics in response to GSK-3 inhibition. GSK-3 and vimentin were shown to associate with each other in glioblastoma cells, and reduction in vimentin phosphorylation was observed suggesting it may be a novel substrate of GSK-3. We showed that vimentin is highly expressed in patient glioblastoma samples and higher levels of vimentin are associated with poorer prognosis. Vimentin knockdown also reduced glioblastoma cell migration. The mechanism of action of GSK-3 inhibition in the context of glioblastoma invasion and the potential of developing a therapeutic strategy based on these observations will be discussed.

S4 - Session1 1015-1030

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Bone marrow derived immune cells and their role in tumor heterogeneity

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The role of bone marrow derived cells (BMDC) in tumor neo-vascularization remains controversial. We previously demonstrated recruitment and migration of distinct subpopulations of BMDCs to Glioblastoma Multiforme (GBM), and association with the GBM vasculature in a highly tumor region dependent manner. Continuation of this work has focused on establishing the molecular alterations generated in BMDC as a consequence of interaction with the GBM tumor microenvironment. Our second goal has been to establish whether the tumor microenvironment influences differentiation and contribution of BMDC in GBMs. Intracranial xenograft models were created in chimeric mice generated by reconstituting the bone marrow with fluorescent (gfp or dsred) BM. BMDC were isolated from the GBM microenvironment using FACS sorting of the fluorescent tag at early and late stages of GBM growth, in addition to following treatment with RTx and AATx. We demonstrate that VEGF inhibition though indirect mechanisms, RTx, and direct mechanisms, VEGFTRap, can alter the differential recruitment of pBMDCs observed through normal tumor progression. It is known that inhibition of VEGF leads to an increase in ANG2 signal, which may in turn be linked to the recruitment of pBMDCs. Through addition of an ANG2 inhibitor we can show that through concomitant VEGF and ANG2 inhibition, pBMDC recruitment can be prevented. These results suggest that BMDC contribute through distinct mechanisms to tumor invasion and neo-vascularization and thereby targeting the specific cascade of angiogenic and invasion factors will prevent the pro-tumoral contribution of BMDC in supporting tumor growth and aiding in response to therapy.

S5 – Session1 1100-1115

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Role of hexokinase 2 (HK2) in modulating tumor metabolism and response to therapy in glioblastoma

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Glioblastoma (GBM), similar to many other cancers, exhibits enhanced aerobic glycolysis with concomitant lactate production, a phenomenon known as the Warburg effect. We have demonstrated that preferential expression of Hexokinase 2 (HK2) is a critical mediator of metabolic reprograming in GBMs and its inhibition is a potential therapeutic strategy for sensitization of GBM tumors to radiation (RAD) and/or temozolomide (TMZ**).** Our results indicate that conditional HK2 inhibition disrupts energy homeostasis and sensitizes GBMs to radiation and chemotherapy. In GBM xenografts, conditional HK2 loss sensitizes GBM tumors to concomitant RAD/TMZ and results in a significant survival benefit in the mice. Moreover, loss of HK2 resulted in GBM remodeling with HK2 knockdowns showing increased necrosis, hypoxia, inflammatory infiltration and reduced vascularization. We demonstrate that targeting a key metabolic enzyme involved in the Warburg effect might improve the efficacy of current therapeutic regimen and provide a unique paradigm for the management of GBMs.

S6 – Session1 1115-1130

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Classifying medulloblastoma into molecular subgroups: Means, motive, and opportunity

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Current medulloblastoma protocols stratify patients based on clinical features: patient age, metastatic stage, extent of resection, and histological variant. Stark prognostic and genetic differences between the four subgroups suggest that subgroup-specific molecular biomarkers could improve patient prognostication. Method: Molecular biomarkers were identified from a discovery set of 673 medulloblastomas from 43 cities around the globe.

Combined risk stratification models were designed based on clinical and cytogenetic biomarkers identified by multivariate Cox proportional-hazards analyses. Identified biomarkers were tested using FISH on a non-overlapping medulloblastoma tissue microarray (n=453), with subsequent validation of the risk stratification models. Results: Subgroup information improves the predictive accuracy of a multivariate survival model compared to clinical biomarkers alone. Most previously published cytogenetic biomarkers are only prognostic within a single medulloblastoma subgroup. Profiling a six-pack of FISH biomarkers (GLI2, MYC, 11, 14, 17p, and 17q) on FFPE tissues, we can reliably and reproducibly identify very low-risk and very high-risk patients within each of SHH, Group3 and Group4 medulloblastomas. Conclusions: Combining subgroup and cytogenetic biomarkers with established clinical biomarkers substantially improves patient prognostication, even in the context of heterogeneous clinical therapies. The prognostic significance of most molecular biomarkers is restricted to a specific subgroup. We have identified a small panel of cytogenetic biomarkers that reliably identifies very high-risk, and very low-risk groups of patients, and which will make an excellent tool for selecting patients for therapy intensification and therapy de-escalation in future clinical trials.

S7 – Session1 1130-1145

Abstract withdrawn.

S8 – Session1 1145-1200

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An *in vitro* mouse model of GBM with abrupt and predictable onset

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Glioblastoma (GBM) is a fatal cancer which harbors multiple genetic alterations, many of which are thought to be passenger mutations. Those that involve receptor tyrosine kinase signaling and the p53 and RB pathways are found in most newly diagnosed GBMs and are thought to be 'drivers' of this cancer. Here we report a PDGF-A-linked *in vitro* mouse model of GBM in which malignant transformation appears to occur abruptly, and the responsible genetic events can be studied. Cells from the subventricular zone (SVZ) of p53-null, adult mice were dissected and cultured as spheres in serum-free media supplemented with either EGF/FGF or PDGF-A. p53-null SVZ cells cultured continuously in EGF/FGF proliferated rapidly but remained growth factor dependent and non-tumorigenic. In contrast, PDGF-A cultured SVZ cells grew poorly over 3-4 months until passage 8, whereupon sphere formation and size accelerated abruptly in multiple independent cultures. These transformed cells proliferated rapidly in the absence of PDGF-A, and unfailingly, generated tumors with a striking resemblance to GBMs when implanted into the striatum of immunocompetent, p53 wild-type mice. EGFR, PDGFRα, Olig2 and NG2 were expressed in EGF/FGF and PDGF-A cultures in early to late passages (≤P1-P15). Increased nestin expression was observed in PDGF-A transformed cultures only, whereas GFAP expression decreased in both. This model recapitulates other systems in which PDGF-A-driven glioma formation has been achieved *in vivo* in p53-null mice, but may have these advantages: low cost, easy accessibility to sequential molecular interrogation, and suitability for screening of libraries of potential inhibitors of gliomagenesis.

S9 – Session2 1400-1415

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Targeting oncofetal high mobility group A2 (HMGA2) to increase sensitivity to temozolomide (TMZ) in glioblastoma (GB) cells

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The Base Excision Repair (BER) pathway facilitates the removal of temozolomide (TMZ) induced alkylated DNA bases. We previously identified the non-histone chromatin binding protein and DNA minor groove binder HMGA2 as a novel member of BER that directly interacts with APE1, a key BER member. We showed that the AP/ dRP lyase activity, located within the AT-hook DNA binding domains of HMGA2, protects stem cells and cancer cells against alkylating drugs. The in-vivo interactions of HMGA2 with Ataxia telangiectasia and Rad3-related kinase (ATR) and checkpoint kinase 1 (CHK1) result in sustained activation of the ATR-CHK1 signaling pathway, prolonged G2/M