identified that treatment resistance may be due to glioma-driven dendritic cell dysfunction. We observed significant down regulation of co-stimulatory markers on tumor-associated dendritic cells in mice that escaped ACT and subsequent capacity to activate tumor-reactive T cells.Method: KR158B bearing mice received HSC and tumor reactive T cells one day before 9 Gy irradiation followed by 3 does of BMDC vaccine. Tumor associated DCs were sorted from tumors evaded from ACT and co-cultured with primary KR158B reactive T cells or escaped KR158B reactive T cells. T cell proliferation and interferon gamma were detected for comparison. PCR array was performed to identify dysregulated functional genes.Results and CONCLUSIONS: Functional evidence demonstrated that dendritic cells from resistant tumor had significantly decreased capacity in activating tumor-reactive T cells against either primary KR158B glioma cells or T cells generated to target tumor cells that evaded ACT. Gene expression data showed this may be due to significant decreases in genes associated with co-stimulation, T cell engagement, and antigen presentation.

TMIC-14. DIFFUSE P16^{INK4A} EXPRESSION IS ASSOCIATED WITH TUMOR CELL SENESCENCE AND SENESCENCE-ASSOCIATED SECRETARY PHENOTYPE RELATED TO IMMUNE MICROENVIRONMENT IN GLIOBLASTOMAS.

Tae Hoon Roh[1], Young Hwa Kim[1], So Hyun Park[1], Soyeong Eom[1], Tae Jun Park[1], Jang-Hee Kim[1], and Se-Hyuk Kim[1]; [1]Ajou University School of Medicine, Suwon, Republic of Korea

Cellular senescence (CS) is a state of irreversible cell cycle arrest, and the expression of p16^{INK4a} in cells is one of the reliable markers for CS. However, senescent cells are metabolically active with the senescence-associated secretory phenotype (SASP), which can influence the tissue microenvironment by paracrine signaling to the adjacent tumor, non-tumor, and immune cells. In the present study, we evaluated p16^{INK4a} expression in glioblastoma and investigated its association with CS and SASPs. We analyzed the expression of p16^{INK4a} in 73 glioblastomas by immunostaining. To examine the association of p16^{INK4a} expression and CS, we performed senescence-associated β-galactosidase (SA-β-Gal) staining, a standard marker of CS, using glioblastoma cell lines and fresh frozen tumor tissues. For SASPs analysis, RNA sequencing with Gene Set Enrichment Analysis (GSEA) was performed. Among 73 glioblastomas, twenty-eight cases (38.4%) revealed diffuse strong p16^{INK4a} expression in tumor cells. The glioblastoma with diffuse p16^{INK4a} expression (GMDP) patients were younger (52.4 vs. 59.2 years) and showed prolonged overall survival (median: 25.5 vs. 12.3 months) compared to those harboring negative expression. In vitro analysis, p16^{INK4a} over-expressed glioblastoma cell line showed increased expression of SA-β-Gal which indicates CS. In addition, fresh frozen tissues from GMDP also revealed SA-β-Gal positivity. RNA sequencing analysis revealed that splicing or protein biosynthetic genes were enriched in GMDP, and GSEA showed significant enrichment of SASP genes (false discovery rate < 0.05), especially chemokines associated with monocytes and macrophages. Our data suggest that increased expression of p16^{INK4a} in glioblastoma is associated with tumor cell senescence, and SASPs from senescent tumor cells could be one of the crucial modulators in the tumor immune microenvironment.

TMIC-15. AGE-RELATED MARKERS FOR SENESCENCE INCREASE IN THE OLDER ADULT EXTRATUMORAL MOUSE BRAIN DUE TO GLIOBLASTOMA

April Bell[1], Margaret Johnson[2], Erik Ladomersky[3], Miri Kim[3], Junfei Zhao[4], Jason Miska[5], Lijie Zhai[6], Kristen Lauing[6], Evridiki Asimakidou[3], Prashant Bommi[7], James Chandler[8], Xinlei Mi[1], Masha Kocherginski[8], Rimas Lukas[3], and Derek Wainwright[1]; [1]Northwestern University, Feinberg School of Medicine, Chicago, IL, USA, [2]The Preston Robert Tisch Brain Tumor Center, Duke University Medical Center, Durham, USA, [3]Northwestern, Chicago, USA, [4]Department of Systems Biology at Columbia University, New York, NY, 10032, USA, New York, NY, USA, [5]Northwestern University Feinberg School of Medicine, Chicago, USA, [6]Northwestern University, Feinberg School of Medicine, Chicago, USA, [7]Northwestern University, Feinberg School of Medicine, Chicago, Chicago, USA, [8]Northwestern University, Chicago, USA

OBJECTIVE: The median age of onset for glioblastoma (GBM; IDHwt) is 68-70 years. Age is a strong prognostic factor for GBM patient outcomes such that overall survival in older adults is less than their younger counterparts – even after adjustment for MGMT promoter methylation status. Aging is associated with increased levels of senescence in the brain. Several age-related neurological disorders have been shown to improve with senolytic treatments. Here, we explored the effects and therapeutic neutralization of syngeneic brain tumors on increasing senescence levels in the extratumoral brain (ie. outside of the brain tumor) of young and old mice. METHODS: General RNA-sequencing, as well as single-cell (sc) RNA-sequencing was performed on extratumoral tissue from young (8-12 weeks) and older adult (80-90 weeks) C57BL/6 mice with or without GL261 and key markers were validated with RT-PCR. The combined effects of the senolytics, dasatinib and quercetin, with radiation, anti-PD-1 mAb, and IDO enzyme inhibitor treatment, was also investigated. RESULTS: General- and sc-RNA sequencing revealed a distinct gene expression profile in the extratumoral brain of older mice with syngeneic GL261 as compared to all other groups. RT-PCR results confirmed that the brain tumor increased gene expression for senescence levels, p53, and NFkB signaling in the older adult extratumoral brain. Expression of the senescence marker p16^{INK4A} was primarily localized to oligodendrocyte progenitor cells in the older adult brain. The combinatorial treatment of senolytics with RT, anti-PD-1 mAb, and IDO enzyme inhibitor led to a synergistic survival benefit in older adult mice with GL261 as compared to the treatment with senolytics, or immunotherapy, alone. CONCLUSIONS: The data suggest that the extratumoral brain may be responsible in-part for the poorer outcomes of older adults with GBM and that treatment approaches that target senescent cells may provide clinical benefit.

TMIC-16. INTRATUMORAL THROMBOSIS INITIATES HYPOXIA AND NECROSIS TO DRIVE TUMOR PROGRESSION THROUGH DRAMATIC TUMOR MICROENVIRONMENTAL ALTERATION

Steven Markwell[1], Cheryl Olson[2], Jiabo Li[2], Ling-Kai Shih[2], James Ross[3], and Daniel Brat[4]; [1]Northwestern University, Chicago, USA, [2]Northwestern University, Chicago, IL, USA, [3]Emory University, atlanta, USA, [4]Department of Pathology, Northwestern University, Chicago, IL, USA

All GBM molecular subsets share the common trait of accelerated progression following necrosis which cannot be adequately explained by cellular proliferation arising from accumulated genetic alterations. We suggest that development of necrosis is much more than a passive phenomenon related to rapid growth but rather is a driving force behind TME restructuring responsible for sustaining accelerated expansion. Current tumor models fail to adequately mimic the magnitude of post-necrotic restructuring within the microenvironment and remain overly reliant on post-mortem analyses, obligating researchers to extrapolate causal relationships between necrosis and progression phenomena during tumor evolution. In GBM (WHO grade 4), the most malignant primary brain tumor, vascular pathology and central necrosis precede rapid, radial expansion. Mechanisms enabling selective fitness within a hypoxic/anoxic GBM setting remain poorly understood. Nanostring GeoMX spatial profiling demonstrates M1-like TAM enrichment in non-necrotic human samples and M2-like TAMs in perinecrotic regions. Our immunocompetent RCAS/tv-a model aptly captures events seen in human gliomas, exposing dynamic temporal and spatial changes that facilitate GBM progression, incorporating unique microenvironmental stressors typically absent from GBM animal models, specifically emerging central necrosis. Simultaneously, our *in vitro* models scrutinize how hypoxia-dependent signaling between GBM cells, microglia and monocytes alter TAM accumulation and function in the TME. TAMs increase dramatically with the onset of necrosis, with a preferential localization to the hypoxic zone in the perinecrotic niche, which supports their survival. Flow cytometry on digested pre- and post-necrotic GBMs, showed increased TAM accumulation at 6 weeks vs. 2 weeks (necrosis emerges in week 4-5). Our *in vivo* data along with in silico analysis of Ivy GAP and TCGA datasets suggests GBM cells within the peri-necrotic niche attract and polarize TAMs through MIF secretion, necrotic DAMP (adenosine, S100B) release, and arginase secretion to suppress T cells.

TMIC-17. DISCOVERING DOMINANT TUMOR CHARACTERISTICS IN CYSTIC GLIOBLASTOMA

Zhihua Chen[1], Yifeng Miao[2], Shun Yao[3], Denis O'Meally[4], David Frankhouser[4], Biao Tang[5], MingHui Li[5], Paul Frankel[4], Mari Shahmanyan[4], Yongming Qiu[2], and Russell C. Rockne[4]; [1]City of Hope National Medical Center, Shanghai, China (People's Republic), [2]RenJi Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China (People's Republic), [3]The First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China (People's Republic), [4]City of Hope National Medical Center, Duarte, USA, [5]Sinotech Genomics Ltd co., Shanghai, China (People's Republic)

BACKGROUND: As a rare subtype of glioblastoma (GBM), the genetic features and outcomes in cystic GBM (cGBM) are largely unknown. This study aimed to evaluate the survival of cystic features, investigate genomic patterns and the immune microenvironment in patients with cGBM. METHODS: Gd T1-weighted, T2-weighted, T2-Flair, DWI or ADC images were used to classify cGBM or noncystic GBM (non-cGBM). Clinical information from IvyGAP, EGA databases and Chinese cohort were used for survival analysis. Fresh cystic fluid and residual tumor tissue were collected to evaluate immunological components, pathological and genomic differences between cGBM and non-cGBM. Data from IvyGAP were included to determine difference of molecular feature among substructure. RESULTS: 143 cases with cGBM and non-cGBM were screened to compare survival outcomes. Cystic features were to confer a survival benefit. It was an independent prognostic factor