several tumor types. To date, a clear hypoxia gene signature has not been specifically described for GBM. We hypothesize that specific cellular pathways are differentially regulated in hypoxic tumor niches and can serve as novel actionable targets for treatment-resistant tumor cells in GBM. Over the past 3 years, we have administered PIMO to 35 patients with primary IDH1/2 wild-type GBM and isolated PIMO-positive and PIMO-negative tumor cells by laser capture microdissection using a PIMO-specific antibody on frozen tumor specimens. Total genomic DNA was isolated and subjected to DNA methylation profiling using the Illumina Infinium Methylation EPIC array. Our preliminary results suggest that PIMO-positive (hypoxic) tumor cells display a distinct DNA methylation profile that corresponds to changes in expression of a set of genes associated with immune regulation, angiogenesis, and proliferation. Furthermore, multiple CpG sites within the promoter of some genes are differentially methylated in hypoxic cells, suggesting these genes may be epigenetically regulated under hypoxia. In conclusion, our results indicate that hypoxia is associated with distinct epigenetic alterations in tumor cells which may alter how these cells respond to low oxygen levels and can further be utilized to uncover the epigenomic vulnerabilities of hypoxic tumor cells in GBM.

ECOA-5. INTEGRATIVE 3D SPATIAL CHARACTERIZATION OF GENOMIC AND EPIGENOMIC INTRATUMORAL HETEROGENEITY IN GLIOBLASTOMA

Radhika Mathur[1], Qixuan Wang[2], Patrick Schupp[1], Stephanie Hilz[1], Chibo Hong[1], Ivan Smirnov[1], Marisa Lafontaine[1], Devika Nair[1], Sriranga Iyyanaki[2], Joanna Phillips[1], Susan Chang[1], Yan Li[1], Janine Lupo[1], Michael Oldham[1], Feng Yue[2], Joseph Costello[1]; [1]University of California San Francisco, San Francisco, CA, USA. [2]Northwestern University, Evanston, IL, USA

Treatment failure in glioblastoma is often attributed to intratumoral heterogeneity (ITH), which fosters tumor evolution and selection of therapy-resistant clones. While genomic alterations are known contributors to ITH, emerging studies highlight functional roles for epigenomic ITH which integrates differentiation status, stochastic events, and microenvironmental inputs. Here, we have established a novel platform for integrative characterization of genomic and epigenomic ITH of glioblastoma in three-dimensional (3-D) space. In collaboration with neurosurgeons and biomedical imaging experts, we utilize 3-D surgical neuro-navigation to safely acquire ~10 tumor samples per patient representing maximal anatomical diversity. We conduct whole-exome sequencing, RNA sequencing, and assay for transposase-accessible chromatin using sequencing (ATAC-Seq) on each sample. The spatial location of each sample is mapped by its 3-D coordinates, allowing 360-degree visualization of genomic and epigenomic ITH for each patient. We demonstrate this approach on 8 patients with primary IDH-WT glioblastoma (83 spatially mapped samples), providing unprecedented insight into their spatial organization at the genomic and epigenomic levels. We link genetically defined tumor subclones to patterns of open chromatin and gene regulation, revealing underlying transcription factor binding at active promoters and enhancers. We also identify ITH in whole-genome doubling and focal oncogene amplification events in multiple patients, which we then link with epigenomic ITH. Further, to study microenvironmental inputs and their contribution to epigenomic ITH, we conduct deconvolution of RNA sequencing and ATAC-Seq data by analyzing feature co-variation. We resolve the 3-D spatial organization of immune, neural, and other nontumor cell types present in glioblastoma, characterizing their functional states and interactions with tumor cells. This work provides the most comprehensive spatial characterization of genomic and epigenomic ITH to date in glioblastoma. As a resource for further investigation, we have developed an interactive data sharing platform – The 3D Glioma Atlas – that enables 360-degree visualization of both genomic and epigenomic ITH.

ECOA-6. GENOMIC AND TRANSCRIPTOMIC ANALYSES REVEAL DIVERSE MECHANISMS RESPONSIBLE FOR DEREGULATION OF EPIGENETIC ENZYME/MODIFIER EXPRESSION IN GLIOBLASTOMA

Marta Maleszewska[1], Bartosz Wojtas[1], Bartlomiej Gielniewski[1], Shamba Mondal[1], Jakub Mieczkowski[1], Michal Dabrowski[1], Janusz Siedlecki[2], Mateusz Bujko[2], Pawel Naumann[2], Wieslawa Grajkowska[3], Katarzyna Kotulska[3], Bozena Kaminska[1]; [1]Nencki Institute of Experimental Biology, Warsaw, Poland. [2]The Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland. [3]The Children's Memorial Health Institute, Warsaw, Poland

Malignant gliomas represent over 70% of primary brain tumors and the most deadly is glioblastoma (GBM, WHO grade IV), due to frequent dysfunctions of tumor suppressors or/and oncogenes. Recent whole genome studies of gliomas demonstrated that besides genetic alterations, epigenetic dysfunctions contribute to tumor development and progression. Alterations in genes encoding epigenetic enzyme/protein or aberrations in epigenetic modification pattern have been found in gliomas of lower grade, yet no epigenetic driver was identified in GBM. We sought to identify different mechanisms driving aberrant expression of epigenetic genes in GBM.

We analyzed gene expression and coding/non-coding regions of 96 major epigenetic enzymes and chromatin modifiers in 28 GBMs, 23 benign gliomas (juvenile pilocytic astrocytomas, JPAs, WHO grade I) and 7 normal brain samples. We found a profound and global down-regulation of expression of most tested epigenetic enzymes and modifiers in GBMs when compared to normal brains and JPAs. For some genes changes in mRNA level correlated with newly identified single nucleotide variants within non-coding regulatory regions. To find a common denominator responsible for the coordinated down-regulation of expression of epigenetic enzymes/modifiers, we employed PWMEnrich tool for DNA motif scanning and enrichment analysis. Among others, we discovered the presence of high affinity motifs for the E2F1/E2F4 transcription factors, within the promoters of the epigenetic enzyme/modifier encoding genes. Knockdown of the E2F1/E2F4 expression affected the expression of a set of epigenetic enzymes/modifiers. Altogether, our results reveal a novel epigenetic-related pathway by which E2F1/E2F4 factors contribute to glioma pathogenesis and indicate novel targets for glioma therapy. Supported by a National Science Centre grant 2013/09/B/NZ3/01402 (MM).

ECOA-7. CONSERVED CELL-LINEAGE CONTROLLED CHROMATIN ACCESSIBILITY IN HUMAN AND MOUSE GLIOBLASTOMA STEM CELLS PREDICTS FUNCTIONALLY DISTINCT SUBGROUPS

Xi Lu, Naga Prathyusha Maturi, Malin Jarvius, Linxuan Zhao, Yuan Xie, E-Jean Tan, Pengwei Xing, Mårten Fryknäs, Lene Uhrbom, Xingqi Chen; Uppsala University, Uppsala, Sweden

There is ample support for developmental control of glioblastoma stem cells (GSCs), and a deeper knowledge of their epigenetic regulation could be central to more efficient glioblastoma (GBM) therapies. For this purpose, we analyzed the chromatin-accessibility landscape of nine mouse GSC cultures of defined cell of origin and 60 patient-derived GSC cultures by assay for transposase-accessible chromatin using sequencing (ATAC-seq). This uncovered an epigenetic variability of both mouse and human GSC cultures that differed from transcriptome clusters. Both mouse and human chromatin accessibility-guided clusters were predominantly determined by distal regulatory elements, displayed unique sets of transcription factor motif enrichment, and exhibited different functional and drug-response properties. Cross-species analysis of distal regulatory element regions in accessible chromatin of mouse and human cultures revealed conserved epigenetic regulation of GSCs.

ECOA-8. LUNG ADENOCARCINOMA BRAIN METASTASIS PREDICTION USING TUMOR DNA METHYLATION PROFILING

Jeffrey Zuccato[1], Yasin Mamatjan[1], Kenneth Aldape[2], Gelareh Zadeh[1]; [1]Princess Margaret Cancer Centre, Toronto, ON, Canada. [2]National Cancer Institute, Bethesda, MD, USA

INTRODUCTION: The development of brain metastases from primary cancer profoundly impacts patient prognosis. Up to one quarter of lung cancers develop brain metastases and subsequent median overall survival is one year. Although clinical factors do not reliably predict brain metastasis development, DNA methylation signatures have been recently shown to predict outcomes in other cancers. It is hypothesized that DNA methylation signatures predicting brain metastasis development from lung cancer will be identified. This work may allow for treatment strategies that prevent brain metastasis development in high risk lung cancer patients. METHODS: DNA methylation profiling was undertaken on N=124 lung adenocarcinoma patients. In a randomly selected 70% training cohort, differentially methylated CpG sites between patients developing and not developing brain metastases were identified and used to build a generalized boosted regression model. Patients in the independent 30% testing cohort were assigned brain metastasis risk scores by the model. RESULTS: Brain metastases developed in 49/124 (39.5%) of patients and 2.3K CpG sites were significantly differentially methylated between patients developing and not developing metastases. Methylation-based brain metastasis risk scores predicted time to brain metastasis development in the testing cohort (Univariate cox: HR=3.2, 95% CI 1.1–9.4, p=0.03). A multivariate cox analysis assessing tumor size and nodal positivity together with methylation scores as covariates identified methylation scores as the only independent predictor of brain metastasis development in the testing cohort (HR=4.3, 95%CI 1.1–17, p=0.038). CONCLUSIONS: DNA methylation signatures in lung adenocarcinomas predict brain metastasis development independently from the non-metastatic components of cancer stage. Future work developing a comprehensive nomogram utilizing methylation scores together with clinical factors to determine patient specific risk values may aid in treatment decisions and patient prognosis counselling.