OMRT-15. MULTIDISCIPLINARY MANAGEMENT OF NON-SMALL CELL LUNG CANCER PATIENTS WITH LEPTOMENINGEAL METASTASIS IN THE TKI ERA

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BACKGROUND: leptomeningeal metastasis (LM) is a devastating scenario in patients with non-small cell lung cancer (NSCLC), with an estimated median overall survival (OS) of 4–6 months from diagnosis. Several studies have clarified the prognosis of treatment modalities after LM. However, just a few studies have clarified the prognosis of LM patterns. We evaluate the prognosis based on various patterns of LM under multidisciplinary treatment (MDT). METHOD: This retrospective study evaluated NSCLC patients treated at National Taiwan University Hospital between 2007–2019 with brain metastases (BM) and LM. LM was classified into LM only, LM concurrent with BM, and LM after BM. Treatments including systemic therapy, whole-brain radiotherapy (WBRT), stereotactic radiosurgery (SRS), and intrathecal chemotherapy with Methotrexate (IT MTX) were recorded. BM excision was done by a neurosurgeon using minimally invasive neurosurgery. The MDT was done according to patients' clinical situations. Kaplan-Meier methodology was used to describe overall survival OS. Multivariate Cox regression model was used to access prognostic factors. RESULT: One hunderd patients with NSCLC CNS metastasis was included in this study. Median OS in patients with single, oligo and multiple BM was 42.0 months (95% CI= 0.12–83.89), 58.1 months (95% CI= 13.00–103.26), and 21.3 months (95% CI= 16.93–25.73), respectively. The median OS of all LM patients was 9.8 months. The median OS of LM after BM, concurrent BMLM, and LM only was 8 months (95% CI= 2.58–13.56), 41.5 months (95% CI= 0.00–94.36), and 18.5 months (95% CI=3.68–33.32), respectively. Multivariate Cox regression analysis showed only IT MTX (p= 0.010, HR= 0.392, 95%CI= 0.19–0.80) was associated with survival. CONCLUSION: MDT in the TKI era has led to a dramatic improvement of OS in patients with LM (4–6 months vs. 9.8 months). NSCLC patients with LM only and concurrent BM LM has a better prognosis and longer survival, and thus are worth receiving intensive MDT care.

FINAL CATEGORY: OMICS OF TUMOR EVOLUTION AND HETEROGENEITY

OTEH-1. ALTERNATIVE RNA SPLICING MODULATES COMPOSITION OF RIBOSOMES AND DETERMINES SPATIAL PHENOTYPE OF GLIOBLASTOMA CELLS

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Glioblastoma (GBM) is an extremely heterogeneous tumor and its different regions are populated with phenotypically distinct types of cancer cells. However, it is still unclear how multiple GBM populations arise from the originally homogenous group of tumor precursor cells. Here we showed that GBM cells from the core and edge of the tumor have different composition of ribosomes due to the alternative RNA splicing of multiple ribosomal genes with highest differences observed for RPL22L1. We found that cells at the edge of the tumor express classical isoform of RPL22L1 (RPL22L1a) while core cells have a novel RPL22L1b isoform. RPL22L1b appears due to low pH condition at the core of the tumor. It allows cells to survive during acidosis, promotes more aggressive phenotype in vivo and correlate with worse patient outcome. Mechanistically, RPL22L1b binds to lncRNA MALAT1 in the nucleus and induces its degradation enhancing stemness of GBM cells. On the other hand, RPL22L1a interacts with ribosomes in cytoplasm and upregulates p53 translation favoring less aggressive edge phenotype of GBM. The splicing switch between RPL22L1 isoforms is regulated by SRSF4 proteins. We identified a small molecule compound that inhibits SRSF4 and impairs splicing of RPL22L1, inducing apoptosis of GBM cells and decreasing tumor growth in vivo. Altogether, our data unraveled the mechanism by which less aggressive edge-like GBM cells acquire more malignant core-like phenotype during tumor growth. It may also explain discrepancies between proteome and transcriptome of GBM cell populations. Targeting this pathway may help to decrease tumor heterogeneity and eliminate therapy resistant cells at the tumor core.

Work was supported by the Russian Science Foundation grant 19-44-02027.

OTEH-2. HIGH-DIMENSIONAL ANALYSIS OF SPATIAL IMMUNE CELL HETEROGENEITY IN GLIOBLASTOMA REVEALS DIFFERENCES BETWEEN CONTRAST-ENHANCING AND NON-CONTRAST-ENHANCING TUMOR RIMS

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BACKGROUND: Glioblastoma (GBM) is the most common primary malignant brain tumor in adults. GBM remains an incurable disease, with a median survival ~20 months. Complex intercellular interactions within the tumor microenvironment and spatial heterogeneity have challenged and impeded therapeutic efficacy. The non-contrast-enhancing (by T1-weighted MRI) rim of GBM is not always safely resectable and represents a major source of recurrence. We hypothesized that differential immune infiltration is an underlying factor of spatial heterogeneity in GBM, particularly in the non-contrast-enhancing tumor rim. METHODS: Five patients with newly diagnosed GBM (ages 53–84) were recruited to a device feasibility study (NCT04545177) utilizing an intraoperative high-resolution MRI-based navigation system coupled with the NICO Myriad (a non-ablative semi-automated resection tool) and a coupled automated biological Tissue Preservation System (NICO APS) to sample spatially mapped regions of tumors in a reproducible and minimally destructive manner. We obtained brain tumor tissue from: (a) tumor core, (b) contrast-enhancing tumor rim and (c) non-contrast-enhancing tumor rim. Downstream processing consisted of digestion of tumor tissue (Miltenyi human tumor digestion kit) for subsequent single-cell isolation, viability assessment and immediate staining for multiparametric flow cytometry for immune profiling. RESULTS: Viability varied across sampled regions (median 85%, range 52–100%). With the exception of 1 sample, viability was >70% in all specimens. High-dimensional analysis with 26 marker flow cytometry revealed spatial heterogeneity in the frequency of myeloid-derived suppressor cell subsets, regulatory T cells, CD8+ T cells, as well as expression of T cell activation and exhaustion markers. CONCLUSIONS: Semi-automated, spatially mapped intraoperative sampling of GBM with high viability of specimens is feasible and reproducible with the NICO Myriad and APS devices. High-dimensional analysis of immune cells in the GBM microenvironment captured the spatial heterogeneity of GBM. Future studies will expand on these observations by analyzing more patient specimens in combination with multiple omics assays.

OTEH-3. TARGETED GENE-EXPRESSION ANALYSIS DURING MALIGNANT TRANSFORMATION IN PRIMARY AND SECONDARY MALIGNANT MENINGIOMA

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BACKGROUND: Malignant meningiomas comprise 2–5% of all meningiomas. The process of malignant transformation when benign meningiomas (WHO grade I-II) become malignant (WHO grade III) has not previously been investigated in sequential tumour surgeries. Upregulation of FOXM1 expression and DREAM-complex repression have shown phenotypical subgroups correlating with WHO grade and aggressiveness. We investigated the RNA expression of 30 genes central to meningioma biology and 770 genes involved in neuroinflammatory pathways in primary and secondary malignant meningioma patients who underwent one to several operations. METHODS: We identified a cohort of consecutive malignant meningioma patients treated at Rigshospitalet, Copenhagen from 2000–2020 (n=51) and gathered their malignant tumours and previous WHO grade I/II tumours. The malignant cohort (MC) was counter matched with a benign cohort (BC) where patients had no recurrences during follow-up. RNA expression signatures from 140 samples from the MC and 51 samples from the BC were analysed with the Nanostring Neuroinflammation panel customized with 30 genes known to be relevant in meningioma phenotypes. RESULTS: 49% of MC patients had a previous grade I/II meningioma making them secondary malignant meningioma patients. Progression-free survival calculated from first malignant surgery to first recurrence or death showed no significant difference in the

primary vs. secondary patients. Preliminary results of single-gene analysis of MC tumours showed FOXM1, MYBL2, TOP2A, BIRC5 expression was higher in WHO grade III samples. Gene-expression signatures in the individual patients and gene ontology enrichment analyses are in process. CONCLUSIONS: FOXM1, MYBL2, TOP2A, BIRC5 RNA expression levels seem to rise during malignant progression across patients. Gene-expression analysis using the Nanostring technology is feasible and a potentially powerful tool to distinguish meningiomas prone to malignant transformation from truly benign meningiomas.

OTEH-4. DEEPER INSIGHT INTO INTRATUMORAL HETEROGENEITY BY MRI AND PET-GUIDED STEREOTACTIC BIOPSIES FROM GLIOBLASTOMA PATIENTS

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Glioblastoma is one of the most aggressive cancers, but the molecular evolution is still not fully understood. We used PET imaging combined with deep sequencing of glioblastoma biopsies at both the RNA and DNA levels to get a deeper insight into molecular evolution. In the clinical setting, PET imaging provides information about metabolically active tumor areas, but the molecular interpretation is unclear. Our primary objective was to perform an intratumoral spatial comparison of biopsies from potentially aggressive and less aggressive areas in glioblastomas according to PET scans. Additionally, tissue from the tumor periphery was included. We used MRI, ^{11}C-methionine(MET) PET, and ^{18}F-FDG PET was used in combination to obtain a series of neurosurgical stereotactic biopsies from tumor areas with high MET and ^{18}F-FDG uptake (hotspot), low MET and ^{18}F-FDG uptake (coldspot), as well as tumor periphery of six glioblastoma patients that were processed for whole genome, exome, and transcriptome sequencing. Differential gene expression and gene ontology analysis showed that hotspots were enriched in gene sets associated with DNA replication, cell cycle, and ligand receptor interaction. Genome and exome analysis suggested hotspots and coldspots to have similar mutational profiles. However, a limited number of hotspot-specific mutations and fusion transcripts indicated that hotspot tumor cells developed from coldspot cells and point at the potential role of hotspot driver genes in glioblastoma. Our findings reveal that hotspots in glioblastomas represent a more advanced stage of molecular evolution than coldspots.

OTEH-5. CHARACTERIZATION OF LONG-NON CODING RNA ASSOCIATED CERNA NETWORK HUB GENE INVOLVED IN GLIOBLASTOMA MULTIFORME LIPID METABOLISM

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BACKGROUND: Glioblastoma multiforme (GBM) are the major death contributor in primary brain tumour. Despite having an improved diagnostic criterion by integrating both histological and molecular features such as Isocitrate Dehydrogenase (IDH) detection, the prognosis of GBM patients still remain poor. Lipid metabolism is an essential pathway that fuel GBM aggressiveness. IDH1 one of the key enzyme that regulates it. Long non-coding RNAs (lncRNAs) act as competing endogenous RNAs (ceRNAs) in tumour initiation and progression. In parallel, miRNA-mediate ceRNA crosstalk between lncRNAs and mRNAs. In this study, we aim to investigate the IDH1 subgroup lncRNA associated ceRNA network hub gene responsible in the coordination of glioblastoma multiforme lipid metabolism using bioinformatics approach. METHODS: TCGA-GBM dataset consist of 168 GBM RNA-seq (159 IDH1 wt and 9 IDH1 mutation) were downloaded. Differentially expressed genes (DEG) were then obtained using Limma. Gene sets related with lipid metabolism from GSEA-MSigDB were overlapped with DEG using Venn diagram to identify the DEmRNA that are related with lipid metabolism. Construction of mRNA-miRNA and lncRNA-miRNA interaction networks were performed using miRNet. The ceRNA interaction network were later combined in the Cytoscape software. Potential lncRNA hub genes were identified by CytoHubba analysis. RESULTS: From 1389 DEG, 67 genes were identified to be significant in the regulation of lipid metabolism. By analysing the lncRNA-miRNA-mRNA interaction network, candidate hub lncRNAs consists of three genes with highest connective nodes; CYTOR, LOXL1-AS1 and HOTAIR. These genes are significantly upregulated in glioma. LOXL1-AS1 serve as an excellent prognostic biomarker for both glioma and glioblastoma as the effect of high and low LOXL1-AS1 expression on patients' survival is significant ($p<0.05$). CONCLUSIONS: Data mining and bioinformatics approach guided the identification of the potential hub lncRNAs associated ceRNA network in GBM lipid metabolism. This allows us to uncover the novel role of lncRNA in GBM tumorigenesis.

OTEH-6. ALGORITHMIC APPROACH TO CHARACTERIZE POST-TREATMENT RECURRENT GLIOMA USING RNA SEQUENCING AND QUANTITATIVE HISTOPATHOLOGY

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INTRODUCTION: Distinguishing between tumor and treatment effect in post-treatment glioma, although crucial for clinical management, is difficult because contrast-enhancing regions are mixtures of recurrent tumor and reactive tissue, and definitive histopathological criteria do not exist. This study disentangles the marked intra-tumoral heterogeneity in the treatment-recurrent setting by developing an unsupervised framework to algorithmically categorize intraoperative MRI-localized biopsies into three clinically-relevant tissue clusters based on joint analysis of RNA sequencing and histopathological data. METHODS: A retrospective cohort of 84 MRI-localized biopsies from 37 patients with post-treatment recurrent glioblastoma underwent mRNA extraction and quantification via PLATEseq protocol. For 48 of 84 biopsies, a neighboring piece of tissue underwent quantitative histopathology based on labeling index (LI) for SOX2, CD68, NeuN, Ki67, and H&E. Correlation between LIs and gene expression for these 48 samples was performed. Genes significantly correlated ($p<0.05$) with ≥1 marker were used for hierarchical clustering of correlation matrix, identifying three mutually-exclusive tissue-specific gene sets. These sets were then used to perform ssGSEA to categorize each of 84 biopsies into one of three tissue types. RESULTS: Correlation analysis identified 7779 genes significantly correlated with ≥1 histopathological marker. Clustering revealed three gene sets associated with specific markers: SetA-3688 genes associated with SOX2/Ki67/H&E; SetB-2418 genes associated with CD68; SetC-1673 genes associated with NeuN. ssGSEA using these sets categorized each biopsy into one of three tissue types: 27 biopsies enriched in SetA, 28 in SetB, and 29 in SetC. CONCLUSIONS: Using MRI-localized biopsies with both RNAseq and histopathological data, this algorithmic approach allows development of three orthogonal tissue-specific gene sets that can be applied to characterize the heterogeneity in post-treatment recurrent glioma: SetA: genes correlated with SOX2/Ki67/H&E, representing recurrent tumor; SetB: genes correlated with CD68 (microglia) representing reactive tissue consistent with treatment effect; SetC: genes correlated with NeuN (neurons), representing infiltrated brain.

OTEH-7. MOLECULAR CHARACTERIZATION OF TUMOR STIFFNESS IN GLIOBLASTOMA

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