ETMR-02. NOVEL CIC-LEUTX FUSION IN CNS EMBRYONAL TUMOR: A CASE REPORT AND REVIEW OF THE LITERATURE

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INTRODUCTION: Central nervous system (CNS) embryonal tumor is a group of rare, poorly differentiated neuroepithelial malignant neoplasm predominantly occurs in pediatrics. Herein, we firstly report a CNS embryonal tumor harboring pathogenic CIC-LEUTX fusion. METHODS: Immunohistochemistry (IHC), Fluorescence in Situ Hybridization (FISH) and Next Generation sequencing (NGS). RESULTS: A 2-year-old male was found to have solid and cystic mass in left temporal lobe-basal ganglia and left parietal lobe (maximum diameter=75mm). The pathological diagnosis was CNS embryonal tumor (NEC) after totally resection. The tumor was poorly differentiated embryonal neoplasms of neuroectodermal origin that lacked the specific features and rosettes. IHC showed Syn was strongly/diffusely positive and Ki67 proliferation index was high (50%+), and copy number at the 19q13.42 C19MC locus showed no alterations. NGS showed pathogenic mutations including a brand new CIC-LEUTX fusion, heterozygous germline NBN c.C127T mutation and somatic TSC2 c.G2714A mutation. One month after operation, intracranial tumor recurred (maximum diameter=55mm) and spinal cord implantation metastasis occurred, and then the patient received chemotherapy (CTX+CBP+VCR/DDP+VP-16) and had significant improvement in symptoms and tumor shrinkage (maximum diameter=31mm). Literature review revealed CIC fusion predominantly presented in sarcomas, such as CIC-NUTM1 fusion in rare CNS sarcoma, CIC-LEUTX fusion in epithelioid angiosarcoma and CIC-DUX4 fusion in Ewing-like sarcoma. Hitherto, apart from this case, there were only two cases which had CIC-LEUTX fusion in CNS, including a case of CNS angiosarcoma and a case of anaplastic ganglioglioma. CONCLUSIONS: We firstly found a specific new type in CNS embryonal tumor with distinct molecular-pathological characteristics of CIC-LEUTX fusion.

ETMR-03. THE ROLE OF FOXR2 IN PEDIATRIC BRAIN CANCER

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Forkhead Box R2 (FOXR2) is a transcription factor of the Forkhead Box family that has been correlated with tumorigenesis, aberrant cell growth or tumor progression. Expression of *FOXR2* in pediatric brain tumors is, besides in subsets of medullo-, pineo- and glioblastoma, primarily present in CNS neuroblastoma with FOXR2 activation (CNS NB-FOXR2), a novel entity that we in 2016 identified from the former class of primitive neuroectodermal tumors of the central nervous system (CNS-PNET). Analyzing CNS-NB-FOXR2 tumors we found that *FOXR2* mRNA is expressed in an anti-correlative manner compared to the proto-oncogenes *MYC* and *MYCN*. With immunoprecipitation analyses we show that FOXR2 binds to MYC and MYCN and is thereby stabilizing these proteins. These observations on the interaction and the anti-correlative manner suggest that FOXR2 and MYC(N) may drive tumor formation in a molecularly similar fashion. To investigate this further we stably expressed *FOXR2*, *MYCN* and *MYC* and a combination of *FOXR2* with *MYC(N)* in human neural stem cells (hNSC) and injected these in the striatum of NSG mice. We could show that hNSC itself do not from a tumor, whereas the expression of *FOXR2* and/or *MYC(N)* in hNSC results in tumorigenesis. Tumors expressing both, *FOXR2* and *MYC(N)* were growing faster than tumors with *FOXR2* alone. In addition, tumors are currently being analyzed by ChIP-sequencing for FOXR2, MYC, and MYCN, to better understand the mechanisms how FOXR2 drives tumor formation compared to its interaction partners MYC and MYCN.

ETMR-04. EMBRYONAL TUMOR WITH MULTILAYERED ROSETTES: THE MD ANDERSON CANCER CENTER EXPERIENCE

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BACKGROUND: Embryonal Tumor with Multilayered Rosettes (ETMR) are rare tumors that are molecularly diagnosed by C19MC amplification. Rarity of this tumor has precluded profiling uniform therapeutic strategy. METHODS: Retrospective review after institutional approval, identified 10 pediatric case of ETMR, treated at MD Anderson Cancer Center during the period of 2005 to 2019. RESULTS: Median age of at diagnosis was 4.6 years. Tumor sites include frontal or parietal lobes (3), spine (3) and posterior fossa involving the brainstem (4). All patients received a combination of chemotherapy and radiation. 4 patients had metastasis at the presentation. 9 patients received focal radiation but only 6 of them received Craniospinal irradiation (CSI). Average dose of radiation was 50 Gy. Surgical resection was performed in all cases except the brainstem tumors. 7 children had recurrence including all the patients with metastasis at diagnosis (median time: 9.4 months), 1 passed away secondary to hemorrhage in brainstem and data was not available for 2 patients. 5/6 patients who received CSI had recurrence. CONCLUSIONS: To-date no well-defined treatment regimens exists for these neoplasms, resulting in poor overall survival. Preclinical drug screen have shown the efficacy of topotecan, actinomycin D, and volasertib as potential new therapeutic candidates, though this has not translated successfully into the clinical arena. Given the limited success with current conventional therapeutic methods, molecular interrogation in addition to histopathological diagnosis are essential upfront, as it could provide clues to targeted therapy. Defining molecularly-based treatment with less toxicities and increased survival are warranted.

ETMR-05. SINGLE-CELL RNA-SEQ OF ETMR REVEALS CELL PROGRAMS OF DEVELOPMENTAL HIERARCHY AND CELLULAR DIVERSITY IN THE TUMOR MICROENVIRONMENT

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Embryonal tumors with multilayered rosettes (ETMR) are deadly brain malignancies affecting young children. No standard treatment is available and the median survival is less than 12 months. Molecularly, the disease is characterized by the miRNA C19MC cluster amplification, with the expression of multiples miRNAs related to a stem cell program. The discoveries on the purely molecular mechanisms of the disease did not help to create a bridge for new treatment strategies so far and the cellular diversity of ETMR remains poorly understood. In this study, we used single-cell RNA sequencing of murine and human tumors to describe ETMR cellular heterogeneity. Our findings support that intra-tumoral heterogeneity is mainly characterized by 4 cellular programs defining a developmental hierarchy related to different metabolic states: 1) Early quiescent NSC-like cells supported by fatty-acid oxidation 2) Late NSC and NP-like proliferative cells fueled by glycolytic metabolism; 3) Post-mitotic neuroblast-like cells, relying on oxidative-phosphorylation; 4) NSC-like proliferative cells, with metabolic plasticity and capable of performing the three types of metabolism. Tumor-specific ligand-receptor interaction analysis revealed that ETMR exchange with microglia and vascular mural cells (MC) signals related to extracellular matrix (ECM) organization (*Cxcl12-CxCr4*), stem cell signaling (BMPs-BMP receptors), anti-apoptosis and survival (*Ntf3-Ntrk*), not seen in the control brain. In addition, the vascular MC showed a cancer-associated fibroblast (CAF) phenotype, with potential prognostic implications, as previously demonstrated for other tumors. This study provides new findings to build up a more robust understanding of ETMR biology and opens space for further studies in the field.

ETMR-06. DISSECTING THE MOLECULAR AND DEVELOPMENTAL BASIS OF PINEOBLASTOMA THROUGH GENOMICS

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Pineoblastoma (PB) is an aggressive embryonal brain tumor comprising 1% of pediatric CNS tumors. The clinico-molecular heterogeneity and developmental origins underlying PB are poorly understood; therefore, we have assembled a molecular cohort of histologically defined PBs (n=43) with corresponding outcome data. Methylation profiling revealed four molecularly and clinically distinct PB subgroups, including two novel entities. Mutational and transcriptional analysis identified characteristic molecular features of each subgroup, such as mutations in the miRNA processing pathway or *FOXR2* proto-oncogene overexpression. Furthermore, subgroups exhibited differences in propensity for metastasis, cytogenetics, and clinical outcomes. To dissect PB developmental origins and resolve PB subgroup biology, we have employed a combination of single-cell genomics and genetically engineered mouse modeling. We created a single-cell transcriptional atlas of the developing murine pineal gland across 11 timepoints and are currently integrating these data with single nuclei RNA-seq data of human PB (n=25). Single-cell analysis of the developing pineal gland revealed three distinct populations of pinealocytes, referred to as early, mid and late pinealocytes, which segregate by developmental stage yet lie along a single developmental trajectory. Preliminary results implicate significant associations between PBs and the early pinealocyte population as well as subgroup-specific differences in intratumoral heterogeneity. Furthermore, this knowledge has informed the downstream generation of biologically faithful disease models, including a transgenic mouse model of the PB-RB subgroup. Remarkably, this model shows up-regulation of key markers of PB such as *Crx*, *Asmt* and *Otx2* and substantiates early pinealocytes as the probable cell-of-origin for this PB subgroup.

ETMR-07. ETANTR: A RARE TUMOR IN A RESOURCE-LIMITED SETTING

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INTRODUCTION: Embryonal tumor with abundant neuropil and true rosettes (ETANTR) is a rare aggressive brain tumor with low survival rates. There are about 80 cases reported in literature since 2000 when it was first described. There is no standard treatment scheme for ETANTR yet. CASE REPORT: A 2 years old boy presented with a month-long of headache and inability to hold his head. CT scan and MRI revealed a large mass in the right frontal lobe with midline shift. Subtotal tumor resection was done. Histological and immunohistochemical analyses was consistent with ETANTR in one laboratory and PNET in another. The second opinion suggested by the Center of Pediatric Oncology, Hematology and Immunology in Moscow the diagnosis ETANTR was confirmed. Taking into account certain similarities with medulloblastoma was decided to provide treatment according to HIT-2014 protocol. Control MRI done after 2 cycles of Block SKK Carboplatin/Etoposide found tumor progression and for that reason patient underwent second surgical resection. Considering the age of the child radiation therapy was not expedient and the decision was to continue treatment with HIT 2014 intensified regimen, which includes Cisplatin, Vincristine, Etoposide, Cyclophosphamide and intravenous High dose Methotrexate with intrathecal Methotrexate. Aiming to evaluate the effectiveness of treatment we are planning to perform MRI after this 2nd cycle of intensified regimen. DISCUSSION: There are difficulties in diagnosis of rare types of cancers in Armenia. Since there is no approved treatment for ETANTR, there is a need for ongoing research to improve its prognosis.

ETMR-08. INTERNATIONAL CONSENSUS PROTOCOL FOR EMBRYONAL TUMOR WITH MULTILAYER ROSETTES

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Embryonal tumors with multilayer rosettes (ETMR) are rare and highly-aggressive central nervous system (CNS) neoplasms which occur primarily in young children and carry a dismal prognosis. To date, no large clinical investigations have been conducted to determine the optimal therapy for ETMR. Data from retrospective case series suggest that our most aggressive standard therapies are not sufficient for cure in the majority of cases. New treatment approaches incorporating pre-clinical data and the known biology of ETMR are therefore urgently needed. A German drug screen using the patient-derived ETMR BT183 cell line and its xenograft revealed anti-tumor activity of topotecan, doxorubicin, and actinomycin D; three agents used infrequently for treating infant CNS tumors. Additional results from a small series of ETMR patients suggest that optimization of induction chemotherapy using these active agents may improve response and survival outcomes. In 2019, an international panel of pediatric neuro-oncology experts convened to advance therapy for ETMR. A consensus protocol was developed incorporating maximal safe surgical resection, induction chemotherapy with active pre-clinical agents, intrathecal chemotherapy, radiotherapy, and high-dose chemotherapy. This international consensus protocol represents the first prospective clinical investigation specific to ETMR and will be available through a treatment registry globally and as a clinical trial at select centers. The study aims to improve survival by providing aggressive, optimized therapy for ETMR and will serve as a platform to explore new biologically-promising agents. The investigation will also provide valuable prospective outcome data and correlative biological studies to serve as baseline comparators for future clinical trials.

ETMR-09. THE ROLE OF RADIATION FOR EMBRYONAL TUMOR WITH MULTILAYERED ROSETTES

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BACKGROUND: Embryonal tumor with multilayered rosettes (ETMR) is a challenging tumor. The prognosis of the patients suffering from this tumor is extremely poor. We have survival cases of more than 12 months. However, the status of illness is different. In order to clarify the cause of this difference, we reviewed our treatments in this study. TREATMENT COURSE: We have two cases. Both have relapsed after the same chemotherapy after the same radiation therapy. After the recurrence we used protocols that were included extended resection, second radiation therapy with bevacizumab. METHODS: We compared molecular biological evaluations for the initial and recurrent tumors. The resection rate at the time of second removal and the intensity of radiation therapy intensity were compared. RESULTS: We succeeded to remove the tumors with the confirmation of intraoperative MRI. No apparent differences could be seen in molecular biological characters of tumors before and after treatment. There was a difference between the period until radiation therapy and the irradiation methods. CONCLUSIONS: This tumor is untreatable only by resection. We need the second radiation therapy with bevacizumab. It was presumed that tumor should be irradiated quickly with appropriate irradiation field and dose.

ETMR-10. EARLY FOCAL RADIOTHERAPY AND TEMOZOLOMIDE FOLLOWING COMPLETE RESECTION APPEAR SUPERIOR TO INTENSIVE CHEMOTHERAPY AND DELAYED RADIOTHERAPY IN CHILDREN WITH EMBRYONAL TUMORS WITH MULTILAYERED ROSETTES (ETMR)

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BACKGROUND: Embryonal tumor with multilayered rosettes (ETMR) is a rare, aggressive embryonal central nervous system tumor characterized