ETMR-05. SINGLE-CELL TRANSCRIPTOMICS OF ETMR REVEALS DEVELOPMENTAL CELLULAR PROGRAMS AND TUMOR-PERICYTE COMMUNICATIONS IN THE MICROENVIRONMENT

Flavia W. de Faria[1], Carolin Walter[1,2], Marta Interlandi[1,2], Viktoria Melcher[1], Nicole Riedel[1], Monika Graf[1], Natalia Moreno[1], Melanie Schoof[3,4], Dörthe Holdhof[3,4], Christian Thomas[5], Michael C Frühwald[6], Bruno Maerkl[7], Ben Ho[8], Sarah Sandmann[2], Julian Varghese[2], Martin Ebinger[9,10], Martin Schuhmann[11], Aysegül Canak[9], Annie Huang[12,13], Ulrich Schüller[3,4], Thomas K. Albert[1], Kornelius Kerl[1]; [1]Department of Pediatric Hematology and Oncology, University Children's Hospital Münster, Münster, NRW, Germany. [2]Institute of Medical Informatics, Westphalian Wilhelms-University Münster, Münster, NRW, Germany. [3]Institute of Neuropathology, University Medical Center Hamburg-Eppendorf, Hamburg, HH, Germany. [4]Department of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, HH, Germany. [5]Institute of Neuropathology, University Hospital Münster, Münster, NRW, Germany. [6]Swabian Children's Cancer Center, Paediatric and Adolescent Medicine, University Medical Center Augsburg, Ausburg, Bavaria, Germany. [7]General Pathology and Molecular Diagnostics, Medical Faculty, University of Augsburg, Ausburg, Bavaria, Germany. [8]Department of Cell Biology, Hospital for Sick Children, Toronto, Ontario, Canada. [9]Department Pediatric Hematology/Oncology, Children's University Hospital, Eberhard Karls University of Tuebingen, Tübingen, BW, Germany. [10]German Cancer Consortium (DKTK) partner site Tübingen, Tübingen, BW, Germany. [11]Division of Pediatric Neurosurgery, Department of Neurosurgery, University Hospital of Tuebingen, Eberhard Karls University of Tuebingen, Tübingen, BW, Germany. [12]Division of Haematology Oncology, Department of Paediatrics, Hospital for Sick Children, Toronto, Ontario, Canada. [13]The Arthur and Sonia Labatt Brain Tumour Research Centre, Hospital for Sick Children, Toronto, Ontario, Canada

BACKGROUND: Embryonal tumors with multilayered rosettes (ETMR) are pediatric brain tumors bearing a grim prognosis, despite intensive multimodal therapeutic approaches. Insights into cellular heterogeneity and cellular communication of tumor cells with cells of the tumor microenvironment (TME), by applying single-cell (sc) techniques, potentially identify mechanisms of therapy resistance and target-directed treatment approaches. MATERIAL AND METHODS: To explore ETMR cell diversity, we used single-cell RNA sequencing (scRNA-seq) in human (n=2) and murine ETMR (transgenic mode; n=4) samples, spatial transcriptomics, 2D and 3D cultures (including co-cultures with TME cells), multiplex immunohistochemistry and drug screens. RESULTS: ETMR microenvironment is composed of tumor and non-tumor cell types. The ETMR malignant compartment harbour cells representing distinct transcriptional metaprograms, (NSC-like, NProg-like and Neuroblast-like), mirroring embryonic neurogenic cell states and fuelled by neurogenic pathways (WNT, SHH, Hippo). The ETMR TME is composed of oligodendrocyte and neuronal progenitor cells, neuroblasts, microglia, and pericytes. Tumor-specific ligand-receptor interaction analysis showed enrichment of intercellular communication between NProg-like ETMR cells and pericytes (PC). Functional network analyses reveal ETMR-PC interactions related to stem-cell signalling and extracellular matrix (ECM) organization, involving factors of the WNT, BMP, and CxCl12 networks. Results from ETMR-PC co-culture and spatial transcriptomics pointed to a pivotal role of pericytes in keeping ETMR in a germinal neurogenic state, enriched in stem-cell signalling. Drug screening considering cellular heterogeneity and cellular communication suggested novel therapeutic approaches. CONCLUSION: ETMR demonstrated diversity in the microenvironment, with enrichment of cell-cell communications with pericytes, supporting stem-cell signalling and interfering in the organization of the tumor extracellular matrix. Targeting ETMR-PC interactions might bring new opportunities for target-directed therapy.