Thieme

29th Meeting of Experimental Pediatric Neurooncology

Date/Venue
16. März 2023, Essen

Chair:
Prof. Dr. Matthias Eyrich, Würzburg
Prof. Dr. Pascal Johann, Augsburg

Editorial

After a longer, Corona-induced break, the year 2023 brought a re-launch of the Symposium "Experimental Pediatric Neurooncology":
Prior to the HIT-Tagung (Meeting of the national brain tumor study groups) at the WPE (Westdeutschen Protonentherapiezentrum Essen), researchers from various backgrounds reconvened on 16th of March to shed a light on emerging topics in pediatric brain tumor research.
This comprised a wide spectrum of topics ranging from Bioinformatic research on novel tools to decipher regulatory DNA elements, over molecular characteristics of the blood brain barrier and the tumor environment to current aspects of immune therapy.
Thus, we did not only cover the major entities in pediatric neurooncology (such as Medulloblastoma and Pilocytic Astrocytoma) but also had a plethora of rares entities represented: A number of lectures focused on ETMR (embryonal tumors with multi-layered rosettes) or on Rhabdoid tumors.
Beyond that, research on novel anti-tumor compounds, potentially effective in various tumor types, represented a further focus of the meeting.
Overall, the evolving fields of liquid biopsy and immunotherapy marked a further emphasis on the way towards personalized medicine.
We would like to thank the organizers of the HIT meeting (Prof. Dr. Beate Timmermann, PD Dr. Botzenhardt) for their infrastructural support as well as the Deutsche Kinderkrebsstiftung for financial help.
The next meeting for 2024 is currently still in the planning phase – further information will be released as soon as possible.

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Synergistic drug combinations for the treatment of *MYC* amplified medulloblastoma

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DOI 10.1055/s-0043-1774759

Background Patients with Group 3 medulloblastoma (MB) harboring a *MYC* amplification (subtype II) represent a high-risk subset of MB patients. We have previously shown that class I HDAC inhibition with entinostat is an effective treatment of *MYC* amplified MB cells *in vitro*. We here explore the potential of novel combination therapies with entinostat to further enhance treatment efficacy.
Methods We performed a drug screen of 75 clinically relevant drugs in combination with entinostat in three *MYC* amplified and one non-*MYC*-amplified MB cell line. Synergistic drug interaction of the top six drugs hits in CellTiter-Glo experiments was assessed by the Loewe additivity model using a combination of a ray and a checkerboard matrix design. Validation experiments of the synergistic drug behavior of entinostat and navitoclax included cell count experiments, quantification of apoptosis induction by caspase-3/7-like activity assays and on-target activity confirmation by acetylated Histone H3 immunoblotting and BCL-XL/BAK immunoprecipitation. To explore the mechanisms of action, the impact of entinostat on the proteome of MB cells was evaluated by antibody-based proteomic profiling.
Results 20/75 tested drugs were effective in combination with entinostat in all *MYC* amplified cell lines (combinatorial drug sensitivity score ≥ 10). The combination entinostat and navitoclax showed the most robust synergistic drug interaction, validated by reduced viable cell numbers and an increase in apoptotic cell death. The proteomic profiling revealed a disturbance of diverse vital cellular functions by entinostat.
Conclusion Entinostat and navitoclax show promising synergistic drug interaction in *MYC* amplified MB warranting further testing *in vivo*.

Multi-omics characterization of the blood-brain barrier in molecular groups of ependymoma

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DOI 10.1055/s-0043-1774760

Ependymoma (EPN) represents the third most common pediatric CNS tumor. While recurrence rates remain high, systemic therapies have so far failed to lead to clinical benefit. A better understanding of pathophysiological blood-brain barrier (BBB) characteristics represents an important component in developing effective (pre-)clinical trials.

Our study seeks to increase knowledge of molecular EPN group-specific BBB compositions as a proof-of-concept for other brain tumor entities. Furthermore, we explore the correlation between BBB characteristics and their functional impact to adapt an established *in silico* model that currently predicts drug penetration over the healthy BBB.

T-distributed stochastic neighbor embedding (tSNE)-based clustering analyses using the most relevant tight junction and transporter gene sets revealed distinct molecular EPN group-specific expression patterns. While PDX models (n = 20) showed high similarity with patient tumor samples, IUE mouse models (n = 2) did not fully recapitulate these BBB characteristics. Single-cell analyses and spatial mapping of protein abundance allowed dissection of BBB gene expression patterns in endothelial cells (e.g. Claudin5). Functional validation on protein level showed that coherence of RNA and protein is BBB gene-dependent.

The differences in BBB markers between molecular EPN groups may partly explain drug resistance of aggressive EPN as especially ZFTA fusion-positive tumors are characterized by high tight junction expression suggestive of low BBB permeability. Our multi-omics approach is intended to develop a score that further complements our established *in silico* prediction tool for BBB drug penetration. These findings will be validated in preclinical studies while molecular BBB characterization will be further expanded to other brain tumor entities.

Targetable T-cell epitopes on H3.3K27M altered diffuse midline gliomas

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Introduction Diffuse midline gliomas (DMG) are malignant brain tumors of childhood with unfavorable prognosis. Due to their localization, they are difficult to resect and respond poorly to radiochemotherapy.A hallmark are alterations at the H3K27 locus , either as histone3(H3) gene mutation or EZHIP overexpression, leading to epigenetic alterations, disturbed histone methylation and imbalanced gene expression. In order to exploit immunotherapeutic options in DMG tumors, aim of this project is to decipher the immunopeptidome of DMGs with a special focus on the methylome-based oncogenesis.

Experimental Procedures 21 native tumor tissues, as well as patient-derived primary cell lines were analyzed to cover for > 90 % of the HLA alleles in our population. HLA-I peptides are isolated from digested tumor tissue/cells using monoclonal antibodies. The bound HLA-I peptides are separated from the HLA-I molecules by acid elution, purified and identified by mass spectrometry (MS). Peptide sequences are subsequently validated bioinformatically. With search algorithms (peptide prism), peptides can be peptides/potential tumor-specific Targetable T-cell epitopes can be tracked back to derivatives of natural proteins as well as cryptic peptides from non-canonically translated RNA., binding ability to respective HLA alleles can be predicted by NetMHCpan and a matching with the so called benignome is done (https://hla-ligand-atlas-org). Immunogenicity testing is performed by CD8 + in vitro priming assay and TCR sequencing.

Results A total of 40,380 peptides could be identified in 15 patients, of which 83.6 % were "strong-", 14 % "weak HLA-binder" and only 2.4 % "non-binder"; 3.6 % were cryptic and 96.4 % were classical peptides. A total of 1,832 peptides were classified as tumor-specific, including 27.6 % cryptic and 72.4 % classical peptides. Based on a biological rationale, we selected 11 tumor-specific peptides as particularly promising T cell epitopes. Preliminary results show that tumor-specific peptides of both origins are immunogenic.

Conclusions We demonstrated that adequate amounts of cryptic and classical immunogenic peptides can be detected by mass spectrometry studies in DMG tumors. Furthermore, a recurrent occurrence of especially cryptic peptides were not only recurrently found in DMG patients (4/15), but also in other tumor entities (Recurrence up to 22). Tumor specific epigenetic changes might be related to higher expression of cryptic peptides. To investigate the effects of DMG specific epigenetic changes we are collaborating with the University of Göttingen, using CRISPR/Cas to knock-in/knock-out the H3.3K27M mutation in WT/H3.3K27mutated- DMG cell lines and with AG Vinci (Rome), conducting a pSILAC experiment by treating a H3.3K27M mutant cellline with corin, a dual inhibitor of LSD1 and HDAC to address mutation-related intrinsic changes. Comparative MS analysis will provide insight into mutation-induced immunopeptidome changes.

The role of key pharmacodynamic and pharmacokinetic parameters in drug response prediction of pediatric tumors in the precision oncology registry INFORM

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The first results of the German pediatric precision oncology program INFORM (INdividualized
Therapy FOr Relapsed Malignancies in Childhood) showed the significance of high evidence
levels for successful matched targeted therapy based on molecular diagnostics alone. Only a
small number of patients (8 %, 42/519) actually present with a high evidence target. This numbercould potentially be increased by adding information on drug sensitivity profiling (DSP). The aimof this project is to improve clinical treatment recommendations by investigating the role of keypharmacodynamic and pharmacokinetic parameters as filtering approaches in drug responseprediction of pediatric tumors.

Primary tumors underwent ex vivo drug sensitivity profiling in the INFORM DSP in a 384 well plateformat using a library of 79 clinically relevant oncology drugs. Metabolic activity was measured72h after treatment. Hit selection was based on dose-response curves-derived pharmacodynamic(PD) parameters (AUC and drug-sensitivity score (DSS)) and literature-derived pharmacokinetic (PK) parameters (maximum serum concentration (Cmax)) of the drug. Cell lines with a specific molecular alteration and clinically proven drug response were used as controls.

A ROC curve analysis showed that the AUC and DSS could both predict the matching drug in the cell lines. A statistical pipeline is currently being built and the PD and PK parameters will be combined to improve drug response prediction in cell line models and primary patient samples.

PD parameters could accurately predict sensitivity of drugs directed against high evidence level targets.

targets in cell lines. Further evaluation of the combination of PD and PK is under investigation.

Heliosterol – member of a new class of mpoxysteroids induces apoptosis in malignant brain tumor and cytostatic drug resistant leukemia and other tumor cells

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Due to the ability of steroids to induce apoptosis and to penetrate the CNS, and due to their high volume of distribution, these substances play an important role in chemotherapy against lymphoma and leukemia. However, steroid resistance in antileukemic therapy is causing an increasingly poor response rate. The work presented here summarizes recent discoveries related to the novel epoxy-steroid heliosterol, which overcomes multidrug resistance in solid tumor, lymphoma, and leukemia cells and induces apoptosis in medulloblastoma and glioblastoma cells, even in H3-K27 mutant cells.

Heliosterol induces apoptosis via the mitochondrial pathway, particularly in a ROS-dependent manner, and overcomes multidrug resistance caused by p-glycoprotein, overexpression of Bcl2, or underexpression of caspase-3 in leukemia and lymphoma cells. Treatment with prednisolone, prednisone, methylprednisolone, and dexamethasone-resistant cell lines has shown increased sensitivity to heliosterol compared with non-resistant cell lines.

Heliosterol shows a synergistic effect with various cytostatic agents, especially in multidrug-resistant cell lines. Compared to leukocytes, we demonstrated a direct effect on malignant cells without relevant non-specific cytotoxicity. First *in vivo* experiments show good tolerability in mice with a high distribution in the brain. Further *in vivo* testing is currently underway.

Moreover, since steroids have a high volume of distribution, heliosterol would be an excellent candidate for the treatment of solid tumors with peritoneal or CNS metastases, in addition to malignant brain tumors.

Multiplexed *in vivo* and *in vitro* genetic interaction screens for identification of gene collaboration networks and cancer vulnerabilities

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The development of first chemotherapeutic agents in the first half of the 20th century revolutionized and still determines treatment concepts of many malignant diseases. Exploiting the cancerous cell's increased replication rate at a vulnerability, classic cytostatic drugs achieved tremendous success rates such as combinatorial regimens in treatment of leukaemia and lymphoma. However, for many other malignancies (e.g., metastasized sarcoma, high-grade glioma) powerful chemotherapeutic alternatives are still missing, giving rise to newer and "targeted" therapeutic approaches as with immunotherapy or usage of "small molecules".

Genetic interactions (GI) represent functional interactions between pairs of genes, and situations in which the combined phenotype has a more severe fitness defect than would be expected from single gene deficiency are referred to as negative GI or synthetic sickness. A typical example is the clinically exploited synthetic sickness between BRCA and PARP. Identification of synthetic sickness interactions, may, though, be far from apparent, and CRISPR-Cas screens offer a powerful means to expand the scope of conventional studies. Previously, in a transplantation-based *in vivo* GBM gene perturbation screen, we identified a PRMT5-regulated splicing program as an exploitable cancer vulnerability (*Cancer Cell* (2017)). PRMT5 centric personalized therapies have since entered clinical trials for adult patients (e.g., NTC05094336; NTC03573310). We have since began to elicit multiplexed CRISPR mutagenesis directly in living tissues and organs (Nature protocols, 2022; Nature protocols, 2022; Nature reviews Cancer, 2020) and are heavily invested in the optimization of CRISPR technologies towards *in vivo* application (Nucleic Acids research, 2020). Focusing on transcriptional and RNA-processing pathways, we thereby identified and validated several unprecedented genetic interactions, which provide deep insights into gene collaboration during oncogenesis and emerging molecular vulnerabilities.

Unravelling similarities and differences in methylation patterns of SMARCB1 deficient pediatric tumors

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Introduction SMARCB1 negative tumors are a heterogeneous group of malignancies, comprising tumors from different parts of the body in different age groups. Among them are rhabdoid tumors (RTs): aggressive malignancies occurring predominantly in children under 3 years of age. This type of cancer often develops in the CNS (atypical teratoid/rhabdoid tumors ATRTs) although appearance in other soft-tissue sites is possible (then termed eMRT). While the epigenome of RTs has been studied extensively it is hitherto unclear if different SMARCB1 deficient tumors from various body parts harbor commonalities in terms of the expression of molecular targets.

Methods To unravel similarities and differences between various SMARCB1 deficient entities, we compared SMARCB1 (−) intra- and extracranial RT and other SWI/SNF deficient entities such as renal medullary carcinomas, epithelioid sarcomas and SCOOHT (small cell carcinoma of the ovary hypercalcemic type) by EPIC array methylation profiling.

Results In accordance with previous gene expression data, we found that RMCs separate from intra- and extracranial RT, pointing to a different cell of origin

and that additional genetic aberrations may drive tumorigenesis and thus alter tumor biology. Our analysis of differentially methylated genes by comparing RMCs to other kidney tumors predominantly revealed genes of early nephrogenesis to be hypomethylated in RMCs and showed distinct methylation patterns of genes involved in EMT like *OCD1* and *CEACAM1*.

In summary, we establish RMC as a separate entity with few similarities to eMRT and ATRT at the epigenetic level. Further research is necessary to unravel differences and commonalities in the universe of SWI/SNF deficient entities.

Rapid DNA methylation-based classification of pediatric brain tumors from ultrasonic aspirate specimens

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Background Cavitating ultrasonic aspirator (CUSA) devices are commonly used in neurosurgical procedures to carefully debulk tumor from adjacent healthy brain tissue. Here, we explore the feasibility of using ultrasonic minced tumor tissue to classify otherwise discarded sample material by DNA methylation according to the respective World Health Organization Classification of Tumors of the Central Nervous System using low pass nanopore whole genome sequencing.

Methods 21 ultrasonic aspirated specimens from patients undergoing surgery in the department of pediatric neurosurgery at the Charité –Universitätsmedizin Berlin with either newly diagnosed cerebral lesions or pre-treated lesions were processed by nanopore sequencing to generate copy number profiles and ad-hoc random forest classification. Results were compared to microarray-based routine profiling. Tumor purity was assessed.

Results In 19/21 (90.5 %) samples the minimum amount of 1,000 CpG sites were sequenced. In 20/21 (95.2 %) cases copy number variation profiles could be generated and matched microarray derived copy number profiles, allowing for identification of diagnostically or therapeutically relevant pathognomonic alterations. 12/17 (70.6 %) samples were concordantly classified to the corresponding microarray-based diagnosis by routine neuropathological workup. Applying the recently defined threshold for nanopore-based classification resulted in sensitivity of 64.7 % and specificity of 100 %.

Conclusion CUSA referred sample material of pediatric brain tumors allows for methylation-based classification according to the respective WHO classification of CNS tumors with acceptable sensitivity and high specificity. Hereby, a promising opportunity for accurate classification of pediatric brain tumors by a time- and cost-efficient advanced molecular technique is offered from otherwise discarded tumor tissue.

Identifying precision medicine opportunities for paediatric high grade brain tumours

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Background Childhood brain cancers are the leading cause of cancer- related death in children. Despite a better understanding of tumour characteristics and behaviour, the overall survival remains poor and current treatment options result in long-life sequelae and threatening treatment side effects. This project aims to find new therapeutic targets, to optimize childhood brain cancer therapy and to improve the patient's outcome.

Methods 82 paediatric brain cancer cell lines including AT/RT (Atypical Teratoid Rhabdoid Tumor) (n = 15), DMG /DIPG (Diffuse Midline Glioma) (n = 41), MB (Medulloblastoma) (n = 11) and pHGG (paediatric High-Grade Glioma) (n = 15) were analyzed for molecular characteristics using RNA and DNA sequencing methods. In addition, drug screens and CRISPR screens were performed to search for novel therapeutic opportunities. Statistical analysis was performed using a cohort-based method for the total group and a lineage-specific analysis for Medulloblastoma (MB) cell lines. This work was done in the context of the Childhood Cancer Model Atlas (CCMA).

Results In total, 32 drugs showed selectivity in the cohort. In a Heatmap, these drugs showed two clusters in which one cluster had an up-regulation of 83 genes. MB cell lines showed a dependency on the gene TOP1. MB showed sensitivity to Irinotecan ($p = 0.00023$), as well as to HDAC inhibitors (Belinostat, $p = 0.00468$) and led to cell death compared to the total cohort.

Conclusion Our results show that the grouping of different cancer types is mandatory for investigating new drug targets in brain tumours. The lineage-specific approach defined potential drugs having a promising effect in treating Medulloblastoma in vitro. However, it remains to be seen if in vitro data reflect in vivo effectivity sufficiently.

Inhibition of PARP results in highly effective radiosensitization of Gr. 3 medulloblastomas

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Medulloblastoma (MB) is the most frequent high-grade brain tumor of childhood and adolescence. Multimodal therapy of MB consists of combined surgery, chemotherapy and radiotherapy. Biologically, MB is a heterogeneous disease with at least four different sub-groups: WNT, SHH-TP53 mutant, SHH-TP53 wildtype and non-WNT/non-SHH (Gr. 3/4), with significant implications for prognosis. Despite the high importance of RT for disease control in MB, mechanisms underlying response and resistance to RT are incompletely understood. Furthermore, the development of new treatment approaches to increase radiosensitivity is highly important.

For the analysis of cellular radiosensitivity and DNA repair MB cell lines and ex vivo slice cultures of patient samples, patient-derived xenograft (PDX) and genetically engineered mouse models (GEMM) were used. The SHH medulloblastoma-derived cell lines were the most radioresistant strains, whereas the Gr. 3/4 cell lines demonstrated profoundly higher cellular radiosensitivity. Analysis of residual DSBs demonstrated a significant correlation between DSB repair capacity and cellular survival in vitro. In line with this, ex vivo analysis revealed elevated numbers of residual irradiation-induced DSBs in Gr. 3 samples as compared to those from SHH subgroup.

In first radiosensitizing approaches we treated the Gr. 3 MB cells and tumor slice cultures with the PARP inhibitors olaparib and pamiparib before irradiation. Twenty-four hours after treatment cell lines and tumor slice cultures displayed elevated residual DSB indicating compromised DNA repair. Moreover, Gr. 3 MB cell lines showed increased cellular radiosensitivity, when they were treated with PARP inhibitors before irradiation, demonstrating that PARP inhibition is an effective strategy to radiosensitize Gr. 3 MB cells.

Individualized multimodal immunotherapy improves overall survival of adults with IDH1 wild-type GBM

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A synergistic activity between maintenance temozolomide (TMZm) and individualized multimodal immunotherapy (IMI) during/after first-line treatment has been suggested to improve the overall survival (OS) of adults with IDH1 wild-type MGMT promoter-unmethylated (unmeth) GBM. We expand the data and include the OS of MGMT promoter-methylated (meth) adults with GBM. Unmeth (10 f, 18 m) and meth (12 f, 10 m) patients treated between 27/05/2015 and 01/01/2022 were analysed retrospectively. There were no differences in age (median 48y) or Karnofsky performance index (median 80). The IMI consisted of 5-day immunogenic cell death (ICD) therapies during TMZm: Newcastle disease virus (NDV) bolus injections and sessions of modulated electrohyperthermia (mEHT); subsequent active specific immunotherapy: dendritic cell (DC) vaccines plus modulatory immunotherapy; and maintenance ICD therapy. There were no differences in number of vaccines (median 2), total number of DCs (median 25.6x10e6), number of NDV injections (median 31) and number of mEHT sessions (median 28) between both groups. The median OS of 28 unmeth patients was 22m (2y-OS: 39 %) confirming previous results. OS of 22 meth patients was significantly better (logrank: p = 0.0414) with 38m (2y-OS: 81 %). There were no major treatment-related adverse reactions. The addition of IMI during/after standard of care should be prospectively

Preventing recurrence: targeting molecular mechanisms driving tumor growth rebound after MAPKi withdrawal in pediatric low-grade glioma

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Pediatric low-grade gliomas, the most common primary brain tumors in children, are mainly driven by alterations in the MAPK pathway. While patients often benefit from MAPK-inhibitors during treatment, tumor rebound may occur once treatment is stopped, constituting a significant clinical challenge.

BT-40, patient-derived cells with molecular features of pleomorphic xanthoastrocytoma ($BRAF^{V600E}$, CDKN2Adel), were used to model the rebound growth *in vitro*, based on viable cell counts in response to treatment and withdrawal of the clinically relevant $BRAF^{V600E}$-specific inhibitor dabrafenib and standard-of-care chemotherapy as a reference. Based on the observed cell-regrowth and MAPK signaling reactivation pattern (WB- and qPCR-analysis), key-timepoints during withdrawal were identified and further analyzed through RNAseq.

BT-40 cell regrowth was faster after dabrafenib withdrawal compared to chemotherapy withdrawal. MAPK pathway activity showed a transient overactivation upon treatment withdrawal before going back to baseline. Furthermore, single-sample geneset enrichment analysis and GO-Term analysis of upregulated genes upon dabrafenib treatment and withdrawal showed significant enrichment of cytokine-related signaling. This is associated with increased expression of 37 cytokines. Phospho-/proteomics analyses to validate this finding is currently underway.

The earlier cell regrowth after dabrafenib withdrawal compared to chemotherapy withdrawal matches clinical observations, making the model suitable to study the rebound. Gene expression analysis showed enrichment of cytokine activity upon dabrafenib treatment and withdrawal, potentially driving cell rebound growth. Protein expression and secretion of significantly upregulated cytokines is being investigated to identify potential rebound-breaking targets, which will be further investigated *in vitro* and *in vivo* using BT-40 as well as additional PXA models.

Revana: a comprehensive tool for regulatory variant analysis and visualization of cancer genomes

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Motivation As non-coding driver mutations move more into the focus of cancer research, a comprehensive and easy-to-use software solution for regulatory variant analysis and data visualization is highly relevant. The interpretation of regulatory variants in large tumor genome cohorts requires specialized analysis and visualization of multiple layers of data, including for example break-

points of structural variants, enhancer elements and additional available gene locus annotation, in the context of changes in gene expression.

Results We introduce a user-friendly tool, Revana (REgulatory Variant ANAlysis), that can aggregate and visually represent regulatory variants from cancer genomes in a gene-centric manner. It requires whole-genome and RNA sequencing data of a cohort of tumor samples and creates interactive HTML reports summarizing the most important regulatory events.

Availability and implementation Revana is implemented in R and JavaScript. It is available for download as an R package under <https://github.com/KiTZ-Heidelberg/revana>. Sample results can be viewed under <https://github.com/KiTZ-Heidelberg/revana-demo-report> and a short walkthrough is available under <https://github.com/KiTZ-Heidelberg/revana-demo-data>.

Supplementary information: Supplementary data are available at Bioinformatics online

Oncogenic Dependency of Pediatric Ependymomas on Extracellular Vesicle Pathways

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Introduction The majority of pediatric ependymoma (EPN) comprise either supratentorial EPN characterized by ZFTA fusions (ST-EPN-ZFTA) or posterior fossa group A EPN (PF-EPN-A), for both of which only limited therapeutic options are available. Data from previous transcriptional studies and a cross-species in vivo screen implied aberrant vesicular pathways in ST-EPN-ZFTA prompting further investigation of their putative role in EPN pathogenesis.

Methods We investigated EPN group-specific differences in extracellular vesicle (EV) biogenesis pathways in human EPN transcriptome and proteome datasets. In addition, we characterized isolated EPN EVs and their cargo by mass spectrometry, immunofluorescence staining and western blotting. This allowed for a pre-selection of inhibitors targeting specific EV biogenesis pathways. In vitro proliferation and invasion assays as well as in vivo treatment studies were performed on EPN model systems.

Results Integration of multi-omic data led to identification of ST-EPN-ZFTA-specific EV populations. We could spatially map specific EV markers to the perivascular niche that primarily harbors undifferentiated ST-EPN-ZFTA cell populations. Targeting lipid metabolism pathways of EVs reduced the abundance of released EVs from ST-EPN-ZFTA resulting in altered growth behavior and decreased invasion of tumor cells in vitro. In vivo validation of EV release inhibitors in an orthotopic ST-EPN-ZFTA PDX models significantly reduced tumor growth and increased survival.

Outlook We have leveraged ST-EPN-ZFTA-specific lipid metabolism pathways of EVs as a potential therapeutic vulnerability. Further mechanistic investigations on EPN EV biogenesis, release or uptake is expected to improve our understanding of the cross-talk between tumor cells and cells of the microenvironment and may lead to potential new therapeutic avenues.

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