TMOD-03. A NOVEL MB GR3 TRANSGENIC MOUSE MODEL IS GENERATED BY *MYCN* AND *P53* DEFECTS IN VENTRICULAR ZONE PROGENITORS.

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Medulloblastoma (MB) represents the most common embryonal tumour of the Central Nervous System in childhood. MB occurs in the cerebellum and molecular features dictate the classification into four subgroups. Although Group3 (Gr3) MB tumours are dominated by primitive progenitor-like cells, the cells of origin remain unidentified. Gr3 MB is associated with relatively common MYC family member amplification and overexpression, often combined with p53 pathway defects at relapse. Molecularly stratified treatment is not yet available, causing Gr3 MB and its subsequent relapse to often represent an unstoppable progressive disease. The limited understanding of Gr3 tumorigenesis and targeted therapy development is also due to the lack of faithful *in vivo* models and consequently, their use in preclinical studies. We have now developed a new germline genetically engineered *mouse* model (GEMM), harbouring MYCN amplification in a p53 inactive background (tamoxifen-inducible p53 activation, Trp53ER^{TAM}). The purpose of the GEMM is to investigate the developmental significance of MYC aberration in putative Gr3 MB cells of origin and exploit it in preclinical studies. A LSL-MYCN-Luciferase strain was crossed with mice expressing Cre recombinase under the Blbp promoter and subsequently to Trp53ER^{TAM} inducible mice. As result, the MYCN overexpression alone did not generate tumours, conversely to the combination of MYCN with p53 deregulation. Tumours arise exclusively in the hindbrain of homozygote mice, with a penetrance of 100% and a latency of ~135 days. Pathology report suggests tumours are Gr3 MB with large cell/anaplastic (LCA) histology. Preliminary transcriptional profiling data analysis reveals that tumours share molecular features with human counterparts, clustering with Gr3 MB. Ongoing analysis will explore the tumour cells of origin, followed by tumour progression alteration restoring p53 activity and blood-brain barrier integrity status. In conclusion, we have developed a MYCN/Trp53ER^{TAM} Gr3 MB GEMM arising from ventricular zone progenitor cells and resembling human cancer characteristics.

TMOD-04. IMAGE-BASED DRUG RESPONSE PROFILING FROM PEDIATRIC TUMOR CELL SPHEROIDS USING PATIENT-BY-PATIENT DEEP TRANSFER LEARNING

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Introduction: Image-based phenotypic drug profiling is receiving increasing attention in drug discovery and precision medicine. Compared to classical end-point measurements quantifying drug response, image-based profiling enables both the quantification of drug response and characterization of disease entities and drug mechanisms of actions. In pediatric precision oncology, we aim to study drug response in patient-derived 3D spheroid tumor cell cultures and tackle the challenges of a lack of image-segmentation methods and limited patient-derived material. Methods: We investigate deep transfer learning with patient-by-patient fine-tuning for cell-viability quantification. We fine-tune a convolutional neural network (pre-trained on ImageNet) with many cell-line-specific and few patient-specific assay controls. The method is validated using 3D cell cultures in 384-well microplates derived from cell lines with known drug sensitivities and tested with primary patient-derived samples. Network outputs at different drug concentrations are used for drug-sensitivity scoring; dense-layer activations are used in t-distributed stochastic neighbor embedding and clustering of drugs. Results: Cell-line experiments confirm expected hits, such as effective treatment with BRAF inhibitors in a BRAF V600E mutated brain tumor model and NTRK inhibitors in a cell line harboring an NTRK-fusion, indicating the predictive power of deep learning to identify drug-hit candidates for individual patients. In patient-derived samples, clustering of drugs further confirms phenotypic similarity according to their mechanisms of actions. Combining drug scoring with phenotypic clustering may provide opportunities for complementary combination treatments. Conclusion: Deep transfer learning with patient-by-patient fine-tuning is a promising, segmentation-free image-analysis approach for precision medicine and drug discovery based on 3D spheroid cell cultures.

TMOD-05. GENOME-WIDE DNA METHYLATION PROFILE: A POWERFUL STRATEGY TO RECAPITULATE HETEROGENEITY OF PEDIATRIC BRAIN TUMORS IN PRIMARY CELL LINES

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Background: Development of in vitro models of pediatric brain tumors (pBT) is instrumental for both understanding the contributing oncogenic molecular mechanisms and identifying and testing new therapeutic strategies. Primary cell lines should be established and managed to prevent epigenetic and genetic alterations and thus recapitulating the original tumor. DNA methylation (DM) is a stable epigenetic modification, altered in cancer and recently used to classify tumors. We aim to apply DM and Copy Number Variation (CNV) profiling to characterize pBT primary cell lines and tumors. Methods: We investigated 34 pBT tissues from different histology paired to 52 their derived primary cultures in both 2D and 3D conditions, as stem-cells or in serum-supplemented medium, and both short and long-terms in culture. We studied 18 additional pBT-derived cell-lines, 9 organoids, 5 commercial cell-lines, and 122 pBT tissues from the same histological categories, as controls, for a total of 240 genome-wide DM profiles. We analyzed DM and CNV profiles by using Illumina EPIC-arrays. By means of a bump hunting strategy, we identified differentially methylated regions in faithful vs unfaithful cell lines, and performed a functional characterization using over-representation analysis. Results The 69% (25/36) of cells at early passages retained genetic alteration and the same DM patterns of the original tumors, with no differences related to 2D/3D methods or the presence of serum in media. The 70% (24/34) of primary cell lines analyzed at later passages (>5 or >14 days in culture) diverged from the primary tumor, the totality of those cultured with serum. All divergent cells clustered together acquiring common deregulated epigenetic signature induced by serum culture media, 2D methods and longer time in culture. Conclusions: We have shown that global DM profiles, along with CNV analysis are useful tools to detect the recapitulation of pBT-derived primary cell-lines from the original tumor. Whatever subgroups tested, our results suggest that in vitro models should be passaged as little as possible to retain the epigenetic and genetic alterations of the tumors and thus to be considered relevant for basic and translational biology.

TMOD-06. LOSS OF DICER COOPERATES WITH TUMOR SUPPRESSORS TO INITIATE METASTATIC MEDULLOBLASTOMA

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To determine the role of microRNA regulation in brain tumor development, we incorporated a conditional allele of the microRNA processing enzyme Dicer to a previously characterized glioma mouse model based on inactivation of the tumor suppressors Nf1, Trp53, and Pten using the Nestin-creER^{T2} transgene. Loss of Dicer and tumor suppressors at adult ages led to glioma development; however, mutant mice tamoxifen induced at early postnatal ages developed medulloblastoma instead of glioma. The switch in tumor spectrum occurred with 100% penetrance and tumors were histologically indistinguishable from human medulloblastoma (MB). The minimum genetic mutations required for MB formation were Dicer and Trp53. Nf1 was dispensable, while additional loss of Pten produced more invasive tumors and leptomeningeal metastases. The time window for initiation of tumorigenesis was until the 2nd postnatal week, coinciding with the disappearance of the external granule layer (EGL), where cerebellar