3D GLIOMA-ON-A-CHIP MODELS FOR PERSONALISED MEDICINE IN ORGANOPLATES®

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Introduction Treatment of gliomas is complicated by variable response rates of individual patients’ tumours to therapies. The Department of Neurosurgery of the Erasmus MC has developed a 2D culture platform (GLIOscreen) for screening patient-derived glioma tissues with potential therapeutic compounds. Unfortunately, not all patient-derived glioma tissues are amenable to 2D culture, probably caused by tumour heterogeneity, raising the necessity for additional, complementing culture models.

Material and methods Here we show the development of an organotypic glioma model in the OrganoPlate to establish screenable cellular models for all glioma patients. The OrganoPlate is an easy to use, high throughput microfluidic platform enabling 3D cell culture and co-culture options, creating physiologically relevant models with a minimal requirement of cell material.

Results and discussions The 3D glioma model will be used to culture individual patient’s cancer cells for the screening of potential effective (combinatorial) treatments, such as Temozolomide, a first line therapy in glioma treatment. GLIOscreen-derived glioma cell line GS261 was seeded in BME2 (reduced growth factor ECM), in the OrganoPlate and cultured for 8 days. On day 8 cells were analysed by phase contrast imaging and the live/dead cell viability assay (Life Technologies). The cell viability can also be studied in time with RealTime-Glo (Promega), a non-toxic cell viability assay.

Conclusion Glioma cells can be cultured in the OrganoPlate for up to 2 months and are suitable for high-throughput chemotherapeutic drug screening.

INTERPLAY BETWEEN CODING AND NON-CODING GENOME IN HUMAN PARATHYROID TUMOURS

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Introduction Parathyroid tumours are the second most common endocrine neoplasia in women, after thyroid cancer. Mutations in the oncosuppressor CDC73 are the key event in most carcinomas whereas alterations in the tumour suppressor MEN1 (located at 11q13.1) occur in up to a third of sporadic adenomas. Although lncRNAs play a regulatory role in endocrine cancer pathogenesis, a lncRNAs profiling in human parathyroid tumours is still missing. Here, we identified a ‘molecular signature’ able to distinguish among parathyroid histotypes and a new potential epigenetic role of MEN1 in lncRNAs regulation.

Material and methods Ninety lncRNAs were investigated in 4 parathyroid carcinomas (PCas), 12 adenomas (PADs) and 2 normal glands (PaNs). Hierarchical clustering (HCL) and Significance Analysis of Microarray (SAM) were performed to identify differences in lncRNAs expression. Significant lncRNAs were validated in additional 7 PCas, 26 PADs, 6 atypical PADs (aPADs) and 4 PaNs. CDC73 and MEN1 genes mutations were detected by Sanger sequencing. PADs genomic characterisation was obtained by array Comparative Genomic Hybridization (aCGH). HEK293 cells were transiently silenced for MEN1 expression to analyse MEN1-lncRNAs correlation.

Results and discussions Nine lncRNAs were identified as differentially expressed in parathyroid tissues. Specifically, KCNQ1OT1 and SNHG6 were enriched in PaNs, reduced H1AR1, MEG3, HOX3as and NEAT1 expression characterised PADs, whereas BC200, HOX6as and WT1-AS were upregulated in PCas. HCL identified 3 clusters in which PaNs and PCas were distinctly separated, while aPADs were closer to PCs. Moreover, PADs clustered in a highly heterogeneous way. Notably, PCs and aPADs harbouring CDC73-mutations overexpressed the majority of the lncRNAs, compared to CDC73 wild-type samples. Interestingly, BACE1-AS, KCNQ1OT1, NEAT1 and SNHG6 levels in PADs were positively correlated with MEN1 levels. aCGH analysis revealed that Chr11 loss of heterozygosity (LOH) was the main chromosomal aberration in PADs. Of note, Chr11 LOH was associated with significant H1AR1 upregulation and these data were confirmed in HEK293 cells knocked-down for MEN1.

Conclusion Parathyroid histotypes are characterised by different lncRNAs signatures, suggesting a correlation with tumour aggressiveness and pathogenetic mechanisms. Further, our data highlight that lncRNAs profiles are related to CDC73 gene mutation status, chromosome 11 derangements and MEN1 inactivation.

TARGETED PROTEOMICS TO IMPROVE THERAPY STRATIFICATION OF CANCER PATIENTS

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Introduction The heterogeneity of tumours calls for patient stratification to select the most effective, personalised therapies. The NCT MASTER (Molecularly Aided Stratification for Tumour Eradication Research) program aims at comprehensive characterisation of cancer patients seen at NCT Heidelberg and Heidelberg University Hospital. SNVs, small InDels, CNVs, and gene expression data obtained by whole-genome/
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exome and RNA sequencing are assessed by a molecular tumour board for their potential clinical impact. Variants are assigned to therapeutic baskets (PI3K-AKT-mTOR signalling, RAF-MEK-ERK signalling, tyrosine kinase signalling, DNA damage response, developmental pathways, immune evasion, cell cycle), connecting actionable aberrations with targeted drugs. Evidence-based treatment recommendations are provided to treating physicians and are currently acted upon clinically in ~30% of cases. We here investigated whether targeted proteomics adds valuable information in the therapy stratification process.

Material and methods We analysed 134 tumour specimens, representing 17 entities, from patients enrolled in the NCT MASTER using reverse phase protein arrays (RPPA) technology. 123 antibodies for key proteins and posttranslational modifications (PTMs) mapping into the NCT MASTER therapeutic baskets were incubated, and signal intensities bioinformatically processed. Genomic data were reevaluated by the molecular tumour board to update therapy recommendations and were then integrated with proteomic information.

Results and discussions Heatmaps were generated to display expression/PTM patterns. The heterogeneity of the cohort was reflected in the proteomic data; however, samples did not cluster by entity, tumour cell content, or genomics-based treatment recommendation. Next, 48 proteins reflecting activation of cancer signalling pathways were investigated in detail to evaluate whether proteomic data would add relevant information towards shaping therapy recommendations. Results were compared with recommendations based on genomic data alone. Disparate results were found for many cases mirroring what the heatmaps had suggested.

Conclusion We have systematically evaluated the potential benefit of proteomic profiling in the therapy recommendation process of the NCT MASTER program. The data suggest that targeted proteomics indeed adds an additional, clinically meaningful layer to treatment stratification. Implementation of a proteomic workflow in the NCT MASTER program is in progress.

PO-457 OPTIMISED ARID1A IMMUNOHISTOCHEMISTRY IS AN ACCURATE PREDICTOR OF ARID1A MUTATIONAL STATUS IN GYNAECOLOGICAL CANCERS

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Introduction ARID1A is a tumour suppressor gene that is frequently mutated in clear cell and endometrioid carcinomas of the ovary and endometrium and is an important clinical biomarker for novel treatment approaches for patients with ARID1A defects. However, the accuracy of ARID1A immunohistochemistry as a surrogate for mutation status has not fully been established for patient stratification in clinical trials. Here we tested whether ARID1A immunohistochemistry (IHC) could reliably predict ARID1A mutations identified by next-generation sequencing.

Material and methods Three commercially available antibodies - EPR13501 (Abcam), D2A8U (Cell Signalling) and HPA005456 (Sigma) – were optimised for IHC using cell line models and human tissue, and screened across a cohort of 45 rare gynaecological tumours. IHC was scored independently by three pathologists using an immunoreactive score. ARID1A mutation status was assessed using 2 sequencing platforms. The concordance between ARID1A mutation and protein

PO-456 PROGNOSTIC SIGNIFICANCE OF PROTEIN AND GENE EXPRESSION OF C-MYC, BCL-2, BCL-6 IN DIFFUSE LARGE B-CELL LYMPHOMA DETERMINED BY DIGITAL IMAGE ANALYSIS

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Introduction Due to its heterogeneity in appearance and clinical behaviour, numerous studies have attempted to further subclassify this object into significantly different groups. We determined the prognostic significance of immunohistochemical (IHC) and molecular markers (FISH probes) using digital pathology in patients with DBLCL.

Material and methods The study included data on 54 patients with diffuse large-cell B-cell lymphoma aged 18 to 77 years (mean age 50±15 years). In 46% of cases, localised forms of the disease were identified (stage I-II), 54% - the general stage (III-IV stage). As therapy, patients received 5–8 courses of chemotherapy R-CHOP. IHC parameters were assessed to diagnose DBCL: bcl-2, bcl-6, pax-5, CD5, CD3, CD19, CD20, CD30, CD10, Ki67. Hans algorithm was evaluated in 100% of cases. The subtype of the GCB was established in 43% of cases, and not in the GCB in 57%. We made FISH for BCL2, BCL6, C-MYC genes and estimated their prognostic value. All patients slides were quantified using computer quantitave IHC and FISH algorithms. The results were measured in terms of overall survival, response, or relapse after a complete response. Statistical analysis was carried out using the statistical software R.

Results and discussions In the study group, 2 year survival without progression was 81% [95% CI 1.85–2.3]. Survival without progression was significantly lower in patients with B-symptoms (29% vs. 90%, OR 3.7 [95% CI 0.9–13, p<0.05]); the protein expression of the c-myc (26% vs. 39%, OR 1.7 [95% CI 0.25–0.9], p<0.05); the expression of the bcl-2 (64% vs. 83%) OR 1.9 [95% CI 1.4–2.0], p<0.05). Protein expression of bcl-6 has better outcomes (p<0.05). We found good correlation between c-myc protein expression and gene translocation C-MYC (p<0.05). GC/non-GC phenotype made according to Hans’ algorithm had no prognostic impact. BCL6 is the most frequently translocated gene, followed by BCL2 and C-MYC (14%, 12% and 3% respectively). The most frequent gene involved in amplification was BCL2, followed by BCL6 and C-MYC (9%, 6%, and 4% respectively).

Conclusion DLBCL represents a clinically and genetically heterogeneous group of lymphoma. Analysis of gene and protein levels of BCL2, C-MYC and BCL-6 alterations and clinical information provide important prognostic information to help identify a high-risk group of patients.

PO-455 ABSTRACT WITHDRAWN