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Program
13th Annual Meeting Dutch Society for Stem Cell Research
April 16th 2021

13:00-13:10 Welcome

13.10-13.30 Else Driehuis *Hubrecht Institute; Oncode Institute; Utrecht Medical Center (UMC) Utrecht, Dep. of Pathology, Utrecht, The Netherlands*
Patient-derived tumoroids as a therapy compass: can organoids indicate if and how to treat HNSCC patients?

13.30-13.50 A. Sabogal Guaqueta *University of Groningen, Molecular Pharmacology, Groningen, Netherlands*
Functionalty of human differentiated microglia and integration in 3D environment

13.50-14.10 Jelte van der Vaart Oncode Institute, Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences and University Medical Center, Utrecht, The Netherlands
Generation of adult stem cell derived organoid cultures from thyroid follicular cells

14.10-14.30 Antonio Tomasso *Hubrecht Institute-KNAW (Royal Netherlands Academy of Arts and Sciences), 3584CT Utrecht, The Netherlands*
Sustained ERK activity drives regeneration by promoting blastema formation and extracellular matrix remodeling

14.30-14.50 Sanne van Neerven *Experimental Oncology and Radiobiology, Experimental and Molecular Medicine, Cancer Center Amsterdam and Amsterdam Gastroenterology and Metabolism, Amsterdam University Medical Center, Amsterdam, The Netherlands*
***Apc*-mutant cells act as supercompetitors in intestinal tumour initiation**

14.50-15.10 Break

15.10-15.30 Albert Blanch Asensio *Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, The Netherlands*
STRAIGHT-IN: A platform for high-throughput site-specific integration of large DNA payloads into human pluripotent stem cells

15.30-15.50 Dennis Nahon *Anatomy & Embryology, Leiden University Medical Center, Leiden, The Netherlands*
Modular 3D NVU-on-a-chip model for studying vascular dementia

15.50-16.10 Giulia Campostrini *Anatomy and Embryology, Leiden University Medical Center (LUMC), Leiden, The Netherlands*
HiPSC-derived cardiomyocytes in tricell-type cardiac microtissues express post-natal SCN5A isoform revealing the mutant phenotype

16.10-16.30 Bahareh Rajaei *Department of Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands*
Towards a GMP-Compliant Protocol for the Differentiation of Human Pluripotent Stem Cells to β-like Cells for the Treatment of Type 1 Diabetes

16.30-16.50 Atilgan Yilmaz *The Azrieli Center for Stem Cells and Genetic Research, Department of Genetics, The Hebrew University of Jerusalem, Israel*
The essentialome of human pluripotent stem cells and their differentiated germ layers

16.50-16.55 Break
16.55-17.00 Award ceremony
17.00 End

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Abstracts presentations

PATIENT-DERIVED TUMOROIDS AS A THERAPY COMPASS: CAN ORGANOIDS INDICATE IF AND HOW TO TREAT HNSCC PATIENTS?

Else Driehuis[1,2*], Rosemary Millen[1*], Willem de Kort[2], Mandy Koomen[1], Remco de Bree[3], Lot Devriese[4], Robert van Es[3], Stefan Willems[5] and Hans Clevers[1,6]

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Organoids are three-dimensional stem cell-derived structures that can be grown from patient material. These miniature organs recapitulate disease characteristics when established from a wide range of pathological specimens, including cancer tissues. Tumor organoids have been shown to predict therapy response in patients. Head and neck squamous cell carcinoma (HNSCC) patients are treated with surgery with or without (chemo)radiotherapy. Here, chemotherapy is given as a radiosensitizer. Treatment side effects are significant and relapse rates are high. Therefore, selecting patients that can be spared harsh treatment side-effects would be of high value to clinical practice.
To assess if organoids can help guide therapy decisions in HNSCC patients both in primary and post-operative setting, we started an observational clinical trial. 60 tumoroid cultures have been biobanked and are currently analyzed for therapy sensitivity. 20 organoid cultures (confirmed tumor by DNA sequencing) have been screened so far, of which 8 (40%) could be used to correlate to patient response. Study inclusion criteria have since then been refined to enrich for patients likely to receive post-operative treatment. Therapy screening of patient-derived tumoroids was standardized by including quality control steps including single nucleotide polymorphism matching to patient material and the inclusion of 'housekeeping' organoid cultures to control for assay variability. Preliminary analysis of this small set reveals that organoid treatment response strongly correlates with patient relapse.
Our in vitro screening set-up allows us to assess the interaction of radiotherapy and chemotherapy. Chemoradiation of organoids confirmed that cisplatin and carboplatin serve as radiosensitizers *in vitro*, enhancing the effect of radiotherapy. Importantly, our results indicate that anti-EGFR antibody Cetuximab (a clinically used alternative to cisplatin or carboplatin) does not enhance the effect of radiotherapy and even might serve as a radioprotector. As Cetuximab is currently given concomitant to radiotherapy in patients, these results may hold clinical implications.

FUNCTIONALITY OF HUMAN DIFFERENTIATED MICROGLIA AND INTEGRATION IN 3D ENVIRONMENTS

A. Sabogal Guaqueta[1], A. Thiruvalluvan[2], F. Foijer[3], L. Barazzuol[4] , B. Eggen[2] , E. Boddeke[2], A. Dolga[1]

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Microglia, the brain-resident innate immune cells, are increasingly implicated in neurodegenerative diseases, such as Alzheimer's and Parkinson's disease. Our goal is to generate a human disease model to study how human microglia communicate with other brain cells in conditions of altered inflammatory signaling. First, we have generated and characterized microglia differentiated from an iPSC and ESC lines. Immunostaining of CD11b, TMEM119, and Iba-1 demonstrated the maturation of iPSC/ESC derived microglia in culture. The capacity of phagocytosis was tested both in premature and mature microglia in culture, showing an increased phagocytic capacity in mature microglia compared to progenitor cells as measured by Incucyte live-cell imaging using pHrodo E. Coli BioParticles conjugates. In addition, to mimic inflammatory pathways and to test whether the human iPSC-derived microglia react differently to LPS compared to primary mouse microglia, we performed real-time cell impedance measurements using an xCELLigence system. Microglia from both human and mouse increase cell index after LPS and alpha-syn stimuli. Finally, we investigated the integration of GFP-labeled microglia into brain organoids to mimic the 3D environment of the brain. Application of fluorescent-labeled mature microglia to 3-month-old cerebral organoids for a period of 10 days showed a significant integration based on the morphology of cells. We are currently determining the transcription profiles of microglia cultured in 2D and 3D in the presence of inflammatory stimuli (LPS & alpha-syn). These experiments will provide a characterization of human microglia in a "mini-brain" environment, which could be used to investigate underlying mechanisms and potential treatments of different neurological disorders.

GENERATION OF ADULT STEM CELL DERIVED ORGANOID CULTURES FROM THYROID FOLLICULAR CELLS

Jelte van der Vaart[1], Lynn Bosmans[1], Harry Begthel[1], Kèvin Knoops[2], Jeroen Korving[1], Willine J. van de Wetering[2], Peter J. Peters[2], Hanneke M. van Santen[3,4], Menno R. Vriens[5], Hans Clevers[1,3]

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The thyroid is essential for maintaining systemic homeostasis by regulating thyroid hormone concentrations in the bloodstream. Due to the limited number of representative model systems, there is limited understanding of fundamental thyroid biology as well as thyroid carcinogenesis. To fill the caveats in the understanding of thyroid cell biology, we aimed to develop an adult stem cell-derived three-dimensional (3D) organoid culture system using murine and human thyroid follicular cells (TFCs). We have succeeded to grow such an organoid culture system that harbours the complete machinery of hormone production visualised by the presence of colloid in the lumen and essential transporters and enzymes in a polarised cell layer. Both the established murine as human thyroid organoids express canonical thyroid markers PAX8 and NKX2.1/TTF1. Moreover, the thyroid hormone precursor thyroglobulin is expressed in both cultures to similar levels as in tissue. Extensive characterisation furthermore identifies known and new biological insights in TFC subclassification, subcellular organisation and hormone production using state-of-the art techniques like single cell RNA sequencing, transmission electron microscopy and genome editing. These 3D *in vitro* cultures allow for a variety of thyroid-related studies including the progression of wild type cells towards cancer. Additionally, due to the success of generating patient-specific tumour organoids of primary differentiated thyroid carcinoma and metastasis, insights in drug resistance and metastases can be identified. In short, this newly developed organoid culture of murine and human wild type TFCs as well as tumour tissue opens up an extensive area of research that will help understand the drivers for growth and development of thyroid (cancer) cells and enable studies upon drug responsiveness.

SUSTAINED ERK ACTIVITY DRIVES REGENERATION BY PROMOTING BLASTEMA FORMATION AND EXTRACELLULAR MATRIX REMODELING

Antonio Tomasso[1], Kerstin Bartscherer[1] and Ashley W Seifert[2]

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Regenerative ability diverges enormously across animal phyla and even closely related species respond differently to injury. While most mammals, including laboratory mouse strains (*Mus*) heals wounds with scarring, African spiny mice (*Acomys*) regenerate complex tissue structures, such as hair follicles, glands, nerves, muscles and cartilage upon injury. However, the mechanisms underlying this unique regenerative potential are poorly understood. The identification of core pathways driving regenerative rather than fibrotic healing in mammals are of a great significance. Here we show that MAPK/ERK signaling acts as a master regulator of regeneration. Despite ERK was activated immediately after injury in both *Mus* and *Acomys*, its activation was sustained only in *Acomys*, during blastema formation and regeneration. Upon ERK inhibition, re-epithelialization and regeneration were independently impaired in *Acomys*. Interestingly, the expression of markers characteristic of a pro-regenerative wound epidermis was reduced and we observed a downregulation of canonical Wnt signaling and an activation of TGF-β signaling, a pro-fibrotic extracellular matrix (ECM) profile and markers of inflammation. Thus, these data indicate that ERK inhibition skewed *Acomys* regeneration towards a *Mus*-like fibrotic response. Conversely, to partially recapitulate the *Acomys* regenerative healing in *Mus*, ERK activation was triggered by the ectopic injection of a specific growth factor mix. This treatment stimulated key features of regenerative healing, including cell proliferation, hair follicle neogenesis and pro-regenerative ECM remodeling. Our data demonstrate that ERK activation is a critical molecular hub that controls the delicate balance between regeneration and fibrosis at mammalian wounds. Understanding how ERK orchestrates wound response and regeneration might pave the way to activate regenerative programs in non-regenerating mammals.

***APC*-MUTANT CELLS ACT AS SUPERCOMPETITORS IN INTESTINAL TUMOUR INITIATION**

Sanne van Neerven, Nina de Groot, Lisanne Nijman, Brendon Scicluna, Milou van Driel, Edward Morrissey, Nicolas Léveillé, & Louis Vermeulen

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Colorectal cancer (CRC) development is characterized by the step-wise accumulation of mutations over time. Almost 90% of all CRCs present with early mutations in the tumour suppressor gene *APC*, leading to overactivation of the Wnt pathway and the formation of pre-malignant polyps. Previously, we have shown that *Apc*-mutant intestinal stem cells (ISCs) have a competitive advantage over healthy ISCs. Consequently, *Apc*-mutant ISCs frequently outcompete all healthy stem cells within a crypt, thereby reaching clonal fixation in the tissue and initiating cancer formation. However, the mechanism by which they outcompete the wild type cells remained unresolved. Now, we show that *Apc*-mutant ISCs act as supercompetitors by secreting Wnt antagonists that induce differentiation in neighbouring wild type ISCs. Downstream pharmacological activation of the Wnt signalling pathway by GSK3β inhibitor lithium chloride rendered wild type cells resistant to the Wnt antagonists and prevented expansion of the *Apc* mutant clones. We propose that boosting the cellular fitness of healthy cells to prevent expansion of mutant cells could provide a novel strategy to prevent CRC development in high-risk individuals such as familial adenomatous polyposis (FAP) patients.

Key words: cell competition – colorectal cancer – tumour initiation – Wnt signaling

STRAIGHT-IN: A PLATFORM FOR HIGH-THROUGHPUT SITE-SPECIFIC INTEGRATION OF LARGE DNA PAYLOADS INTO HUMAN PLURIPOTENT STEM CELLS

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Abstract

Inserting large DNA payloads (>10 kb) into specific genomic sites of human pluripotent stem cells (hPSCs) is still challenging. Applications ranging from synthetic biology to drug screening and the functional annotation of disease-associated variants would greatly benefit from tools that facilitate this process. Here, we merged the strengths of different classes of site-specific recombinases and combine these with CRISPR/Cas9-mediated homologous recombination to develop a strategy for stringent site-specific replacement of genomic fragments >50 kb in human induced pluripotent stem cells (hiPSCs). This platform, termed Serine and Tyrosine Recombinase Assisted Integration of Genes for High-Throughput INvestigation (STRAIGHT-IN) enables a genomic locus to be repeatedly modified not only rapidly, but also efficiently. We demonstrate the versatility of this approach by; 1) inserting a combination of fluorescent reporters as readouts for different parameters (action potential, calcium flux and contraction) of the excitation-contraction coupling cascade in hiPSC-derived cardiomyocytes, and; 2) simultaneously targeting multiple KCNH2 variants associated with arrhythmic disorders into a pool of hiPSCs, followed by clonal recovery of 11 of the 12 variants targeted. Furthermore, we confirmed the expected electrophysiological phenotype was observed for one of the variants introduced. In summary, STRAIGHT-IN offers a precise approach to generate panels of hiPSC lines containing either multiple combinations of transgenes or potential disease variants in the same cell line and genomic context both efficiently and cost-effectively.

MODULAR 3D NVU-ON-A-CHIP MODEL FOR STUDYING VASCULAR DEMENTIA

Dennis Nahon[1],*, Amy Cochrane[1] , Mees de Graaf[1] , Xu Cao[1] , Oleh Halaidych[1] , Francijna van den Hill[1] , Gisela Terwindt[2] , Laurent Roybon[3] , Christine Mummery[1] , Valeria Orlova[1]

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Neurodegenerative diseases are an increasing burden on society. Accumulating evidence indicates a fundamental role of the dysfunction of the brain vasculature in these pathophysiological conditions, resulting in vascular dementia. Here we aimed to develop a 3D multicellular model that would enable investigation of the complex interplay between the multiple cell types composing the neurovascular unit (NVU). We have previously established robust protocols to efficiently differentiate human induced pluripotent stem cells (hiPSCs) towards endothelial cells (ECs) [1], vascular smooth muscle cells (vSMCs) [2] and macrophages [3]. In the current work a novel protocol is used to generate forebrain neural progenitor cells (NPCs). These NPCs were differentiated towards astrocytes using a conventional 2D protocol or a 3D co-culture approach. In the 3D co-culture approach the hiPSC-derived cell types were combined in a hydrogel of choice and injected into a 3D microfluidic chip (AIM Biotech). Microvascular network formation and NPC differentiation were quantified in triple cultures of hiPSCderived ECs, vSMCs or primary human brain vascular pericytes (HBVPs), and hiPSC-derived NPCs. The formation of the perfusable 3D vasculature was achieved within 3-4 days after the cells were combined in a microfluidic chip. Examination of the detailed structural architecture using confocal microscopy showed that the cells oriented themselves similarly as found in vivo. hiPSC-derived NPCs supported 3D vasculature formation and differentiated towards astrocytes in prolonged cultures. In conclusion, we were able to robustly generate a 3D NVU-on-a-chip model comprised of hiPSC-derived cells. Future work using patient derived hiPSCs will enable us to model the vascular aspects of neurodegenerative diseases.

References

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HIPSC-DERIVED CARDIOMYOCYTES IN TRICELL-TYPE CARDIAC MICROTISSUES EXPRESS POST-NATAL SCN5A ISOFORM REVEALING THE MUTANT PHENOTYPE

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are potentially a powerful human cardiac *in vitro* model, but their immaturity is an obstacle in using them to study post-natal cardiac disease. *SCN5A* encodes the cardiac sodium channel Nav1.5 and undergoes fetal-to-adult isoform switch postnatally, regulated by alternative splicing. Mutations in *SCN5A* have been previously associated with severe cardiac arrhythmia, but those in the adult isoform of *SCN5A* are of difficult evaluation in immature hiPSC-CMs. We have shown that combining hiPSC-CMs in 3-dimensional microtissues together with hiPSC-derived cardiac fibroblasts and endothelial cells induced maturation in gene expression and functional properties, including electrophysiology. Here, we investigated whether this system could be used to reveal the functional phenotype of a mutation in the adult isoform of *SCN5A*. We derived hiPSC-CMs from a patient carrying two compound mutations in *SCN5A:* p.W156X in exon 4 and p.R225W in the adult splicing variant of exon 6 (exon 6B). Using CRISPR/Cas9, we corrected the exon 4 mutation to investigate specific effects of exon 6B mutation. ddPCR analysis showed a small fraction of exon 6B-containing transcripts in hiPSC-CMs, accompanied by no effect of exon 6B mutation on the sodium current, as assessed by single-cell patch clamp electrophysiology. Engineering the hiPSC isogenic pair by excising exon 6A with CRISPR/Cas9 also did not increase adult *SCN5A* expression, rather it resulted in splicing impairment and strongly reduced sodium current. However, maturing hiPSC-CMs in cardiac microtissues promoted *SCN5A* exon 6B expression and revealed the contribution of both *SCN5A* mutations to the cellular disease phenotype. These results show the utility of hiPSC-CM maturation in cardiac microtissue to induce expression of SCN5A adult isoforms and postnatal phenotypes, opening the possibility to study other developmentally regulated cardiac genes and to decipher the mechanisms underlying genetic cardiac arrhythmia in adults.

TOWARDS A GMP-COMPLIANT PROTOCOL FOR THE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO Β-LIKE CELLS FOR THE TREATMENT OF TYPE 1 DIABETES

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Background: β-cell replacement therapy by allogeneic pancreas or islet transplantation is a promising approach for patients with type 1 diabetes. However, there is a scarcity of organ donors. The generation of insulin-producing pancreatic β-cells from human pluripotent stem cells (hPSC) *in vitro* would provide an unlimited cell source for drug discovery and cell replacement therapy for diabetes. Here we aim to generate β-cells under GMP (Good Manufacturing Practice)-compliant conditions that can be used for clinical application.
Methods: We applied a modified seven-stage (30-day) differentiation protocol to generate hPSC-derived insulin-producing β-like cells in a 3D microwell culture system. Next, in order to scale up the production of hPSC-derived β-cells, we adapted the protocol to spinner flask culture. Differentiation was assessed by qPCR, immunocytochemistry and flowcytometry for stage-specific marker expression. β-cell function was evaluated *in vitro* by GSIS (Glucose-Stimulated Insulin Secretion), and *in vivo* by intraperitoneal glucose tolerance tests after transplantation of stage-7 cell clusters in immunodeficient mice and human C-peptide secretion was determined by ELISA.
Results: In the microwell set-up, cells acquired a definitive endoderm phenotype at stage 1 (day 3), characterized by expression of SOX17 (87.2% ± 4.6; n=5), FOXA2 (82.8% ± 4.6; n=3), co-expression of c-KIT and CXCR4 (93.3% ± 5.6; n=4), and silencing of pluripotency marker OCT4 (2.8% ± 6; n=5). At the end of stage 4 (day 12), pancreatic progenitor cells were identified by the co-expression of PDX1/NKX6.1 (46.7% ± 3.5; n=3). Finally, at the end of stage 7 (day 30), C-peptide-positive β-like cells (49.5% ± 10.5; n=3) and glucagon-positive α-like cells (18.4%± 6.1; n=3) were present. The stimulation index upon glucose stimulation was 4.4 ± 3.1(n=5). Following transplantation of day-30 clusters into mice, stimulated human C-peptide levels reached 46 pmol/L (n=21), 70.5 pmol/L (n=19) and 327.4 pmol/L (n=11) at day 14, 28 and 60 respectively, indicating further maturation of the cells *in vivo* after transplantation. Initial experiments in spinner flasks indicate that comparable differentiation efficiency can be reached in larger cultures.
Conclusion: We can generate functional hPSCs-derived β-like cells in GMP-compliant conditions *in vitro*. hPSCs-derived β-like cells are a promising future alternative to donor islets for the treatment of type 1 diabetes.

THE ESSENTIALOME OF HUMAN PLURIPOTENT STEM CELLS AND THEIR DIFFERENTIATED GERM LAYERS

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Maintenance of pluripotency requires coordinated expression of a set of essential genes. A thorough identification of these essential genes is vital for gaining a better understanding of the pluripotency state, as well as of the differences between this unique cellular state and differentiated states. To this end, we generated a genome-wide loss-of-function library in haploid human pluripotent stem cells (hPSCs) that we recently isolated and characterized. For this screening platform, we utilized CRISPR/Cas9 technology targeting more than 18,000 coding genes, using over 180,000 sgRNAs. This library enabled us to define the genes essential for the normal growth and survival of undifferentiated hPSCs. By defining the essential genes for hPSCs, we could allude to an intrinsic bias of essentiality across cellular compartments, uncover two opposing roles for tumour suppressor genes and link autosomal-recessive disorders with growth retardation phenotypes to early embryogenesis. More recently, we set out to map the essential genes for the differentiation of hPSCs into the three embryonic germ layers by using our loss-of-function library. Through the analysis of essential genes for the differentiation of hPSCs into ectoderm, mesoderm and endoderm, we defined the essentialome of each germ layer separately and also identified commonly essential genes for the transition from pluripotency stage into differentiated cells. Interestingly, the latter group was enriched by genes localized within the endoplasmic reticulum-Golgi network and regulate membrane and secreted molecules, highlighting the key role of signaling events during these dynamic cell state transitions. Overall, our work sheds light on the gene networks regulating pluripotency and early gastrulation events in human by defining essential drivers of specific embryonic germ layer fates and essential genes for the exit from pluripotency.

These studies were recently published:
Yilmaz et al. *Nature Cell Biology* (2018)
Yilmaz and Benvenisty *Cell Stem Cell* (2019)
Yilmaz et al. *Cell Stem Cell* (2020)