Developmental Origins of Paediatric Cancer symposium – book of abstracts

09:55-18:00, 8th June 2026
Francis Crick Institute, London NW1 1AT

Talks:

Natalie Andersson, The Wellcome Sanger Institute, Cambridge, UK
Clonal Bottlenecks in Normal and Predisposed Paediatric Liver Development

Introduction/Background
Hepatoblastoma is the most common paediatric liver tumor, with a median age at diagnosis of 18 months. It is frequently driven by CTNNB1 mutations and Wnt pathway activation, yet the earliest steps of tumor initiation remain poorly understood. Emerging evidence from Wilms tumor suggests that some childhood cancers arise from premalignant clonal expansions within histologically normal tissue, challenging the classical model of tumorigenesis. Whether hepatoblastoma develops through a similar mechanism remains unknown.

Methods
We performed multi-regional profiling of both tumours and surrounding histologically normal liver from six patients with hepatoblastoma and one patient with a liver metastasis from adrenal cortical carcinoma. In total 155 samples were included with a mean of 22 samples per patient from both tumour and macroscopically normal liver tissue. The samples were analyzed using whole genome sequencing, RNA sequencing, and DNA methylation profiling to characterize genetic, transcriptional, and epigenetic alterations associated with early tumorigenesis and liver development.

Results
Extensive genetic mosaicism was observed within histologically normal liver across all patients, including widespread clonal expansions spanning distinct regions. Phylogenetic analyses revealed that the hepatoblastomas arose from a subset of these clonal fields, providing direct evidence for premalignant precursor lesions within normal-appearing liver. In contrast, no such premalignant clonal fields were detected in the liver harbouring metastatic disease.

Livers from patients with Beckwith–Wiedemann syndrome (BW) exhibited significantly higher numbers of somatic mutations and increased intra-sample genetic diversity, compared to non-BW livers. These differences were accompanied by increased inter-regional heterogeneity, indicating more extensive clonal expansion and diversification in BW liver tissue. In contrast, non-BW livers showed fewer mutations and more limited clonal structure.

Transcriptomic analysis revealed that BW livers display a distinct molecular state, characterized by increased expression of GPC3, activation of Wnt signaling, and upregulation of RN7SK, alongside enrichment of imprinted genes. Pathway analysis showed activation of developmental and oncogenic programs, including PI3K–AKT, NOTCH, and extracellular matrix interactions, whereas non-BW livers were enriched for metabolic pathways. Recurrent alterations affecting chromosome 11p, including loss of heterozygosity or imprinting defects at 11p15.5, were identified in five of six hepatoblastomas and were detectable at subclonal levels in adjacent liver tissue in two BW patients. All hepatoblastomas showed reduced expression of H19.

Conclusion/Implications

These findings demonstrate that hepatoblastoma can originate from premalignant clonal fields within the developing liver and that the extent of clonal expansion and associated transcriptional reprogramming is influenced by the underlying tissue context, particularly in Beckwith–Wiedemann syndrome. This challenges the traditional single-cell origin model and highlights a previously unrecognized role for clonal liver mosaicism and developmental signaling states in pediatric tumorigenesis. Understanding these early events may inform strategies for early detection, risk stratification, and targeted intervention.

Caleb C. Reagor & Igor Adameyko, Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden
Self-Supervised Learning Uncovers a Shared Mesenchymal Landscape Across Development and Cancer

Introduction/Background

Mesenchymal transitions reshape diverse tissues across both embryonic development and cancer metastasis. During development, numerous epithelial-mesenchymal transitions (EMTs) convert 2D epithelia into highly motile 3D aggregates across all three germ layers. A small collection of core EMT transcription factors (EMT-TFs) such as Snail, Slug, Twist, and Zeb govern cells' progression from epithelial to mesenchymal states, including dissolution of cell-cell junctions, establishment of front-back polarity, and acquisition of motility. However, these EMT-TFs can also promote mesenchymal transitions across cancers including both carcinomas and non-epithelial malignancies such as gliomas, neuroblastoma, and melanoma. Although these transitions are highly context dependent and rely on combinations of EMT-TFs, they also display striking similarities in their final mesenchymal phenotypes. Here, we present a computational approach based on self-supervised learning to uncover a shared mesenchymal landscape across development and cancer.

Methods

To facilitate our identification of shared gene expression programs across mesenchymal transitions, we first collected publicly available single-cell RNA-sequencing datasets to construct a mesenchyme development meta-atlas that contained epithelial, mesenchymal, and intermediate cell states across embryonic development. We then performed pseudotime analysis to identify modules of genes whose expression varied as cells progressed towards mesenchymal fates such as lateral plate mesoderm, presomitic mesoderm, and cranial mesenchyme. These trajectories were subsequently used to train MesenCoder, a custom autoencoder model that learned a shared latent space for mesenchymal cell states and transitions. We used these embeddings to construct a shared mesenchymal landscape that included trajectories for gastrulation, posterior somitogenesis, and neural crest cell (NCC) migration. Lastly, we used this landscape to identify shared mechanisms driving mesenchymal transitions across diverse adult and pediatric malignancies in the curated cancer cell atlas (3CA; Tyler et al., 2025).

Results

We used MesenCoder to identify shared early and late gene modules across gastrulation, posterior somitogenesis, and cranial mesenchyme development. These signatures demonstrated transcriptional biases towards early and late programs in epiblast- and NCC-derived cells, respectively. Moreover, we discovered that Pou5f1 expression was highly predictive of early module expression across gastrulation and posterior somitogenesis. We subsequently screened >100 tumor datasets in 3CA for the expression of shared early and late signatures, which led to the identification of many expected diseases such as triple-negative breast cancer, head and neck squamous cell carcinoma, and adult and pediatric gliomas. Notably, we observed that cells from these tumors predominantly mapped to the NCC trajectory on the shared landscape and almost never to the gastrulation trajectory. Nearly all malignant cells expressed POU5F1, and numerous gliomas showed strong expression of its upstream factor, SOX2. Orthogonal validation using TCGA cohorts suggested that POU5F1 was a context-dependent driver gene across tumors undergoing mesenchymal transitions.

Conclusion/Implications

While previous studies have suggested that gastrulation represented the best developmental paradigm for understanding cancer EMT, our results instead suggest that NCC delamination provides a better framework for understanding this process across many malignancies. Our results further suggested that SOX2 and POU5F1 may represent proximal causes for mesenchymal transitions and local invasion in adult and pediatric gliomas, therefore constituting two important targets for preventing progression and mortality across these aggressive malignancies.

Lena Kutscher, Junior Group Leader, German Cancer Research Center
Developmental Determinants of Male Bias in Group 3/4 Medulloblastoma

Introduction/Background

Boys have an overall increased incidence of several childhood cancers relative to girls, including Group 3/4 medulloblastoma where the boy:girl ratio is >2:1. Although part of the bias can be attributed to X-linked genes, most cases cannot be explained by known genetic factors. As tumor initiation is believed to occur during fetal development, this sex bias likely reflects prenatal sex-specific developmental processes.

Methods

We used a multidisciplinary approach, integrating large-scale cancer genomics, developmental biology, and single-cell multi-omics in advanced mouse and human models.

Results

To elucidate the developmental basis of the sex bias observed in Group ¾ medulloblastoma, we generated a sex-matched single-cell RNA-seq atlas comprising over 300,000 cells from male and female murine cerebellum across seven developmental timepoints. At E16, GC_UBC progenitors giving rise to Group 3/4 medulloblastoma reach peak abundance, and male cells were more abundant and more cycling. To validate these findings, we isolated E16 GC_UBC progenitors using a novel genetically engineered mouse model and performed single-cell Multiome analysis. This dataset revealed differentially accessible chromatin regions that indicated intrinsically higher susceptibility to transformation in male cells. To determine whether the sexual dimorphism of this progenitor arises from intrinsic or extrinsic factors, we used the Four Core Genotype mouse model, which decouples presence of gonads from sex chromosome complement. Although presence of XY contributed to the abundance phenotype, the predominant effect was driven by presence of male testes. To assess the relevance of these findings in human cells, we generated human cerebellar organoids from XX and XY transgenic iPSC lines modified to carry a reporter for the lineage of origin of Group 3/4 medulloblastoma. Following treatment with dihydrotestosterone (DHT), estrogen or vehicle control, DHT-treated organoids harbored more LMX1A+ progenitor cells. Single-cell RNA seq of isolated LMX1A+ cells revealed a more progenitor-like state in the DHT-treated condition.

Conclusion/Implications

Altogether, we found that the male progenitor cells giving rise to Group 3/4 medulloblastoma were more abundant and more proliferative, providing a plausible biological explanation for the sex bias of this aggressive tumor type. Outcomes from this study may inform novel treatment strategies delivered according to sex and are likely to be broadly applicable to other sex-biased malignancies arising in early life.

Sumana Shrestha, University College London
Mapping The MYCN-Driven Oncogenic Transformation of Neuroepithelial Stem Cells to Embryonal Tumour with Multilayered Rosettes (ETMR)

Introduction/Background

Embryonal Tumour with Multilayered Rosettes (ETMR) is an aggressive paediatric brain malignancy with a five-year survival rate below 30%. A significant barrier to understanding the developmental ontogeny of ETMR is the lack of preclinical models that accurately represent its cell of origin. The transcription factor MYCN is a regulator of neurogenesis during embryonic development and is frequently overexpressed in ETMR. However, it remains unknown if MYCN is sufficient to initiate this disease. Human neuroepithelial stem (NES) cells are the early progenitors for the majority of neuronal and glial populations in the developing brain. These cells are regulated by genetic, epigenetic, and signalling pathways, such as WNT and SHH, to maintain a precise balance between proliferation and differentiation. Since ETMR displays characteristics of early neural development, we hypothesised that MYCN expression in NES cells is sufficient to alter normal developmental programs toward tumorigenesis. This study establishes a model to investigate how MYCN interacts with the lineagespecific features of human neural progenitors to produce ETMR.

Methods

We generated stable NES cell lines with doxycycline-inducible overexpression of a stabilised MYCN mutant (MYCNT58A) using a Tet-On system. These human neural progenitors were injected intracranially into immunocompromised NSG mice to assess their in vivo tumorigenic potential. Tumour initiation and progression were monitored non-invasively using high-resolution MRI. Upon reaching clinical endpoints, tumours were harvested for comprehensive analysis. This included histopathology and immunohistochemistry for ETMR-specific markers to verify lineage commitment. To confirm the developmental and molecular accuracy of the model, we performed RNA-sequencing, global DNA methylation profiling, and ATAC-seq to map the epigenetic state of the resulting tumours.

Results

MYCN overexpression in NES cells resulted in the formation of aggressive, high-grade tumours with 100% penetrance and a median survival of ~50 days. These tumours developed in multiple locations, including both supratentorial and infratentorial regions, which reflects the anatomic distribution observed in clinical ETMR. Histologically, the tumours showed high cellular density and primitive neuroepithelial characteristics. Molecular analysis and global DNA methylation profiling showed that these tumours clustered with clinical ETMR samples and were distinct from other paediatric brain tumour entities. Furthermore, gene expression analysis and ATAC-seq confirmed the activation of embryonic transcriptional programs and an open chromatin state at loci associated with early neural development.

Conclusion/Implications

In conclusion, increased MYCN expression in NES cells is sufficient to transform these human neural progenitors into ETMR. This inducible system allows for the dissection of the temporal role of MYCN in ETMR origin and the identification of the specific developmental windows where these progenitors are most vulnerable to transformation. By using xenografts and tumour spheroids, we can map the mechanisms that disrupt normal neural differentiation in ETMR. Our findings provide a robust model to investigate the developmental arrest characteristic of these tumours and identify therapeutic targets that specifically address the unique biology of early embryonic neural progenitors.

Harry Leitch, UCL Great Ormond Street Institute of Child Health
On the Developmental Origins of Paediatric Germ Cell Tumours

Introduction/Background

Germ cell tumours (GCTs) are among the most common childhood cancers and remain a major cause of morbidity and mortality. While platinum-based chemotherapy has improved overall survival, outcomes for poor-risk or relapsed patients are unacceptable, and current treatments carry significant long-term side effects. There have been no substantial therapeutic advances for decades, reflecting the lack of relevant pre-clinical models. GCTs arise from primordial germ cells (PGCs), the earliest precursors of sperm and eggs. Recently, there have been major recent advances in our understanding of PGC potency and in the in vitro tools that can be used to study human PGC biology. However, these advances have yet to be applied to answer questions relevant to GCT pathogenesis.

Methods & Results

We recently developed the first defined culture system to convert human PGC-like cells (PGCLCs) to pluripotent stem cells (PSCs) - capturing a key element of GCT pathogenesis (PMID: 41418783). We have begun to characterise this transition using single-cell transcriptomics to profile the transitions from the hPSC state to hPGCLCs and back, DNA methylation profiling to study the overall reversibility of epigenetic states, and multi-omics gene regulatory network analysis to identify key drivers of the cell state transitions. We aim to use this as the basis of an in vitro germ cell tumorigenesis (ivGCT) platform which will enable systematic dissection of pathogenic mechanisms and thus allow us to test longstanding assumptions regarding the developmental origins of GCTs.

Conclusion/Implications

Our ultimate goals is the generation of advanced human ivGCT models, which will serve as robust pre-clinical platforms to facilitate the discovery of new therapies in the future. By bridging developmental biology, stem cell biology, and cancer research, we hope to lay the foundations for future innovation and improved outcomes for children with GCTs.

Irina Poverennaya, Medical University of Vienna
RNA Modifications Connect Fate Selection in Neural Crest and Prognosis in Neuroblastoma

Introduction/background

Ribosomal heterogeneity may contribute to translational regulation and, in turn, influence cell fate decisions during embryonic development. Cranial neural crest cells (CNCCs) generate diverse lineages, including skeletogenic ectomesenchyme and neuroglial derivatives, yet the molecular mechanisms that bias CNCCs toward one fate over another remain poorly defined. Notably, aberrant reactivation of embryonic mesenchymal programs is often observed in aggressive, therapy-resistant neuroglial tumors such as neuroblastoma.

Methods

Using Smart-seq2 single-cell RNA sequencing on E8–E10 mouse embryos, we mapped the transcriptional programs associated with CNCC fate specification. We validated the identified pro-mesenchymal program in vivo using CRISPR/Cas9-mediated Tsr3 knockout mice. We also analyzed human neuroblastoma transcriptomic cohorts and performed functional assays on neuroblastoma cell lines following TSR3 knockdown.

Results

We identified a pro-mesenchymal gene signature that biases cranial NC toward a skeletogenic ectomesenchymal fate. Notably, this signature is enriched for genes involved in rRNA modification and ribosome assembly. Consistent with this, we detected a targeted hypermodification ($m^1acp^3\psi$) at the highly conserved U1248 position of the 18S rRNA P-site that was specifically enriched in migrating cranial neural crest cells. Genetic ablation of Tsr3, which catalyzes the final step of this modification, led to an almost complete absence of anterior facial ectomesenchyme in mutant embryos. In the context of cancer, high expression of this exact rRNA-modification signature acts as a robust predictor of poor survival outcomes and aggressive disease in non-MYCN-amplified neuroblastoma patients. In vitro, reducing TSR3 levels selectively impaired proliferation in aggressive, mesenchymal-like neuroblastoma cell lines.

Conclusions/Implications

Our findings highlight the importance of ribosomal specialization during embryonic development and suggest that rRNA modifications may contribute to cell fate biasing mechanisms. Together, these results reveal a link between ribosome-mediated translational control and both developmental and disease processes, and they point to potentially targetable vulnerabilities in aggressive pediatric cancers.

Jessica Taylor, CRUK Cambridge Institute, University of Cambridge
FOXO3 Suppression Underlies Subtype Diversity in WNT-Medulloblastoma

Introduction

WNT medulloblastoma is classically considered a single molecular subgroup with favourable prognosis. However, clinical variability and emerging genomic data suggest that WNT tumours may comprise biologically distinct entities, potentially reflecting differences in developmental origin and lineage state. We sought to define the molecular heterogeneity of human WNT medulloblastoma and determine how subtype genetics relate to tumour developmental context, with a focus on chromosome 6 alterations and FOXO3 suppression.

Methods

Human WNT medulloblastomas were analysed using integrated genomic and transcriptomic profiling to resolve subtype structure and identify candidate drivers. FOXO3 protein expression was assessed by immunohistochemistry across medulloblastoma subgroups. To test causality and developmental context in vivo, we generated Blbp-Cre driven conditional Foxo3 knockout mice and a complementary model combining Foxo3 loss with stabilised β-catenin. Tumour-free survival and histopathology were assessed across sexes. Mouse tumours underwent bulk transcriptomic and proteomic profiling to define molecular subtype relationships. Integrated single-cell RNA sequencing was combined with cerebellar developmental reference datasets and pseudo time trajectory analysis to infer lineage relationships and tumour origin across models.

Results

Human WNT tumours resolved into four subtypes (WNT1–4) with distinct genetic architectures. WNT1 exhibited mixed CTNNB1 and PTCH alterations, while WNT2 represented a canonical CTNNB1-driven state with frequent chromosome 6 loss and limited additional events. Most tumours fell within WNT3/4, characterised by predominant chromosome 6 loss, recurrent DDX3X mutations, and alterations in chromosome 6 genes, notably FOXO3. FOXO3 mutations were mutually exclusive with PI3K pathway mutations, consistent with convergent selection for FOXO3 suppression through either direct FOXO3 disruption or PI3K-mediated functional inactivation. FOXO3 immunohistochemistry demonstrated marked FOXO3 protein loss in WNT tumours compared with SHH, Group 3, and Group 4 medulloblastomas. In vivo, Foxo3 loss was sufficient to drive medulloblastoma with a pronounced female bias, with female mice showing a median tumour-free survival of 48 weeks, while males rarely developed brain tumours. Combining Foxo3 loss with stabilised β-catenin accelerated disease, reducing median survival in females to 34 weeks, and male median survival now at 45.7 weeks. Multi-omic analyses revealed coherent subtype structure across mouse tumours, including convergence of PI3K-driven β-catenin mutant tumours with Foxo3-deficient β-catenin mutant tumours into a shared transcriptomic state. Importantly, integrated single-cell and developmental mapping positioned Foxo3-driven tumours and Foxo3 plus β-catenin tumours along granule neuron progenitor (GNP)-associated trajectories, branching from postnatal granule lineage programmes rather than the canonical WNT developmental origins typically linked to mossy fibre precursor lineages. These data indicate that WNT-like signalling output can arise within a granule lineage context, uncoupling pathway activation from classical subgroup cell-of-origin assumptions.

Conclusions

These findings support a model in which WNT medulloblastoma heterogeneity reflects convergent genetic routes to WNT-active states implemented within distinct developmental contexts. FOXO3 suppression emerges as a key axis linking subtype genetics to tumour cell

state, and our mouse models provide a platform to dissect how developmental lineage influences tumour identity, signalling dependencies, and stratification beyond CTNNB1 status.

Flash talks/posters:

Thomas Eckhardt, St. Anna Children's Cancer Research Institute
A New Human Stem Cell-Derived Sympathoadrenal Linegae Organoid Model to Study Neuroblastoma Initiation
Poster number 10

Introduction/Background

Neuroblastoma arises from aberrant development of the neural crest-derived sympathoadrenal lineage. Yet the earliest steps of neuroblastoma initiation remain difficult to capture experimentally. Human stem cell-based 2D systems have advanced the field substantially. However, they fail to capture sympathoadrenal development in a three-dimensional and spatially organised context. There is currently no in vitro organoid model of the human sympathoadrenal lineage. To address this, we developed sympathoadrenal lineage organoids (SALOs) as a 3D model to study sympathoadrenal development and early events relevant to neuroblastoma initiation.

Methods

SALOs were generated by adapting an established two-dimensional sympathoadrenal differentiation system to a three-dimensional organoid format. Development was analysed across multiple timepoints by immunohistochemistry and single-cell RNA sequencing. scRNA-seq was performed using Parse Biosciences across six timepoints. To test the system in a neuroblastoma setting, SALOs were generated from engineered cells carrying 1q/17q chromosomal gains together with doxycycline-inducible MYCN. Phenotypes were assessed by immunohistochemistry, image analysis and single-cell transcriptomics in SALOs generated entirely from 1q/17q cells and in mosaic SALOs.

Results

SALOs transition from compact spheroids into organoids with prominent outgrowth-like structures. Immunohistochemical analysis showed that these structures contain the major cell types of the sympathoadrenal lineage, including neural crest cells, Schwann cell precursors, sympathoblast-like cells and chromaffin-like cells. They are connected by a network of peripheral nerves, with SOX10-positive cells localising along these nerve structures. To define this system at higher resolution, we performed Parse Biosciences scRNA-seq across six developmental timepoints. This confirmed the presence of populations with neural crest, Schwann cell precursor, sympathoblast and chromaffin-like signatures, and revealed a shift in cell composition over time from predominantly neural crest/Schwann cell precursor-like populations towards more differentiated sympathoadrenal populations. We then asked how SALOs respond to neuroblastoma-associated alterations. In SALOs generated entirely from 1q/17q cells, PHOX2B expression was lost at both protein and transcript level, in line with previous findings. Surprisingly, a substantial population of cells retained neuronal and sympathoadrenal-associated signatures despite PHOX2B loss. Induction of MYCN in this CNV background caused extensive proliferation, measured by increased organoid area and KI67 expression. Strikingly, a similarly strong proliferative response was also observed in mosaic SALOs. Here, perturbed cells showed behaviours not observed in SALOs generated entirely from 1q/17q cells, including the formation of outgrowths composed almost entirely of transgenic cells.

Conclusion/Implications

SALOs provide a human stem cell-derived three-dimensional model of sympathoadrenal lineage development. They capture key cell types, developmental transitions and spatial organisation of the lineage. This establishes SALOs as a novel and suitable model for studying the earliest steps of neuroblastoma initiation.

Isabella Scott, Institute of Cancer Research
Targeting Chromatin Remodelers in MYCN-Amplified Neuroblastoma
Poster number 12

Introduction

Neuroblastoma is a paediatric cancer of the sympathetic nervous system developing from genetic mutations, such as MYCN amplification. MYCN-amplified neuroblastoma has poor prognosis and high relapse rate, highlighting deficiencies in current treatment. This stresses the need for new therapeutics, however, MYCN is currently considered undruggable. We have sought to identify incompatibilities between MYCN amplification and additional mutations that together cause cell death by mutational burden, known as 'synthetic lethality'. We conducted a screen to identify synthetic lethal dependencies in neural crest cells engineered to overexpress MYCN and identified sensitivity to inhibition of chromatin remodelers ATRX and SMARCA4. We hypothesise ATRX and SMARCA4 have function in modulating MYCN-induced replication stress. Therefore, we will examine ATRX and SMARCA4 loss regarding DNA damage, chromatin remodelling and replication stress in MYCN-amplified neuroblastoma models. The project aims to define synthetic lethal mechanisms to reveal druggable pathways/proteins to improve MYCN-amplified neuroblastoma outcome.

Methods

We validated dependencies of ATRX and SMARCA4 with MYCN amplification using genetic or therapeutic approaches. CRISPR-Cas9 was used to knockout ATRX or SMARCA4 in a panel of neuroblastoma lines and neural crest cells. Camibirstat and ACBI1 (SMARCA4 small molecule inhibitor and degrader respectively) were used to confirm therapeutically induced lethality. The lack of available ATRX small molecule inhibitors prompted us to develop a targeted protein degradation (dTAG) system to study ATRX loss in MYCN-amplified neuroblastoma. ATRX was genetically modified to express a degradation tag, FKBP12F36V, for introduction into MYCN-amplified versus nonMYCN-amplified neuroblastoma lines. CRISPR-Cas9 of endogenous ATRX results in lines solely reliant on FKBP12F36V-tagged ATRX.

Results

ATRX knockout was specifically lethal to MYCN-amplified neuroblastoma lines and SMARCA4 knockout was lethal in MYCN-overexpressing neural crest cells, confirming ATRX and SMARCA4 as potential targets in MYCN-amplified neuroblastoma. MYCN-amplified neuroblastoma lines were significantly more sensitive to therapeutic SMARCA4 inhibition/depletion compared to non-MYCN-amplified lines, and this trend was maintained in the neural crest cells. This confirms the translation of SMARCA4 therapeutics to MYCN-amplified neuroblastoma, as well as validating the neural crest model as a suitable novel system to study MYCN-amplified neuroblastoma.

Conclusions/Implications

Synthetic lethal dependencies in MYCN-amplified neuroblastoma highlight approaches to blocking oncogenic activity of MYCN; loss of chromatin remodelling factors ATRX and SMARCA4 should present targetable opportunities. Incorporation of dTAG will enable genetic approaches to identify the essential ATRX domain responsible for synthetic lethality following introduction of various clinical ATRX mutations. In turn, the dTAG model may lead to identification of druggable ATRX domains, advancing degrader development. Currently, lipofection, nucleofection, and lentiviral transduction all failed to stably express FKBP12F36V-tagged ATRX in neuroblastoma cells, despite being successful in a positive control line. There is potential the size of ATRX (280kDa) is technically challenging in difficult-to-transfect neuroblastoma lines, or there is an underlying biological issue of ATRX overexpression in neuroblastoma causing lethality. In

attempts to overcome this, knock-in of FKBP12F36V to endogenous ATRX is now underway. In summary, the overarching implication is to identify established drugs that recapitulate synthetic lethality from loss of chromatin remodelers that can be clinically implemented to improve MYCN-amplified neuroblastoma prognosis.

Justas Stanislovas, UCL Cancer Institute
Osteosarcoma Cells Undergo Transient Cell Cycle Arrest and Transcriptional Rewiring Which Enable their Survival in Response to Map Chemotherapy in Vitro
Poster number 2

Introduction/Background

Osteosarcoma (OS) is the third most common malignancy in children and adolescents. Despite highly cytotoxic combinatorial standard-of-care chemotherapy, approximately half of the patient population show poor treatment response, as defined by <90% histological tumor necrosis, correlating with poor survival. It is not known why some patients respond poorly to the treatment. An underexplored potential mechanism of chemoresistance in OS is the ability of cancer cells to adjust cell cycling rates to survive chemotherapy. Proliferation dynamics of OS cancer cells during combinatorial chemotherapy regimen (doxorubicin, cisplatin and methotrexate, collectively termed MAP) and after withdrawal of the drug are not fully understood. Here we have set out to understand OS cell cycle behaviour of cells in treatment and their potential to recover.

Methods

To assess OS cell cycling behavior, we have utilized a stable, long-term in vitro label-retention system, doxycycline-inducible mCherry-tagged Histone 2B (iH2B-mCherry), whereby dilution of mCherry signal during proliferation is a marker of the proliferation rate. We have employed iH2B-mCherry to track cell proliferation in the absence of and during combinatorial MAP chemotherapy protocol and performed bulk or single cell RNAsequencing (scRNAseq) to investigate the transcriptional profile of chemoresistant OS cells.

Results

By following the dilution of iH2B-mCherry during cell proliferation, we observe a heterogeneous rate of proliferation across cell populations in established OS cell lines and primary patient-derived tumor cells in basal and treatment conditions. Bulk RNAseq indicates transcriptional differences between slower (mCherry high) and faster (mCherry low) proliferating cells, notably, upregulation of p21 expression and reduced ATP turnover in slower proliferating cells. We show increased retention of mCherry, a marker of non-proliferative cell state, during MAP chemotherapy in a dose-dependent manner. In concordance with in vitro results, we identify cell cycle arrest in scRNAseq of MAP treated population. Furthermore, employing a G0 arrest transcriptional signature, established in Wiecek et al. (2023, Genome Biol.), we detected enrichment of non-proliferative cells in MAP treatment conditions. Following the withdrawal of MAP chemotherapy, cells reverted to a proliferative state, indicating that resistance was mediated by a transient cell cycle exit. MAP treated cells showed upregulation of electron transport chain and autophagy as well as rewiring of ligand-receptor interactions, indicating potential survival dependencies.

Conclusion/Implications

This work uses a novel iH2B-mCherry system in OS to unravel cell proliferation dynamics in response to clinically relevant MAP chemotherapy, identifying cell cycle-dependent and independent survival mechanisms which may present novel therapeutic avenues.

Posters:

Charlotte Jones, University of Edinburgh
Dismantling cPRC1 Complexes to Inform Targeting of Diffuse Midline Glioma
Poster number 1

Introduction

Diffuse Midline Glioma (DMG) is a universally fatal paediatric cancer defined by a dominant mutation from lysine to methionine at position 27 of the H3 histone tail (H3K27M) [1–4]. Survival remains dismally low at just 9–11 months [5], highlighting an area of urgent clinical need. The Brien Lab has previously discovered a functional dependency on a specific type of canonical Polycomb Repressive Complex, containing CBX4 and PCGF4, as it maintains oncogenic gene silencing in the disease [6]. Crucially, other formations of the cPRC1 complex, containing CBX2 or CBX8, and/or PCGF2, were not identified as functionally dependent in DMG cells. This research presents unique therapeutic potential for DMG, but future efforts to target the complex depend on a deeper understanding of cPRC1 assembly and structure. Specifically, we aim to address the distinction between cPRC1 complexes containing PCGF4 and its mutually exclusive paralog, PCGF2.

Methods

We utilised two inducible knockout mouse cell lines, CreERT2 Pcgf2fl/fl and CreERT2 Pcgf4 fl/fl, and differentiated them to the disease-relevant developmental context, neural stem cells (NSCs.) To understand the effects of our knockouts on cPRC1 paralog proportions, we conducted endogenous immunoprecipitation of RING1B coupled with label free mass spectrometry (IP-MS.) This enabled us to examine almost all PRC1 complexes, as it is a predominant, core component of both cPRC1 and vPRC1. To validate IP-MS results we utilised IP-Westerns and glycerol gradient sedimentation to observe protein complex disassembly. Next, we wanted to understand if dynamics observed in the proteomics approaches were driven by changes in the binding or localisation of cPRC1 components to the genome by CUT&RUN-Rx.

Results

We have discovered distinct differences in complex integrity upon PCGF4 knockout compared to PCGF2 knockout. Through both RING1B IP-MS, and RING1B IP-Western Blot, we observed that PCGF4 knockout leads to rapid cPRC1 complex disassembly, while PCGF2 knockout does not. We see the abundance of CBX4-cPRC1 drop upon PCGF4 deletion, but this complex remains stable in the absence of PCGF2. CBX2-cPRC1 and CBX8-cPRC1 also show this destabilisation in the context of PCGF4 knockout (albeit to a lesser extent) while their abundance is unchanged following PCGF2 knockout.

Conclusions and Implications

Despite high sequence similarity, there is increasing evidence that paralogs PCGF2 and PCGF4 form distinct protein complexes. Indeed, we observe that PCGF2 degradation has a markedly lesser effect on complex integrity than PCGF4 degradation. Understanding these differences in complex stability is essential in order to therapeutically target CBX4-PCGF4 cPRC1 which is a functional dependency in Diffuse Midline Glioma. To further probe the interactions of PCGF proteins with other cPRC1 components, we are planning domain swap experiments between PCGF2 and PCGF4 (guided by AlphaFold modelling) which will enable us to probe their interactions with other cPRC1 components. This work is fundamental to fully exploit the genetic dependency on PCGF4-CBX4-cPRC1 and develop new therapies in Diffuse Midline Glioma.

References

1. Schwartzentruber J, Korshunov A, Liu XY, Jones DTW, Pfaff E, Jacob K, et al. Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. Nat Genet. 2012;44(3):251–3.
2. Sturm D, Witt H, Hovestadt V, Khuong-Quang DA, Jones DTW, Konermann C, et al. Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. Acta Neuropathol. 2012;123(4):615–26.
3. Bender S, Tang Y, Lindroth AM, Hovestadt V, Jones DTW, Kool M, et al. Reduced H3K27me3 and DNA hypomethylation are major drivers of gene expression in K27M mutant gliomas. Nat Commun. 2016;7:11185.
4. Lewis PW, Müller MM, Koletsky MS, Cordero F, Lin S, Banaszynski LA, et al. Inhibition of PRC2 activity by a gain-of-function H3 mutation found in pediatric glioblastoma. Nature. 2013;499(7459):434–7.
5. Hoffman LM, Veldhuijzen van Zanten SEM, Colditz N, Baugh J, Chaney B, Hoffmann M, et al. Clinical, radiologic, pathologic, and molecular characteristics of long-term survivors of diffuse intrinsic pontine glioma (DIPG): a collaborative report from the International and European Society for Pediatric Oncology DIPG registries. J Clin Oncol. 2018;36(19):1963–72.
6. Lagan E, Gannon D, Silva AJ, Bibawi P, Doherty AM, Nimmo D, et al. A specific form of cPRC1 containing CBX4 is co-opted to mediate oncogenic gene repression in diffuse midline glioma. Mol Cell. 2025.

Barbara Walkowiak, Wellcome Sanger Institute
Tracing Tumour Transmission in Monozygotic Twins
Poster number 3

Introduction/Background

Tumour transmission between individuals occurs extremely rare in humans, but can take across the placenta between the mother and child or between monozygotic twins in utero. However, very few cases of transmission of solid tumours have been reported and investigated in detail, and therefore their phylogenetic origin and patterns of clonal evolution are not well understood.

Methods

To determine that the tumour spread from one twin to another, rather than being of independent origin in both twins, we performed whole genome sequencing of six samples of normal tissues for each twin. This allowed us to identify early embryonic mutations which were private to either of the twins, and pinpoint the lineage that gave rise to the tumour.

Results

By showing that tumour cells in both twins carried early embryonic mutations private to only one of the twins, we provide unambiguous evidence for tumour transmission, rather than independent origin in both twins. To understand the patterns of clonal evolution during twin-twin transmission, we performed whole genome sequencing of multiple tumour samples from both the donor and the recipient twin. Our analysis suggest that the tumour has undergone an early diversification and parallel evolution in the donor twin. While the metastases within the donor twin were polyclonal, the metastasis to the recipient twin occurred relatively late over the course of its development and likely originated from a single clone.

Conclusion/Implications

Our study provides a high-resolution reconstruction of tumour evolution and metastases in the context of in utero transmission.

Joseph Wragg, University of Birmingham
Rhabdomyosarcoma Tumours Undergo Endothelial Trans-Differentiation to Evade Treatment and Enhance Aggression
Poster number 4

Introduction/Background

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children and adolescents. In the relapse setting 5-year survival is <20%, with few treatment options available. RMS is highly plastic with research to date focussed on myogenic differentiation. We have identified an alternative avenue of plasticity, whereby RMS cells activate an endothelial developmental program, concurrent with the formation of vessel-like structures (vasculogenic mimicry [VM]).

Methods

Cells from 10 RMS tumour lines were profiled for vascular mimetic capacity by visualisation of tumour-cell-derived vessel-like conduits in embryonic zebrafish xenografts and in vitro Matrigel tube formation assays. Response to regorafenib treatment was assessed by MTS and fluorescent tumour growth assessment assays. Matched transcriptomic and secretomic profiling was performed to identify markers of VM in RMS, which were validated by immunofluorescent imaging of cells and tissues.

Results

We have developed techniques to stratify RMS tumour samples by VM capacity using in vivo zebrafish xenografts and found it to be marked by haematopoietic marker CD34 and endothelial specific marker VE-cadherin. Profiling of the VM niche in 40 RMS clinical samples revealed it to be enriched in high-risk metastatic tumours and those that have relapsed from treatment. Profiling of In vitro and in vivo zebrafish xenograft tumour response to the MRTKI regorafenib treatment, revealed VM capacity to be strongly and positively linked to resistance with treatment driving both haematopoietic and myogenic trans-differentiation in distinct tumour cell sub-populations.

Conclusion/Implications

Vascular mimicry in RMS provides a potential route for tumour growth uncoupled from traditional vascularisation, allowing for the greater level of aggression and treatment resistance. Clinically used therapeutics, including regorafenib, can drive this behaviour. There is therefore an urgent need to better understand RMS endothelial plasticity and VM, to stratify patients and avoid detrimental therapeutic regimens, but also to investigate avenues for specific targeting.

James Birch, University of Sheffield
MYCN and Chromosomal Copy Number Alterations Drive Cell Competition during Human Neural Crest Development
Poster number 5

Introduction/Background

Neuroblastoma (NB) is the most common extracranial solid tumour in young children and it is often associated with poor prognosis [1]. The most aggressive NB versions are marked by MYCN overactivation together with chromosomal copy number alterations (CNAs) such as gains of the long arm (q) of chromosomes (chr) 17 and 1 [2,3]. However, the cellular mechanisms underlying MYCN and CAN-driven tumorigenesis are still elusive. CNA/MYCN-driven cell competition has been shown to play a significant role in both embryo development and tumour formation [4,5]. Here, I investigated whether MYCN overexpression and chr17q/1q gains trigger tumour initiation by inducing cell competition during the differentiation of human embryonic stem cells toward trunk NC cells and sympathoadrenal progenitors.

Methods

We developed an in vitro model of human trunk NC development based on the differentiation of isogenic hESCs carrying chromosome 17q and 1q gains and engineered to overexpress MYCN in a doxycycline-inducible manner. Using this platform, I examined cell competition in 'mosaic' cultures composed of wild type (diploid) and chr17q1q gain hESCs in the presence and absence of MYCN overexpression at different stages of differentiation.

Results

My data revealed a strong cell competition phenotype in mixed sympathoadrenal progenitor cultures favouring the expansion of MYCN-overexpressing chr17q1q gain cells and the elimination of their wild type counterparts. This process was driven by cell death and was accompanied by pronounced cell sorting, with populations of distinct genotypes becoming spatially segregated. Moreover, MYCN/17q1q-mediated cell competition was found to be developmental stage-specific, as no competitive advantage was observed in earlier NC cell cultures. I am currently exploring the molecular/signalling drivers underlying these observations.

Conclusion/Implications

My results suggest that MYCN/CAN-mediated cell competition is a key cellular mechanism driving the formation of aggressive NB tumours. This work provides new insights into the biology of NB pathogenesis and may inform the development of novel therapeutic strategies.

1] Louis, U. C., Shohet, M. J. (2015) 'Neuroblastoma: Molecular Pathogenesis and Therapy', Annual
Review of MEdicine', 66, pp.49-63. https://doi.org/10.1146%2Fannurev-med-011514-023121
[2] Pugh, T.J. et al. (2013) 'The Genetic Landscape of High-risk Neuroblastoma', Nature Genetics, 45(3), pp.279-283. https://doi.org/10.1038/ng.2529
[3] Huang, M., Weiss, W.A. (2013) 'Neuroblastoma and MYCN', Cold Spring Harb Perspect Med, https://doi.org/10.1101/cshperspect.a014415
[4] Claveria C, Giovinazzo G, Sierra R, Torres M. Myc-driven endogenous cell competition in the early mammalian embryo. Nature. 2013;500(7460):39–44.
[5] Price, C. J. et. al., (2021) 'Genetically variant human pluripotent stem cells selectively eliminate
wild-type counterparts through YAP-mediated cell competition', Developmental Cell, 56(17), pp.2455-2470. https://doi.org/10.1016/j.devcel.2021.07.019

Eloise Moore, Newcastle University
Establishment of Models of Aberrant ALK Regulation during Neuroblastoma Development
Poster number 6

Background

Neuroblastoma (NB) is a trunk neural crest (TNC) derived tumour of the sympathetic nervous system (SNS). It is the most common extracranial solid tumour in children, accounting for 8-10% of all childhood cancers and is responsible for ~15% of total paediatric cancer mortalities. High risk NB is defined as MYCN amplified NB or metastatic disease in a child over 18 months old, and is associated with less than 50% survival. Genetic aberrations in the ALK (anaplastic lymphoma kinase) gene either mutation or amplification, occur in up to 15% of cases, with higher prevalence at relapse. ALK inhibitors show therapeutic promise, however resistance frequently occurs particularly in ALK-amplified NB. The role of ALK in neuroblastoma pathogenesis remains elusive and requires further study.

Methods

Aim 1) To develop human embryonic stem cell (hESC) lines with inducible mutant and wild-type ALK overexpression.
Aim 2) To differentiate these hESC lines towards trunk NC/sympathoadrenal-lineages.
ALK inducible human (H7) embryonic stem cells (hESC) lines with doxycycline-inducible expression cassettes were generated with WT or mutant ALK-F1174L. Using a previously established differentiation protocol (Frith et al., 2018), these cells were differentiated towards TNCs and SNS derivatives.

Results

Work is currently focused on differentiating ALK aberrant clones towards the TNC and comparing these with doxycycline only controls, and with mutant against WT ALK. Key markers of each stage of differentiation will be identified with qPCR and immunofluorescence and the influence. Preliminary data suggests that there is no effect on TNC markers HOXC9 and SOX10 when WT ALK is induced. Further experiments are ongoing, to investigate influences on apoptosis and proliferation.

Conclusion/Implications

We have established hESC lines with inducible mutant and wild-type ALK overexpression. hESC lines have also been generated with inducible MYCN overexpression and inducible mutant or wild-type ALK which will be used to investigate the effect of inducing ectopic ALK alongside MYCN. This work will enable stage-specific investigation of ALK aberrations during normal human TNC/SNS development and their interaction with MYCN to understand the functional impact on TNC development and neuroblastoma initiation.

Anna Wulf, King's College London
Neuroblastoma-Associated ALK Mutation Leads to Impaired Migration in Human and Mouse Trunk Neural Crest Cell
Poster number 7

Introduction/Background

Neuroblastoma is a trunk neural crest (tNC)-derived childhood cancer, accounting for a total of 6% of all cancers in children. Mutations in the gene Anaplastic lymphoma kinase (ALK) are associated with aggressive tumours and poor prognosis, presenting in approximately 13% of high-risk neuroblastoma cases. Activating mutations in ALK are correlated with migratory and invasive cell behaviours in neuroblastoma cell lines, but whether these phenotypes are cancer cell-specific or are also reflected in the tNC is unknown. Methods: To address this, we first used tNC explants from E9.5 mice, overexpressed either the wild-type receptor or the neuroblastoma-associated ALKF1174L variant and analysed ex-vivo migratory using live imaging. Further, we generated a heterozygous ALKWT/F1174L human embryonic stem cell (hESC) line, analogous to the in-patient genotype, differentiated them into tNC via a neuromesodermal progenitor intermediate, and performed scRNAseq.

Results

In mouse tNC, we observed predominantly filopodia-driven migration, with increased migration dynamics in ALKF1174L overexpressing cells. Intriguingly, only the ALKF1174L variants display morphological changes, presenting increases cytoskeletal protrusions. Following this, we turned to tNC cells derived hESC to emulate transcriptional changes dependent on ALKF1174L expression. Although there was no significant difference seen in the capacity of these cells to generate tNC, scRNAseq data revealed that there was a downregulation of genes involved in pathways associated with adhesion junctions and focal adhesions. Many of these genes have previously been identified to be tumour suppressors, and loss is associated with poor outcomes in neuroblastoma.

Conclusion/Implications

We hypothesise that loss of these genes causes impaired migration, promotion of cancer initiation and subsequently enhance metastatic behaviour. Together, we show that mutant-active ALK affects migration before terminal differentiation, potentially priming cells for neuroblastoma initiation as early as the tNC stage.

Federica Lorenzi, Institute of Cancer Research
Cell State-Specific Factors Enable Alternative Lengthening of Telomeres Induction in ATRX Mutant Neural Crest Cells
Poster number 8

Introduction/Background

Neuroblastoma is a paediatric cancer of the sympathetic nervous system that originates in developing trunk neural crest (NC) cells. ATRX mutations are detected in 11% of high-risk neuroblastomas as either multi-exon deletions with in-frame fusions (IFF, 68%) or missense, nonsense and frameshift mutations causing loss of function (LoF) of ATRX. ATRX mutant neuroblastoma is slow-growing, metastatic and, unlike other high-risk neuroblastomas, is characterised by the activation of alternative lengthening of telomeres (ALT) to elude senescence. In stark contrast to other cancer types, ATRX mutant neuroblastoma is TP53 wild-type, however we and others have previously been unable togenerate ATRX mutations in neuroblastoma cell lines with functional TP53. Given the challenges in recapitulating ATRX mutant neuroblastoma, the role of ATRX in tumour initiation and progression is still largely unexplored. To address this, we aimed to generate more developmentally relevant models of ATRX mutations using human NC cells.

Methods

We adapted previously published human embryonic stem (HES) cell-derived trunk neural crest differentiation protocols (1) to enable the maintenance of NC cells before further downstream sympathoadrenal differentiation. Cas9-inducible H1 HES cells were used to generate models of ATRX knockdown, ATRX IFF and ATRX knockout NC cells. Induction of ALT was assessed by c-circle assay. Single cell-RNA sequencing was performed to further study the function of ATRX in differentiating NC cells.

Results

We show that HES H1 cells can be maintained as NC before downstream differentiation, enabling genetic and pharmacological manipulation at the NC stage. ATRX downregulation in NC cells strongly associated with downregulation of DRG2 (developmentally regulated GTP-binding protein 2) and upregulation of hypoxia-related pathways, a finding that was also recapitulated in ATRX mutant neuroblastoma cells. Immunofluorescence and single cell-RNA sequencing analyses also showed that ATRX knockdown in NC cells does not significantly impair the ability to differentiate into sympathetic precursor cells.ATRX mutations (resulting in ATRX IFF or ATRX LoF), generated at the axial progenitor state, were well tolerated in TP53 wildtype background. Our preliminary data identifies that this tolerance is likely due to cell state-specific differential regulation of TP53 signalling pathway in pluripotent cells. Given that DRG2 downregulation has been associated with both reactive oxygen species (ROS) production and the ALT phenotype (2, 3), we evaluated ROS levels in HES models and identified a higher production of ROS at the NC stage by immunofluorescence analysis. We therefore evaluated ALT status in our models: ATRX alterations did not result in ALT in either day 3 axial progenitor cells or at day 9 of differentiation (the first day that cells become NC cells). However, both ATRX IFF and ATRX knockout mutations induced ALT in NC cells at day 14, after maintenance at the NC stage.

Conclusion/Implications

Our data suggest that multiple factors, including the convergence of 1) decreased TP53 signalling and 2) increased oxidative stress at the NC timepoint result in a permissive environment for ALT to be triggered following ATRX mutations.

1) Frith, T.J.R. et al, 2018. eLife 7, e35786. 10.7554/eLife.35786.
2) Goncalves, T. et al, 2025. Nucleic Acids Research 53, doi.org/10.1093/nar/gkaf061.
3) Vo, M. et al, 2017. Biochem. Biophys. Res. Commun. 486,
http://dx.doi.org/10.1016/j.bbrc.2017.03.154.

Dylan Cameron, King's College London
Hpsc Neuroblastoma Model Reveals ALKF1174l Mediated Sympathetic Neuron Differentiation Block and SCP Enrichmnent
Poster number 9

Introduction/Background

Neuroblastoma (NB) is a trunk neural crest derived malignancy whose cell of origin is currently unknown. NB is clinically and genetically heterogenous making treatment development challenging. Anaplastic lymphoma kinase (ALK) is a tyrosine kinase receptor and is one of the most commonly mutated genes in NB. Gene amplifications and missense mutations within the ALK kinase domain result in aberrant receptor activation. The ALK p.Phe1174Leu (ALKF1174L) variant causes constitutive ligand-independent activation and is found in sporadic cases closely associated with metastatic and refractory disease. Previous studies on ALK F1174L in NB made use of genetically engineered mouse models; however, these failed to recapitulate the metastatic phenotypes of NB, which could be due to timing of transgene activation or differences in cell state transitions between human and mouse sympathoadrenal development.

Methods

We generated three human embryonic stem cell (hESC) lines; a ALKF1174L/WT "knock-in" line mimicking the in vivo genotype of patients and two transgenic overexpressing lines, carrying either the wildtype (ALKWT) receptor or the ALKF1174L allele. We then differentiated these lines towards a sympathetic neuron fate via a trunk neural crest intermediate.

Results

We show that ALK F1174L under endogenous control decreases the prevalence ofperipherin-positive sympathetic neurons, phenocopying the mouse model. Both overexpressing lines generate sympathetic neurons at similar levels to the control line, however the ALKF1174L allele exhibits an axonal elongation defect. To better understand the cellular heterogeneity and cell fate decisions throughout the differentiation, we conducted single cell RNA sequencing (scRNAseq). This revealed the enrichment of a highly proliferative Schwann cell precursor-like population in the ALKF1174L knock-in.

Conclusion/Implications

Together, these data shows that ALK in humans has a highly specific spatiotemporal role in sympathetic neurogenesis. Overexpression of the mutant receptor at very immature stages appears to be able to overcome the neurogenic blockading effect of ALKF1174L. While the same mutation under endogenous control enriches a stem cell like niche which could be transformed and subsequently give rise to tumours. Further characterisation of these SCP like cells when exposed to other NB associated mutations could give insights into the key mechanisms of NB initiation and progression.

Grace Cooksley, University College London, Institute of Child Health
Modelling Diffuse Midline Glioma Using a 3D Hindbrain Organoid Co-Culture System
Poster number 11

Introduction/Background

Diffuse midline glioma (DMG) is a deadly, incurable paediatric tumour, with an overall survival of 9 - 11 months post-diagnosis. Standard treatment of radiotherapy provides temporary benefit, and potential therapeutic interventions fail in clinical trials, highlighting the need for improved pre-clinical models. This work aims to create an organoid-derived model of DMG that encompasses tumour microenvironment components such as vasculature and microglia to explore the potential role of senescence in the underlying mechanisms of radiation resistance. Senescence is the phenomenon where cells undergo cell cycle arrest. Senescent cells have been implicated in promoting tumour growth by secreting protumour signalling proteins and can be induced by radiotherapy treatment. Our organoid-DMG co-culture model will be used to model DMG and its radiation resistance in aid of understanding how resistance is acquired and potential therapeutics that can be used to prevent this.

Methods

Hindbrain organoids were derived from Silvia et al., 2020 and co-cultured with DMG cell lines, DIPG IV and DIPG VII for 7 days. Hindbrain organoids were co-cultured with vascular organoids (Wimmer et al., 2021) for 9 days and interactions were assessed using whole mount imaging. Vascular organoids were also co-cultured with DMG spheres to test the integration of DMG cells within the vasculature without brain tissue. To explore radiation resistance, DMG spheres were irradiated 3 Gy/day for 5 days, left to recover for 3 days, then stained for senescence markers. This radiation protocol was repeated in our organoid-DMG co-culture model.

Results

Our DMG-hindbrain organoid model faithfully recapitulates the diffuse migration and invasion of DMG cells relative to our human embryonic pons co-cultures. The model shows similar expression of key markers of hindbrain and DMG, compared to published single-cell RNA sequencing. Hindbrain organoids were successfully vascularised and showed similar tissue architecture as found in embryonic hindbrain tissue. Preliminary results from the vascular-DMG co-culture showed that DMG spheres did not migrate diffusely as found in the hindbrain organoid cultures, suggesting that the DMG migration is reliant on interactions with hindbrain-specific cell types. Radiation induced a senescent-like signature in our DMG spheres; there was a significant reduction in EdU, a proliferative marker and significant upregulation in p21 and GLB1. However, after 28 days of culture, there was no rebound growth of the spheres. Preliminary results of irradiation response in hindbrain-DMG co-culture showed no impact to the hindbrain tissue. These findings suggest that tumour microenvironment (TME) components such as microglia, are required to model radiation resistance and tumour regrowth.

Conclusion/Implications

In summary, we have created a 3D co-culture model of DMG, recreating cell behaviours to target for therapeutic interventions and elucidated a potential senescence-mediated mechanism behind patient relapse and mortality. This work will be continued, by incorporating TME components such as microglia, to investigate if the model can recapitulate relapse and regrowth post-irradiation.