Karolinska Institutet

ABSTRACT BOOKLET

POSTER SESSION

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Djurönäset

POSTER 1

Systematic identification of differentiation-inducing gene targets in Neuroblastoma using single-cell deep learning models: an ongoing computational and experimental study

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Differentiation therapies are showing promise in treating neuroblastoma, one of the most common and deadly pediatric cancers, by inducing tumor cells into more benign, differentiated states. High risk neuroblastomas are treated with retinoic acid (RA), but the clinical utility is limited due to toxicity and potentially transient differentiation effects, underscoring the urgent need for novel therapeutic strategies. While alternative differentiation-inducing drugs, such as HDAC inhibitors, have shown potential, systematic exploration of gene targets that could similarly induce neuroblastoma differentiation remains largely unexplored.

Leveraging recent advances in artificial intelligence techniques capable of modeling complex biological systems, this project aims to systematically identify novel gene targets capable of inducing differentiation in neuroblastoma. We will perform extensive computational in silico studies, examining both single-gene and combinatorial gene perturbations, to predict differentiation-associated cellular responses at the single-cell level. Specifically, we are adapting a state-of-the-art deep learning model to accurately represent neuroblastoma cellular states and their responses to genetic perturbations while accounting for biological and technical confounders, such as variations in cell type, cell-cycle stages, and experimental batch effects. Following these computational predictions, we will experimentally validate the top candidate genes and gene combinations using CRISPR-based screening techniques combined with single-cell transcriptional analysis.

The project is ongoing, and lessons learned thus far have highlighted methodological challenges, including rigorous data preprocessing requirements, integration of heterogeneous datasets, and careful selection of analytical strategies. Insights gained from reviewing recent foundational models, such as scGPT and scFoundation, have underscored the critical need for explicit approaches to handle confounders, further guiding our current efforts. Addressing these challenges remains central to refining robust models and laying a strong foundation for subsequent experimental validations.

Key words: Neuroblastoma; Differentiation; Deep Learning

POSTER 3

Radiolabeling development and in vitro efficacy of [225Ac]Ac-DOTATATE in neuroblastoma

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Aim/Introduction

Neuroblastoma, the most common extra-cranial solid tumor among children, is today treated with a multi-modal treatment involving chemotherapy, surgery, local radiation therapy and maintenance treatment. Despite this, approximately 50% of patients face relapses [1], with most being metastatic diseases in the bone and/or bone-marrow.

Since the 1980s, 131I-mIBG therapy served as a second-line treatment for high-risk neuroblastoma. Recently, [177Lu]Lu-DOTATATE, which targets somatostatin receptor type 2 (SSTR2), is under clinical investigation for high-risk neuroblastoma (NCT04903899 and NCT03966651). With their superior energy transfer and shorter path length, Alpha-emitters such as Actinium-225 (225Ac) may outperform ß-emitters and effectively eliminate micro-metastatic neuroblastoma.

We aim to establish a robust [225Ac]Ac-DOTATATE labeling protocol suitable for in vivo administration and compare its in vitro efficacy against [177Lu]Lu-DOTATATE in the IMR-32 neuroblastoma cell line.

Materials and methods

We initially attempted previously published labeling protocols for 225Ac-labeled compound but found suboptimal yield and stability at high specific activity [2]. To address this, labeling was further optimized using 1 MBq 225Ac chloride, stabilized and pH-adjusted with sodium ascorbate and sodium acetate, before addition of dotatate acetate. The reaction was heated, followed by formulation with sodium ascorbate, DTPA and additional Dulbecco's phosphate buffered saline. Labeling yield was assessed with instant thin layer chromatography with sodium citrate. Two strips were developed: one strip was cut into two pieces and analyzed in a gamma counter after 30 min (221Fr) and after 4 h (213Bi); the other strip was analyzed the day after on a TLC scanner.

[177Lu]Lu-DOTATATE was prepared according to Lundsten et. al [3].

For in vitro efficacy testing, SSTR2-positive IMR-32 (2000 cells/well) and control SSTR2-negative HEK-293 (1500 cells/well) were plated and different radioactive concentrations of 177Lu- and 225Ac-DOTATATE were added. After 24h, cells were washed to remove unbound radiotracer. Cell viability was measured after 96h using CellTiterGlo and luminescence readout.

Results and conclusions

A robust labeling protocol of [225Ac]Ac-DOTATATE was successfully established, making it suitable for animal injections (1 MBq/5 µg DOTATATE, formulated in DPBS), >95% yield, stable 2d post-synthesis. IC50 was determined for [225Ac]Ac-DOTATATE (0.005 kBq/mL) and for [177Lu]Lu-DOTATATE (4.921 kBq/mL), highlighting the potency of α-emitters and their potential to drive deeper cell death and efficacy.

References

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Key words: Targeted Alpha Therapy; Theranostics

POSTER 4

Gaining spatial insight into patient-derived neuroblastoma tumoroids with Visium HD

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Neuroblastoma is a heterogeneous pediatric cancer with high relapse-and mortality rates among high-risk patients, prompting actions for increased understanding of its biology to develop improved therapies. Thanks to their ability to replicate tumor physiology and cellular heterogeneity, three dimensional tumoroids constitute attractive models for such studies, but detailed characterizations into spatial and transcriptional composition of tumoroids are rare. While single-cell sequencing technologies offer deep transcriptomic insight, they inherently lack spatial context. Similarly, traditional spatial technologies have been limited by low resolution and/or transcriptomic coverage.

To address these limitations, we applied the newly launched Visium HD spatial transcriptomic technology to patient-derived neuroblastoma tumoroids, enabling assessment of over 18 000 genes at a resolution as fine as 2 micrometers. We demonstrated that tumoroids can be processed using standard workflows for formalin fixation and paraffin embedding compatible with the Visium HD technology, yielding high quality transcriptomic data. Notably, the increased resolution generated a significantly more comprehensive and detailed picture of the tumoroids compared to its predecessor Visium V.2, and delivered data quality exceeding that reported of other technologies applied to organoids, such as Stereo-Seq. Similarly, our metrics were comparable to or exceeding that of other human samples analyzed with Visium HD, and displayed a sensitivity on par with publicly reported results from Xenium Prime 5K while capturing expression data for more than three times as many genes. To leverage this comprehensive transcriptome profiling, we co-cultured the same tumoroids with primary NK cells and detected distinct transcriptional signatures from both tumor – and NK cells. We also observed indications of heterogeneous responses among individual tumoroids to NK cells, highlighting the importance of spatially resolved analyses in these contexts. Ongoing investigations aim to determine whether this heterogeneity is linked to tumor cell properties such as differentiation status or cell-cell interactions, and whether these features have spatial dependencies. Further efforts aim to explore additional applications of the technology,

including transcriptional profiling across tumoroids of varying sizes and the potential for generating multilevel characterizations of individual tumoroids.

In summary, our results establish that tumoroids are well suited for Visium HD applications. Leveraging it, we present the first detailed spatial transcriptomic characterization of these patient-derived models, offering high transcriptional coverage at near single-cell resolution. This work lays a foundation for deeper investigations into neuroblastoma biology and offers new opportunities for methodological and therapeutic development in a spatial context.

Key words: Neuroblastoma; Patient-derived tumoroids; Spatial Transcriptomics

POSTER 5

CATALASE FREE RECOMBINANT VARIANTS OF PEROXIREDOXIN 2: PRODUCTION AND PURIFICATION FROM E. COLI

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Human Peroxiredoxin 2 (Prx2) is an important cytosolic peroxidase responsible for the hydrolyses of H_2O_2 and other peroxides and peroxynitrites through catalytic cysteines. It belongs to the typical 2-Cys peroxiredoxins, which contain two active site cysteines: the peroxidatic (C_P) and resolving (C_R) residues. Prx2 exists as a homodimer in a head to tail orientation, but it readily forms larger oligomers. In the catalytic cycle C_P reacts with hydrogen peroxide extremely fast to form sulfenic acid (C_P-SOH), which then condenses with the C_R of the opposing monomer of the functional dimer. The C_P-S-S-C_R disulfide is reduced back to thiol form by the thioredoxin or glutathione system in the final recycling step.

In recombinant peroxiredoxin production and purification is very important to get rid of endogenous *E. coli* catalase in the purification process because its turnover number is extremely high and competes with Prx2 for H_2O_2 in an NADPH independent manner, while the thioredoxin system requires NADPH for Prx2 recycling. For example, determining the activity of Prx2 enzyme by measuring NADPH consumption at A_{340} nm you cannot realize the presence of catalase activity. In addition, the determination of the H_2O_2 sensitivity of Prx2 can be misleading also, if catalase is present in the purified Prx2 stock.

We decided to express human Prx2 and its active site mutants in *E. coli* and purify them to homogeneity for in vitro kinetic study. We were particularly interested in how replacing active site cysteines to selenocysteines modifies the enzyme activity.

Expression constructs encoding His-SUMO-tagged Prx2 variants were designed for protein production in *E. coli*, which allowed the purification of proteins by immobilized metal affinity chromatography (IMAC). After selective binding the His-SUMO-tagged fusion proteins to the HisTrap^{TM}HP (Cytiva) nickel column, they were eluted in two, well separated fractions, Peak1 and Peak2 by applying 40% and 100% elution buffer containing mobile phase, respectively (1st IMAC). After cleavage of His-SUMO-tag by ULP1 protease, catalase-free Prx2 could be gained only from the digestion mixture of Peak2 (2nd IMAC). Thus, catalase could be separated in Peak1 along with a portion of Prx2.

The peculiar chromatographic behavior of Prx2, namely that one part of the same fusion protein can be isolated at 40% and the other part at 100% elution power, is likely due to the

oligomer-forming property of the enzyme, which allows the production of highly purified enzyme.

Key words: Prx2; Immobilized metal affinity chromatography; Selenoprotein

POSTER 6

Exploring the effect of SAMHD1 on tumor suppression in cutaneous melanoma

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Cutaneous melanoma (CM) is one of the most aggressive of all skin cancers and has the highest mortality. Although treatments with immune checkpoint inhibitors (ICIs) have improved the survival rate of CM patients, approximately 50% of these patients still die from the disease. SAMHD1, a key regulator of innate immune responses and DNA damage repair, can act as a tumor suppressor in various malignancies. We hypothesize that SAMHD1 expression confers a survival advantage by enhancing the anti-tumor immune response in CM. We propose that the loss of SAMHD1 in CM promotes tumor progression through dual mechanisms: first, by dysregulating dNTP pools, leading to replication stress and cell cycle disruptions, and second, by hyperactivating the STING pathway, which enhances the expression of immune-suppressive molecules like PD-L1, thereby limiting immune cell infiltration and contributing to immune evasion. This project aims to investigate the impact of SAMHD1 in inhibiting the STING-TBK1 axis in CM, which may result in improved response to immunotherapy in melanoma.

Our data showed that higher SAMHD1 expression is significantly associated with better overall survival (458 CM patients, TCGA data) with a proportion of high-expressers as long-term survivors after adjustment for lymphocyte infiltration. This result supports the hypothesis that the expression of SAMHD1 has a positive effect on survival in CM. Next, we have corroborated the association between loss of SAMHD1 and STING pathway activation in SAMHD1-wt and -KO melanoma cell lines. Indeed, cells lacking SAMHD1 showed hyper-activation of the STING pathway by increased expression of downstream target genes such as ISG15, IFI16, and CXCL10. Moreover, we evaluated if the presence of SAMHD1 can influence the immune response by co-cultures of NK cells from healthy donors and SAMHD1-wt and -KO melanoma cells alone or after treatment with STING inhibitors (STINGi). Our results showed that IFNγ secretion is higher when co-culturing NK cells with SAMHD1-wt cells than SAMHD1-KO cells, and it increases upon treatment with STINGi. Furthermore, SAMHD1-KO cells were more resistant to NK killing compared to SAMHD1-wt cells. Finally, we observed that loss of SAMHD1 can hamper DNA damage response in SAMHD1-KO cells by treatment with a double strand breaks (DSB)- inducing agents. Overall, by exploring the role of SAMHD1 in CM and examining its modulation of the immune landscape and genome instability, we aim to lay the groundwork for novel therapeutic strategies that could improve outcomes for CM patients.

Key words: Precision Medicine; Melanoma; SAMHD1

POSTER 7

GFP-tagged sORF library for systematic discovery of the microprotein repertoire encoded in the human genome

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Microproteins are small proteins (<100 amino acids) encoded by short open reading frames (sORFs) that have been largely overlooked in previous genomic and proteomic studies. Recent evidence suggests that microproteins may play essential roles in the maintenance of cell homeostasis, and therefore their dysregulation could contribute to the development of diseases such as cancer.

To systematically explore this new layer of the proteome, we have developed a lentiviral overexpression library of 8,000 sORFs likely to generate functional microproteins, each tagged with a green fluorescent protein (GFP) to facilitate real-time visualization of intracellular localization. Human melanoma cells were transduced with the library, and over 300 individual clones showing distinct compartment-specific localization patterns were isolated by fluorescence-assisted cell sorting (FACS). We are currently performing DNA sequencing and GFP-pulldown mass spectrometry (MS) to identify the specific microproteins expressed in each clone.

The future aims of this project are:

1) To establish a panel of microprotein-expressing cancer cell models and functionally characterize the contribution of specific microproteins to phenotypes related to cancer progression, such as cell proliferation, epithelial-to-mesenchymal transition (EMT), and chemoresistance.

2) To develop a machine learning model on our validated dataset to predict subcellular localization and infer potential functions of sORF-encoded microproteins, addressing the limitations of current tools trained on larger canonical proteins.

Key words: Microproteins; sORF library; Cancer

POSTER 8

Blood-based biomarkers of survival in patients receiving stereotactic radiotherapy with immunotherapy for oligoprogressive disease

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Purpose: Stereotactic ablative body radiotherapy (SABR) can be useful in oligoprogressive disease to overcome the resistance to immune checkpoint inhibitors (ICI) and extend the clinical benefit (CB) of systemic therapy. However, there are no defined biomarkers to aid in patient selection. Also, the regulation of the ICI+SABR synergy needs to be elucidated. We hypothesize that inexpensive and convenient biomarkers such as cell-free DNA (cfDNA) and the neutrophil-lymphocyte ratio (NLR) may serve for the prediction of the CB, and that such benefit may be primed and marked by DNA methylation redistribution that is traceable in blood.

Methods: Ongoing prospective multicenter study of metastatic patients in oligoprogression to ICI (1-5 extracranial sites) that maintain ICI due to CB (n=61 so far). Patients receive concomitant SABR (35 Gy in 5 fractions, fx) to all oligoprogressive lesions. Blood is obtained before SABR (T1), after the first (T2) and last (T3) fx, 2 months after SABR (T4) and at further progression (TP). Response is evaluated 2 months after SABR and subsequently every 3 months by iRECIST and defined by the objective response rate (ORR) in all lesions (in and out-of-field)– complete (CR) and partial responses (PR) –. cfDNA concentration is measured in plasma across all time points, as well as the cfDNA methylation levels in candidate genes through targeted sequencing. The absolute neutrophil and lymphocyte counts are obtained from T1 and T4 samples. cfDNA and NRL results are correlated with progression-free survival (PFS) and overall survival (OS), while cfDNA methylation results served for building a response classifier.

Results: Most patients had lung cancer (51%) under pembrolizumab (41%) with a single progressing lesion (61%) located in nodal sites (46%). With a median follow-up of 12 months (range, 3-37 months) ORR was 59% (26% CR and 33% PR). Median (m) PFS was 15 months (95% CI, 8 months-NR). mOS times were not reached.

A decrease in cfDNA > 14% from T1 to T4 correlated with better mPFS (NR vs 8 months, p = 0.015). In T3, levels < 0.37 ng/uL also correlated with improved 1-year OS (91% vs 62%, p = 0.031).

Low NLR (< 1.81) at T1 was informative of better mPFS (NR vs 7.5 months, p = 0.034) and 1-year OS (100% vs 70%, p = 0.01).

In a homogeneous fraction for NSCLC, we established 4 different cfDNA methylation signatures, being T2, after the first fx, the moment of highest methylation redistribution. cfDNA methylation levels in 2 genes regulating immune response including B cells, consistently predicted response to ICI+SABR before and during treatment.

Conclusion: The concentration of cfDNA and the NLR are accessible biomarkers in blood that seem to predict CB of ICI+SABR in oligoprogressive disease. Moreover, we have established the first molecular classifier of response to ICI+SABR, with 2

candidate genes to proxy disease evolution in blood. Epigenetic status and rewiring of the immune system show potential for intervening resistance.

Key words: Stereotactic ablative body radiotherapy-immunotherapy; Epigenetics; Non-invasive biomarkers

POSTER 9

The discovery of a novel ferroptosis-inducing biochemical inhibitor of glutathione peroxidase 4 and thioredoxin reductase 1

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Cancer cells rely on antioxidant enzymes, such as glutathione peroxidases isoenzymes GPX1 and GPX4, to maintain redox homeostasis by scavenging excess hydrogen peroxide. Hydrogen peroxide species are reduced in cells by these rapidly cycling, selenoprotein enzymes, preventing oxidative damage or death. GPXs, known to be overexpressed in some cancer types and linked to treatment resistance mechanisms, have emerged as attractive anti-cancer targets. GPX4 uniquely reduces lipid hydroperoxides, counteracting ferroptosis—a form of cell death driven by iron-dependent lipid peroxidation – which growing evidence suggests may play a key role in tumor suppression. Despite the discovery of several ferroptosis-inducing compounds, the redox mechanisms contributing to ferroptosis remain largely unknown. Although ferroptosis inducers are presumed to inhibit cellular GPX4, they require the cellular context for inhibition and fail to directly inhibit recombinant, selenocysteine-containing GPX4. To further study this, we conducted a high-throughput screening of both repurposed and novel molecules against recombinant GPX1 and GPX4. Among the repurposed candidates, Compound A was the sole molecule identified that both directly inhibited GPX4 and induced ferroptotic cell death, which was rescued by the lipid peroxide scavenger, ferrostatin-1. Compound A, like other known ferroptosis inducers such as RSL3 and ML-162, also inhibits thioredoxin reductase 1. Interestingly, unlike these compounds, Compound A biochemically inhibits GPX4 in a manner enhanced by pre-incubation with glutathione, which is present in high concentrations in cells, and reflects physiological conditions. Cellular thermostability assays further revealed that compound A, like RSL3 and ML-162, destabilizes GPX4 in A-549 cells, suggesting that Compound A alters GPX4 stability through direct or indirect engagement. The combined biochemical activities of this novel ferroptosis-inducing compound highlight the intricate redox mechanisms driving ferroptosis and suggest that GPX4 inhibition occurs through distinct, compound-specific mechanisms in a cellular context. Understanding these mechanistic differences may provide deeper insights into GPX4's role in ferroptosis and inform future strategies in targeting GPX4 in the potential treatment of cancer.

Key words: Ferroptosis; Oxidative Stress; Redox

POSTER 10

Deciphering the immune microenvironment of intrahepatic cholangiocarcinoma using spatial proteomics

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Introduction

Curing patients with cholangiocarcinoma and achieving long-term survival is still a challenge. Apart from surgical resection, oncologic therapies are extremely limited, and immunotherapy has not shown a benefit in curable stages. Therefore, there is a strong need to understand the pro- and anti-tumourigenic immune microenvironment of cholangiocarcinoma. We hypothesize that the cellular composition and spatial architecture of cholangiocarcinoma, with focus on the immune compartment, are associated with prognosis.

Methods

Liver tumour samples obtained from patients undergoing curative surgery at Karolinska University Hospital are stored in a research biobank. Clinical data on patients with intrahepatic cholangiocarcinoma between 2008 and 2022 was collected. Samples from patients with extremely short and long recurrence free survival were selected (n=12/group), stratifying for sex, age and tumour characteristics. Spatial proteomics at single-cell level are analysed using MACSima, an ultra-high-plex immunofluorescence staining and imaging platform. This platform is one of the most advanced techniques within spatial proteomics and performs dozens of sequential staining and imaging cycles on the same tissue section. Thus, tumour, stromal and immune cells, their functional status, immune checkpoint expression and presence of tertiary lymphoid structures can be characterized while preserving the natural tissue architecture. Raw data is processed by bioimage analysis software and downstream analysis includes area-dependent cell frequencies, cell-cell distances and neighbourhood analysis. The results from spatial image analysis will be correlated with patient outcome with the goal of identifying an immune cell-based signature for prognosis.

Results

In the initial optimization phase, smaller antibody panels were validated and optimized on both healthy liver tissue and immune-cell rich tonsil tissue using the MACSima platform. After optimization, we are now able to identify all immune cell subsets of interest (T-cell subsets, B-cells, NK cells, MAIT cells, macrophage subsets, dendritic cell subsets, neutrophils), tertiary lymphoid structures and their maturation characteristics, several immune checkpoint molecules, blood and lymphatic vessel structures, fibroblast subsets, and parenchymal and cancer cells. Raw data preprocessing and cell segmentation techniques are established. Recently, the final panel including 60 fluorescently labelled antibodies was successfully tested and is currently applied to the cancer tissue samples from both prognosis groups.

Conclusion

Despite similar tumour stages, some patients with cholangiocarcinoma suffer an early recurrence, while others achieve long term survival. The MACSima platform will allow us to map the spatial organisation of the tumour microenvironment in both breadth and depth, with the goal to increase our understanding of failing immune control mechanisms in disease recurrence.

Key words: Cholangiocarcinoma; Spatial Proteomics; Tumour Microenvironment

POSTER 11

Inhibition of ROCK2 offers a novel approach to target metastatic spread in neuroblastoma

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Background:

Neuroblastoma displays a high rate of metastases, often already at diagnosis. Consequently, new therapeutic agents that can control both tumour growth and metastasis, are urgently needed. We have previously demonstrated that targeting the Rho/ROCK-signalling axis is an attractive therapeutic approach in neuroblastoma. Functions of Rho/ROCK signalling in cytoskeletal remodelling and actin dynamics are well established and various processes associated with metastatic spread depend on ROCK.

Aim:

This work aims to investigate the effects of KD025 (Belumosudil), an FDA-approved ROCK2-specific inhibitor, on metastatic processes in neuroblastoma.

Methods:

We applied in vitro migration and invasion assays using different neuroblastoma cell lines grown as monolayers and tumour spheroids utilising the Incucyte® Live-Cell Analysis System. Additionally, the effects of KD025 under hypoxia were assessed.

Results:

Our results demonstrated that KD025 more potently impaired neuroblastoma growth under hypoxic conditions than normoxia. Furthermore, KD025 reduced neuroblastoma cell migration and blocked cell invasion in neuroblastoma cell lines grown in monolayer. Moreover, KD025 suppressed 3D tumour cell invasion into a Matrigel matrix in a neuroblastoma spheroid model, in a dose-dependent manner. Our findings form the basis for further studies investigating the effects of KD025 on neuroblastoma metastasis in vivo.

Conclusion:

High-risk neuroblastoma patients have a high frequency of metastatic disease and hypoxia is an important environmental stressor contributing to metastatic tumour progression. Our results propose that inhibition of ROCK2 can target tumour growth and invasive capacity in

neuroblastoma, especially under hypoxic conditions. Thus, inhibition of Rho/ROCK signalling may offer a promising treatment approach to target metastatic spread in neuroblastoma.

Key words: Neuroblastoma; Metastasis; ROCK2

POSTER 12

Fibroblast supernatant modulates treatment response in oropharyngeal cancer cell lines

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The incidence of oropharyngeal squamous cell carcinoma (OPSCC) is increasing, primarily due to human papillomavirus (HPV) infection. While HPV-positive (HPV+) OPSCC generally has a more favorable prognosis than HPV-negative (HPV−) OPSCC, both subtypes are commonly treated with chemoradiotherapy, which remains insufficient for a subset of patients. We previously demonstrated that targeted therapies, such as PI3K inhibitors, effectively reduce the viability of both HPV+ and HPV− OPSCC cell lines. In this study, we aimed to better replicate the in vivo tumor microenvironment by investigating the influence of fibroblast-derived factors, as one of the most abundant cells in the microenvironment, on treatment response.

HPV+ (CU-OP-2, CU-OP-20) and HPV− (CU-OP-17) OPSCC cell lines were treated for 72 hours with inhibitors targeting PI3K, FGFR, CDK4/6, and AKT, as well as with the chemotherapeutic agents cisplatin and docetaxel. Treatments were administered with or without conditioned media from the BJ-hTERT fibroblast cell line or primary CAFs (KS35). Treatment responses were evaluated in both 2D cultures (assessing viability, proliferation, and gene expression) and 3D spheroid models (assessing growth and viability).

While the CAF supernatants influence on the sensitivity to treatment was highly variable, the fibroblast supernatant decreased the efficiency of the drugs, especially the inhibitors targeting PI3K, FGFR, and CDK4/6. This reduction in drug sensitivity was evident across multiple assays, including cell viability, proliferation, and spheroid growth. Although the fibroblast supernatant affected both HPV+ and HPV- cells, it had an especially strong effect on the sensitivity of CU-OP-20 cells. However, this resistance could be overcome by increasing drug concentrations in the 3D spheroid model.

These findings show that fibroblast-secreted factors can modulate therapeutic responses in OPSCC cell lines, and highlighting the importance of incorporating tumor microenvironment components when evaluating and optimizing new treatment strategies.

Key words: Oropharyngeal squamous cell carcinoma; Targeted therapy; Fibroblasts

POSTER 13

Control of transcription factor regulation through the thioredoxin system in cancer development

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Monitoring the cross talk and regulation of signaling mechanisms of different transcription factors is of high importance in cancer-related research. However, tools for understanding their complex regulation within single cells and how their activities are intertwined in cellular contexts are still limited. We recently developed a new reporter (called pTRAF, for plasmid for transcription factor reporter activation based upon fluorescence) that enables simultaneous single-cell resolution monitoring of three separate redox biology and cancer related transcription factors: NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), STAT3 (signal transducer and activator of transcription 3) and Nrf2 (nuclear factor E2–related factor 2), that is a derivative of a former variant of this reporter tool[a]. By detecting fluorescence emitted by three separate fluorescent proteins linked to the activation of each transcription factors using flow cytometry, their activation pattern can be monitored at single cell level. With the help of the reporter plasmid, and other fluorescent reporter tools that enable the dynamic measurement of H_2O_2 levels in living cells, we were able to study the intertwined regulation of the thioredoxin system and the Nrf2 signaling pathway. In the other focus of our research is the analysis of TXNL1, a redox-active thioredoxin-like protein with chaperone functions[b], related to Nrf2 signaling, and how its expression modulates the answer of cells to oxidative stress. The single cell resolution data received by the newly described pTRAF variant, and other fluorescent reporter tools can be used to simultaneously monitor the impact of diverse redox related perturbances of signaling pathways in relation to the thioredoxin system.

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Key words: Redox biology; TXNL1; pTRAF

POSTER 14

Targeting replication stress tolerance in MYCN-amplified neuroblastoma

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Neuroblastoma (NB) is the most common cancer among infants, with a devastating survival rate of approximately 50% for high-risk patients. A key driver of aggressive NB is the amplification of the MYCN oncogene, which is associated with poor prognosis and orchestrates a wide array of biological processes including cell proliferation, apoptosis, senescence, and protein synthesis. MYCN also represses neuronal differentiation genes, thereby promoting tumorigenesis. Notably, MYCN overexpression also induces replication stress (RS) fueling genomic instability. However, the molecular mechanisms of MYCN-driven RS and its downstream consequences remain incompletely understood.

We previously demonstrated that the multimeric SCF-FBXL12 ubiquitin ligase provides a protective function in cancers with high levels of RS by targeting CHK1-phosphorylated FANCD2 for proteasomal degradation, indicating that FBXL12 could be an attractive target in cancers exhibiting RS.

Importantly, high expression of FBXL12 is a significant predictor of poor outcomes in NB patients, independent of other clinical variables. This suggests that FBXL12 may play a functional role in the progression of NB by alleviating MYCN-driven RS.

Consistent with this, depletion of either FBXL12 or its substrate FANCD2 in MYCN-amplified NB cell lines leads to reduced proliferation and elevated RS markers. This phenotype is recapitulated in vivo using a NB xenograft mouse model, underscoring the relevance of this pathway in tumor growth.

Intriguingly, both FANCD2 depletion and FBXL12 overexpression result in a marked reduction of MYCN protein levels. Mechanistically, both MYCN and FANCD2 localize to replication forks, and FANCD2 levels are significantly diminished upon MYCN depletion in an FBXL12-dependent manner. This implies that MYCN may protect FANCD2 from degradation, forming a regulatory axis that sustains replication fork integrity. Furthermore, MYCN-driven RS is in part due to the increased occurrence of transcription-replication conflicts (TRCs), which lead to mitotic entry with underreplicated DNA and necessitate activation of mitotic DNA synthesis (MiDAS) to prevent mitotic defects - a process that involves FANCD2.

Our ongoing studies are focused on further dissecting the MYCN-FBXL12-FANCD2 axis, particularly its role in resolving TRCs and implications for therapeutic response. Collectively, our findings highlight FBXL12 as a novel and actionable target in MYCN-amplified neuroblastoma, with potential implications for overcoming therapy resistance.

Key words: Neuroblastoma; MYCN; Fanconi Anemia

POSTER 15

The tumour microenvironment influences long-term tamoxifen treatment response in breast cancer patients

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Background: The tumor microenvironment (TME) influences treatment response in breast cancer. This study examines whether immune and stromal cell composition predicts long-term survival.

Methods: We analyzed 513 ER+ HER2− patients from the Stockholm Tamoxifen (STO-3) trial, which randomized postmenopausal, lymph node-negative breast cancer patients to tamoxifen or no endocrine therapy. Gene expression data assessed TME composition via the ConsensusTME deconvolution algorithm, identifying 18 immune cell types. Correlation analysis revealed distinct patterns between stromal (endothelial, fibroblast) and immune components, prompting separate analyses. Patients were categorized into tertiles (low, intermediate, high) based on immune, endothelial, and fibroblast scores. Survival analyses were performed using Kaplan-Meier and multivariable Cox models, adjusting for clinical factors. To explore transcriptional differences, we performed differential expression and pathway enrichment analyses across tertiles using limma and fgsea with Hallmark gene sets.

Results: Endothelial and fibroblast components showed no correlation with immune cells, justifying separate evaluation. Univariable survival analysis showed that in tamoxifen-treated patients, a low immune score was significantly associated with an improved distant recurrence-free interval (DRFI) ($p = 2e{-}04$). An intermediate endothelial score ($p = 0.00036$) and low/intermediate fibroblast scores ($p = 0.042$, $p = 0.0091$) were also linked to better DRFI. Multivariable models confirmed these associations: low immune score (HR = 0.17, 95% CI [0.08−0.40]), intermediate endothelial score (HR = 0.21, 95% CI [0.09−0.51]), and low/intermediate fibroblast scores (HR = 0.50, 95% CI [0.27−0.93]; HR = 0.36, 95% CI [0.17−0.77]). Individual immune cell types showed similar trends. Differential expression and pathway enrichment analyses revealed that low relative immune and fibroblast tumours were enriched for MYC targets, oxidative phosphorylation, and estrogen response, which were downregulated in endothelial-intermediate tumours. Additionally, low immune and fibroblast groups showed interferon gamma response and allograft rejection downregulation, while endothelial-intermediate tumours exhibited epithelial-mesenchymal transition enrichment.

Conclusions: Immune and stromal components significantly impact long-term survival in ER+ HER2− breast cancer tamoxifen-treated patients. Lower immune, intermediate endothelial, and low/intermediate fibroblast relative abundances improve long-term survival, underscoring

the TME's prognostic value. The distinct molecular patterns among these groups may aid in patient stratification and guide personalized treatment approaches.

Key words: Breast Cancer; Tumour Microenvironment; Translational Research

POSTER 16

High-Throughput Profiling of Transcription Factor Binding Dynamics in Response to Epigenetic Modulation

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Epigenetic drugs (or epidrugs) can reshape transcriptional programs by altering the chromatin landscape, yet their global impact on transcription factor (TF) binding remains poorly understood. While we know that epidrugs affect chromatin association and transcription factor (TF) function, we lack detailed information on specific TF changes when chromatin is disrupted by epidrugs. This issue is more complex for TFs with similar motifs or those not directly binding DNA. However, current methods are inadequate to test individual TFs at the necessary scale.

In this project, we aim to systematically evaluate how a selected epidrug influences TF-DNA interactions across the genome. To achieve this, we employ a cell library produced by High-throughput Insertion of Tags Across the Genome (HITAG) comprising more than 250 HEK293T derived clones4. Each clone expresses a unique FLAG-tagged transcription factor (TF), facilitating the parallel analysis of binding profiles for multiple individual TFs within a single experiment.

Our experimental approach will combine in situ reverse transcription with nanoCUT&Tag5 on the 10x Genomics platform. This strategy will provide single-cell-level data for FLAG-TF identification and TF occupancy in a high-throughput manner. Multiplexed profiling of TF binding at this scale has not been previously achieved and holds the potential to reveal the effects of chromatin remodelers on TF occupancy across a broad range of specific targets

Key words: Transcription-Factor; Epidrug; Chromatin

POSTER 17

Overcome chemotherapy resistance by harnessing replication stress tolerance pathways in osteosarcoma

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Despite progress in treating childhood cancers, outcomes for osteosarcoma (OS) - the most common malignant bone cancer in children - have remained largely unchanged for over 40 years, with advanced disease still associated with a poor prognosis. Consequently, chemotherapy and targeted therapies have shown limited success, underscoring the need to identify critical vulnerabilities and regulatory mechanisms driving treatment resistance in OS. Although OS generally responds poorly to immunotherapy, a subset of tumors with high immune infiltration demonstrates improved responses to standard treatments and better overall prognosis. Therefore, therapies that disrupt pathways suppressing immune signaling in OS may enhance anti-tumor immune responses.

While focal amplifications of c-MYC and CCNE1 are common in OS, no single driver gene fully accounts for its development. Instead, OS is characterized by a complex landscape of mutations affecting genes involved in cell cycle regulation and DNA repair/checkpoint pathways. Notably, recent studies have identified activation of the Fanconi Anemia (FA) pathway, a key regulator of the replication stress response and DNA repair, as a driver of chromothripsis. This catastrophic fragmentation of mis-segregated chromosomes trapped in micronuclei is a hallmark of OS and contributes to cancer genome evolution and acquired treatment resistance.

In this project, we will investigate whether targeting replication stress tolerance pathways can enhance the effectiveness of anti-cancer therapies in OS, including immune checkpoint blockade (ICB) therapy. Our recent work (Brunner et al.) identified a critical dependency on FBXL12–FANCD2 signaling for the survival of cancer cells under high replication stress. In OS, elevated MYC expression is strongly associated with poor patient outcomes. Preliminary analyses reveal that high MYC–FBXL12–FANCD2 activity, combined with low expression of SAMHD1, a key regulator of nucleotide metabolism, correlates with reduced immune cell infiltration in OS tumors.

Our findings suggest that SAMHD1 loss activates the FBXL12–FANCD2 pathway to stabilize replication forks, which may otherwise be compromised due to impaired fork processing. Supporting this, SAMHD1 knockdown increases FANCD2 levels in OS cells, potentially suppressing activation of the cGAS–STING–interferon (IFN) signaling pathway. Furthermore, disruption of FBXL12–FANCD2 signaling induces markers of cGAS–IFN pathway activation, an effect that is amplified in the context of oncogene-induced replication stress in OS cells.

In future studies, we will investigate whether the MYC–SAMHD1–FBXL12–FANCD2 signaling axis represents a potential vulnerability and therapeutic target in osteosarcoma.

Key words: Osteosarcoma; SAMHD1; FBXL12

POSTER 18

Gold nanoparticles enhance radiosensitivity in glioblastoma cells

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Background: Glioblastoma multiforme (GBM) is a highly aggressive primary brain tumor with poor patient prognosis. Standard of care treatment includes maximal surgical resection followed by radiotherapy and temozolomide administration. Despite this multimodal strategy, patients fail to achieve an effective response due to resistance to radio-treatment, allowing the tumor to recur. Therefore, there is a compelling need to discover new radiosensitizer approaches to overcome radioresistance and improve response and survival of patients affected by glioblastoma. A novel approach is represented by nanoradiosensitizers such as gold nanoparticles (AuNPs), able to enhance the effectiveness of radiotherapy.

Methods: In this study, we assessed the biological interaction of AuNPs with ionizing radiation (IR) in both immortalized and primary, patient-derived glioblastoma cell lines.

Results: We observed that the combinatory effect of AuNPs with IR decreased cell viability and increased necrosis, and apoptosis compared to cells treated only with IR or untreated cells. Additionally, our results showed an increase in the sensitization enhancement ratio (SER) of cells treated with AuNPs and IR. Furthermore, AuNPs showed a tumor-specific effect, since it did not seem to support the effects of radiation on GBM healthy counterpart: normal human astrocytes (NHA) cell line.

Conclusion: Our results suggest that the use of AuNPs can improve radiotherapy efficacy by increasing the radiosensitivity of the targeted cells.

Key words: Gold Nanoparticles, Radioresistance, Glioblastoma

POSTER 19

Age and tumour presentations differ between HPV type 16 positive and other high-risk HPV type-positive oropharyngeal squamous cell carcinomas in a Swedish cohort of 2000-2022

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The incidence of human papillomavirus positive oropharyngeal squamous cell carcinoma (HPV+ OPSCC) has increased the past decades. Initial reports disclosed that HPV type 16 (HPV16) was predominant and that patients with HPV+ OPSCC were generally younger and had a better prognosis than those with HPV negative (HPV-) OPSCC. However, recent reports suggest that age differences between patients with HPV+ and HPV- OPSCC are less pronounced and that other high-risk HPV types (HR-HPV) are becoming more common. Here we therefore investigated whether there were age differences between patients with HPV16-positive and other HR-HPV type positive OPSCC. During 2000-2022, in the Stockholm-Gotland-Region, Sweden, 1,681 patients, with OPSCC tested for presence of common mucosal HPV-types were included. Among these, 1,180 cases had a HR-HPV type infection, with 1,032 identified as HPV16 and 148 as other HR-HPV types; one with a low-risk HPV type infection; and 500 classified as HPV-. Patients with HPV+ OPSCC were significantly younger than those with HPV- OPSCC (mean 63 vs. 66.5, $p< 0.001$). Among patients with HPV+ OPSCC, those diagnosed with HPV16 were significantly younger than those with other HR-HPV types (mean 61.1 vs. 64.5, $p< 0.001$). These age differences were present irrespective of sex, but patients with HPV16-positive OPSCC were significantly more likely to present with smaller tumours upon diagnosis ($p=0.002$). Moreover, notably, statistically significant age differences between HPV16 and other HR-HPV types were mainly observed in the more recent years (2010–2022). To conclude, the increasing influence of HR-HPV types other than HPV16 in OPSCC warrants further investigation.

Key words: HPV type; Oropharyngeal cancer; Age

POSTER 20

Integrative epigenome and transcriptome profiling of diffuse midline gliomas

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Diffuse midline gliomas (DMGs) are highly aggressive tumors arising in midline structures such as the thalamus, brainstem, and spinal cord, affecting both pediatric and adult populations. These tumors are unified by hallmark molecular alterations, most notably H3K27M mutations, yet display heterogeneity in anatomical location, age of onset, and clinical outcome. While genomic and epigenomic profiling has revealed subgroups within DMGs, the molecular mechanisms driving tumor development and progression—particularly within the context of a heterogeneous tumor microenvironment—remain incompletely understood.

In this study, we leverage recent advances in single-cell and spatial epigenomic technologies to address these gaps. We collected DMG samples from diverse anatomical sites, all harboring H3F3A K27M mutations and additional alterations in oncogenes such as PDGFRA and TP53. Using Spatial CUT&Tag, a technology based on deterministic barcoding in tissue for spatial omics sequencing (DBiT-seq), we profiled chromatin features and transcriptomes at near-cellular resolution. This approach enabled robust, genome-wide mapping of histone modifications (H3K4me3 and H3K27me3) and gene expression from each spatial pixel. On average, we obtained approximately 3,000 unique fragments for each histone modification and detected 300 genes per spatial spot. Ongoing integrative analyses with single-cell multiomic data from the same tumors aim to annotate cell types and elucidate their roles in DMG pathogenesis, providing new insights into the spatial and molecular landscape of these lethal gliomas.

Key words: Diffuse midline gliomas; H3K27M mutation; Spatial epigenomics

POSTER 21

Predicting Endocrine Therapy Benefit in Premenopausal Breast Cancer Using Whole-Slide Images

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Background

Premenopausal breast cancer patients have a significant long-term risk of distant metastasis. Endocrine therapy remains one of the most important treatments for ER-positive breast cancer, yet reliable markers for predicting endocrine therapy benefit are lacking.

Methods

We analyzed 576 ER-positive patients from the Stockholm Tamoxifen trial (STO-5, 1990-1996). Digitalized whole slide images where tiled and processed using a foundational deep learning model (UNI), tiles were then combined using an attention-based network and using this an individualized endocrine therapy benefit score was computed. Patients were grouped into three strata: no, low, and high predicted benefit. 20-year distant recurrence-free interval (DRFI) was compared between control and endocrine treated arms.

Results

Patients in the high-benefit group (n=321) had a 14 percentage absolute improvement in 20-year DRFI, contrasting endocrine therapy versus control (ET: 73.5%, 95% CI 67.2%-78.7%, control: 59.1%, 95% CI 47.9%-68.7%). For the low-benefit group (n=159) a 5 percentage improvement in DRFI was observed (ET: 63.8%, 95% CI 54.3%-71.8%, control: 59.0%, 95% CI 42.6%-72.2%). In the no-benefit group (n=96), the opposite trend was observed with higher risk for the endocrine treated group, although confidence intervals were wide (ET: 61.0%, 95% CI 49.3%-70.7%, control: 71.4%, 95% CI 40.6%-88.2%).

Conclusion

In this study we aim to predict endocrine therapy benefit in premenopausal patients. These patients have a significant long-term risk of metastasis and face considerable side-effects from the treatment. Ongoing work will extend these analyses with multivariable analyses, investigate which tumor features influence endocrine benefit, and further validate the findings.

Key words: Endocrine Therapy; Breast Cancer; Digital Pathology

POSTER 22

Melanoma metabolism links membrane fluidity and cell migration

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Cancer metastasis is a major cause of mortality, occurring when cancer cells migrate away from the primary tumour. Despite substantial genomic and proteomic heterogeneity, most cancer cell types are capable of metastasis. To identify a unifying principle of melanoma cell migration, we investigated how plasma membrane (PM) remodelling and associated biophysical properties contribute to migratory capacity. PM fluidity controls many cellular functions, including signalling and cytoskeleton rearrangement – key mechanisms involved in cell migration.

Using live-cell imaging with PM fluidity probes, supplementation with L-lactate, and inhibition of glycolysis and oxidative phosphorylation, we examined the relationship between PM characteristics, cellular metabolism, and migration across six melanoma cell lines. We observed that cell lines with higher PM fluidity exhibited greater migratory capacity in wound-healing (scratch) assays. Cell lines inherently dependent on oxidative phosphorylation migrated less effectively, and experimentally shifting cells towards oxidative phosphorylation similarly impaired migration. In contrast, promoting glycolysis through L-lactate supplementation increased both PM fluidity and wound closure efficiency, while inhibition of glycolysis reduced both.In vivo, systemic lactate administration in mice increased tumour growth and metastatic spread following melanoma cell injection.

These findings suggest that the metabolic state of melanoma cells predicts their migratory potential, and that this capacity is linked to PM fluidity. Future work will focus on how glycolytic metabolism drives changes in lipid composition underlying PM remodelling, and how this interacts with the upregulation of metastasis-associated genes. Finally, targeting lactate metabolism may represent a therapeutic strategy to limit metastatic progression.

Key words: Membrane fluidity; Metabolism; Metastasis

POSTER 23

Biological Traits in Primary Breast Tumors Associated with Late Metastasis – Insights From the STO Randomized Trials

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Introduction: Estrogen receptor (ER)-positive breast cancer patients remain at risk for distant metastasis for several decades after primary diagnosis. Furthermore, premenopausal patients have a worse prognosis in contrast to postmenopausal patients. It is therefore essential to identify primary tumor biological characteristics that influence risk to develop late metastasis. In this study, we compared primary tumors from premenopausal and postmenopausal patients that developed late metastasis to patients with early metastasis using the well-annotated and unique Stockholm tamoxifen trials with over 20 years of follow-up.

Results: First, using clinicopathological features we show that both premenopausal and postmenopausal patients with late distant metastasis are significantly more likely to have primary tumors of smaller tumor size, lower tumor grade, lower proliferation and lymph node-negative tumors, as compared to patients with early metastasis.

Second, primary tumors of premenopausal and postmenopausal patients with late metastasis as compared to early metastasis had an upregulation of epithelial-mesenchymal-transition (EMT), hypoxia, and estrogen signaling.

Further, while primary tumors of premenopausal patients with late metastasis in contrast to early metastasis revealed a downregulation of immune-related processes such as interferon signaling, the postmenopausal group showed an increased inflammatory profile. This was seen using gene set enrichment analysis (GSEA) of the predefined MSigDB cancer hallmarks and gene ontology terms analyzing gene expression data as available for the patients in the STO-trials.

Finally, using whole tumor slide images (WSIs) of H&E stainings, we applied HoverNext to classify single cell nuclei and investigate spatial biology. Our findings suggest an increase of stromal and normal epithelial cells in primary tumors of premenopausal patients with late metastasis compared to patients with early metastasis. Proximity analysis between nuclei revealed reduced immune-immune and increased immune-stromal interactions in tumors of premenopausal patients with late metastasis compared to those of patients with early metastasis, while decreased stromal-neoplastic cell interaction is seen for postmenopausal patients.

Conclusion: This knowledge can help guide long-term care, prognosis and improve treatment strategies for patients with ER-positive breast cancer more effectively.

Key words: Breast cancer; Late metastasis; Biomarkers

POSTER 25

Systematic profiling uncovers new interactions of chemoresistance factor SAMHD1 with commonly used drug active metabolites

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Objectives: Nucleobase and nucleoside analogues are a widely used class of chemotherapeutic agent which are standard-of-care in a range of solid and haematological malignancies. These drugs that are administered as prodrugs and thus require intracellular activation. Their active metabolites, however, can be deactivated by the deoxyribonucleoside triphosphate hydrolase (dNTPase) SAMHD1. We have previously demonstrated that SAMHD1 inactivation of ara-CTP, the active metabolite of the deoxycytidine analogue cytarabine (ara-C), contributes to poor treatment outcome in acute myeloid leukaemia (AML). SAMHD1 is a promiscuous dNTPase, and other nucleoside analogue drugs have since been implicated to be under SAMHD1 control. However, a complete understanding of which antimetabolite chemotherapies are controlled by SAMHD1 is currently lacking. We aimed to address this knowledge gap by performing a comprehensive characterisation of more than twenty active metabolites of clinically used nucleobase and nucleoside analogues as SAMHD1 interactors.

Methods: We used biochemical and biophysical methods to identify activators, substrates, and inhibitors of SAMHD1. This includes enzyme activity assays (high-throughput biochemical endpoint assay & continuous NMR-based kinetic assay), a competitive binding assay to study allosteric site affinity as well as assays to study SAMHD1 oligomerisation (chemical crosslinking, mass photometry).

Results: In addition to confirming previously reported SAMHD1 interactors, we identified several new nucleotide analogues as allosteric activators and substrates of SAMHD1. By benchmarking them against physiological ligands, we show that some nucleotide analogues mimic or even surpass endogenous nucleotides as enzyme activators and substrates.

Conclusions: This comprehensive assessment of SAMHD1 interactors is a first step in enhancing our understanding of SAMHD1's role in chemotherapy resistance and potentially improving patient outcomes by designing more effective cancer treatments. By identifying new allosteric modulators and substrates of SAMHD1, we lay the foundation for further studies in disease models.

The results from the SAMHD1 interactor screen also improve our knowledge about SAMHD1 allosteric and catalytic site specificities, broadening our understanding of how chemical modifications affect allosteric or catalytic site binding of nucleotide analogues. A more comprehensive understanding of SAMHD1 allosteric and catalytic site specificity will allow us to better predict interactions with other nucleotide analogues.

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Key words: SAMHD1; Nucleoside Analogues; Chemotherapy

POSTER 26

Examining cellular response to purine antimetabolite nelarabine to gain insight into the mechanism-of-action in T-ALL

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T-cell acute lymphoblastic leukemia (T-ALL) is a T-cell malignancy with a relatively poor 5-year event-free survival (~80%) compared to other leukemic cancers. The only T-ALL specific drug in clinical practice is nelarabine. It was rationally designed for the selective treatment of T-cell malignancies and is the prodrug of ara-G, a deoxyguanosine (dGuo) analogue. Although used in the clinic since 2005, knowledge gaps persist in the molecular mechanisms of this therapy, limiting optimal use in the clinic1. In this project we focus on examining the intracellular metabolism and the molecular mode-of-action of ara-G in T-ALL cell lines. Common in-vitro readouts for cell survival are not in agreement with each other after treatment with Ara-G. We examine which readout to use and the reason for these differences. The mechanism behind this could be relevant for the efficacy of Ara-G and other compounds. We performed proliferation assays with ara-G in 6 T-ALL cell lines with resazurin reduction and CellTiter-Glo® metabolic readouts. These results were in conflict with manual cell count using trypan blue. Therefore, flow cytometry was used to examine the cause of these differences. Proliferation assays were performed with ara-G, ara-C (cytarabine), dGuo and palbociclib, a CDK4/6 inhibitor included for comparison2. Based on these results, 3 cell lines were selected to examine the relationship between mitochondrial content and cell size after Ara-G incubation. Conclusions so far: incubation with Ara-G, Ara-C and dGuo increases cell size in T-ALL cell lines. For Ara-G, the mitochondrial content also increases in a dose-dependent manner. Results differ per cell line and do not correlate to growth speed. This pattern differs from palbociclib response.

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Key words: Nelarabine; Nucleoside analogues; T-cell acute lymphoblastic leukemia

POSTER 27

Exploring the B cell receptor repertoire in tumor tissue from rheumatoid arthritis patients with diffuse large B cell lymphoma

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Purpose

Rheumatoid arthritis (RA) patients are at higher risk of developing diffuse large B cell lymphoma (DLBCL), a germinal center-derived lymphoma that resemble activated B cells. However, it is unknown whether DLBCL development is antigen-dependent. Here we investigated the level of somatic hypermutation (SHM) and whether tumor B cell receptor (BCR) repertoires show variable gene usage bias. Furthermore, we assessed the presence of N-glycosylation sites (N-X-S/T, X cannot be P) in the variable region, which have been implicated in promoting tumor survival via antigen-independent B cell activation.

Methods

Multiplex PCR for the BCR was performed using DNA extracted from paraffin embedded (FFPE) tumor tissues from 10 RA-DLBCL patients included from the Swedish National Autolymphoma study. FFPE tonsil tissue from a healthy donor was included as a control. The BCR was sequenced by Illumina using NextSeq 2000 (2 x 300 bp). BCR assignment was done using the nf-core/airrflow pipeline.

Results

We identified expanded tumor clonotypes and unrelated BCRs from infiltrating B cells in the tumor. The majority of investigated patients had an expanded clonotype that were encoded by a VH3 family variable gene, followed by VH1, without a clear gene bias. The dominant clone displayed moderate or low levels of SHM in four patients (4-13 number of mismatches in comparison to the closest IMGT germline sequence) and were nearly unmutated for three patients (0-3 SHM), but were highly mutated for two patients (>15 SHM). Two patients had N-glycosylation sites in the variable region. One patient had germline encoded N-glycosylation, and the second patient had SHM-induced N-glycosylation.

Key words: Diffuse large B cell lymphoma (DLBCL); Autoimmune disease; B cell receptor (BCR)

POSTER 28

Improving CAR-T cell efficacy in solid tumors using synthetic co-stimulatory receptors

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Chimeric antigen receptor (CAR) T cells have revolutionized the treatment of hematological cancers. However, CAR-T cell therapies have so far shown limited efficacy in treating solid tumors, which is mainly attributed to the immunosuppressive tumor microenvironment (TME) inducing CAR-T cell dysfunction and exhaustion. Synthetic co-stimulatory receptors (SRs), which combine the extracellular domain of an inhibitory receptor with an intracellular co-stimulatory domain, can convert immunosuppressive TME cues into activation signals. Consequently, SRs have emerged as a promising strategy to overcome TME-mediated immunosuppression. However, the pool of potential candidates for SRs is very extensive making individual testing in vivo unfeasible. Therefore, we aim to perform a pooled knock-in screen of a large library of barcoded SRs to identify those that enhance CAR-T cell function and activation in vivo. Using amplicon-seq as well as single-cell RNA-seq, we are investigating the effect of each SR on the proliferation and the phenotype of CAR-T cells in the tumor. Since solid tumors are diverse with distinct TMEs, we are using multiple in vivo mouse models recapitulating different types of solid tumors. This comprehensive approach enables broad screening for SRs improving CAR-T cell functionality across diverse solid tumors. Our findings may significantly contribute to the development of the next generation of CAR-T cells that might eventually be translated into improved cell therapies for solid tumors.

Key words: Cell Therapy; Pooled Screening; Tumor Microenvironment

POSTER 29

Delineating genome instability dynamics in breast cancer by time-course bulk and single-cell copy number profiling

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Hormone receptor-positive (HR+) breast cancer represents the most prevalent breast cancer subtype, comprising approximately 70% of cases. Although copy number alterations (CNAs) are a hallmark of aggressive HR+ tumors and have been proposed as prognostic biomarkers, their temporal dynamics during therapy remain insufficiently characterized. In this study, we performed an integrated analysis of CNA evolution using bulk (n = 168 patients) and single-cell (n = 35,328 cells) DNA sequencing data from the PREDIX Luminal B neoadjuvant clinical trial (NCT02603679). Longitudinal profiling of tumors from two treatment arms (armA and armB) uncovered candidate genomic regions potentially linked to therapeutic response and resistance. These included arm-level gains and losses selectively enriched in post-treatment samples from non-responders. Integration with clinical outcomes further revealed treatment-specific CNA trajectories. Single-cell analyses demonstrated that most patients harbored limited subclonal complexity, and therapy predominantly reshaped the tumor landscape through selective expansion or contraction of pre-existing subclones rather than de novo subclone emergence. Our findings nominate recurrent CNAs as candidate predictive biomarkers of neoadjuvant response and suggest that clonal remodeling underlies inter-patient variability in treatment efficacy.

Key words: Hormone receptor-positive (HR+) breast cancer; Copy number alterations (CNAs); Single-cell DNA sequencing

POSTER 30

Two-step injury response of the liver to metastatic invasion: Diagnostic and therapeutic implications for gastrointestinal cancers

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Background

Metastatic pancreatic and colorectal cancers are devastating diseases, with 5-year overall survival rates of only 3% and 14%, respectively. The liver is the most common host organ for distant metastatic seeding in both tumor types. While the liver microenvironment is known to establish a pre-metastatic niche, its influence on the progression of established metastases remains poorly understood.

Methods

We employed murine models of pancreatic cancer and a histologically well-characterized patient cohort to study phenotypic adaptations within the metastatic liver. Tumor-hepatocyte interactions were analyzed using single-cell RNA sequencing, cellular interaction mapping, and multiplex immunofluorescence. Functional validation was performed via CRISPR/Cas9-mediated genetic perturbation.

Results

Hepatic metastases elicit a two-step injury response in the liver:

(i) Acute phase response: Perimetastatic hepatocytes show a transient acute phase reaction marked by upregulated CRP expression. This response is reversible and spatially restricted to areas of aggressive tumor growth. In a clinical cohort of patients with colorectal liver metastases, elevated perioperative serum CRP levels were negatively associated with overall survival.

(ii) Dedifferentiation response: In a second phase, hepatocytes at the tumor-liver interface undergo dedifferentiation towards a progenitor-like state. High densities of tumor-hepatocyte

contact correlated with poor prognosis. Transcriptomic profiling identified Notch signaling as a key regulator of this process. Tumor-specific CRISPR knockout of the Notch ligand Jagged1 reduced Sox9 activation in adjacent hepatocytes and impaired the invasive capacity of metastases.

Discoveries

Our findings reveal that hepatic metastases exploit liver plasticity through a two-step injury program involving systemic acute-phase signaling and juxtacrine Notch-mediated reprogramming of hepatocytes. These insights identify parenchymal plasticity as a novel vulnerability in liver metastases and suggest that serum CRP and tumor-hepatocyte interactions may serve as prognostic biomarkers and therapeutic targets.

Key words: Liver metastases; Metastatic microenvironment; Tumor-hepatocyte contact

POSTER 31

Cell Cycle-based Replication Timing (2C-RT) reveals hidden drug responses and genome-wide DNA synthesis perturbations

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DNA replication is a fundamental step in cell division intrinsically linked to genome evolution and cancer development. To maintain genetic and epigenetic stability, DNA replication follows a defined temporal sequence known as the replication timing (RT) program. While recent advances have clarified the spatiotemporal relationships among epigenetic marks, transcriptional activity and 3D genome organization, the causal links between RT, its regulation, and genome architecture remain unclear. Here, we propose that the classical definition of RT – inferring S-phase timing from increasing DNA content – can obscure relevant cellular responses to replication perturbations. To address this limitation, we developed 2C-RT, a novel RT sequencing method that uses a DNA content-independent temporal marker. Using 2C-RT, we uncovered drug induced alterations in replication timing that are masked by current methods. We look forward to presenting our latest findings and discussing how 2C-RT can facilitate the discovery of drug resistance mechanisms and genome-wide changes in DNA replication dynamics.

Key words: DNA replication; Genome organization; Multiplex-Sequencing

POSTER 32

Machine-learning of signal-receptor interactions driving immune phenotypes for immune control engineering

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The phenotypes of immune cells are controlled by their signaling environment and receptor profile. Therefore, manipulating immune signal and receptor interactions has the potential to stimulate anti-tumor immunity (by blocking PD1/PD-L1 interaction for example) and reduce autoimmunity. This potential has been demonstrated by the clinical use of immune checkpoint inhibitors (ICI) in cancer treatment. However, ICI fails to achieve long-term progression-free survival in most cancer patients, highlighting our incomplete understanding of which signal-receptor interactions control phenotypic differentiation and tumor progression. Identifying which signal-receptor interactions control immune phenotype remains challenging as we still lack systematic rules underlying this biological process.

Here, we perform a controlled in-vitro time-course experiment to reveal how signal and receptor interaction drive phenotypic activation of T cells. We identify key features of this process by monitoring the temporal changes in receptor and phenotypic marker expression at the single-cell level by flow cytometry in a controlled signaling environment. We propose an initial mathematical model to recapitulate these features and predict the changes in cell phenotypes given their receptor expression profile and their signaling environment. We build upon this initial model by integrating it into a machine-learning model of cell and gene interactions controlling for T cell phenotype.

Applying our machine-learning model on coupled spatial transcriptomic and single-cell sequencing data of diseased tissue could help uncover case-specific novel targets in a more systematic and efficient manner.

Key words: Immune phenotype; Flow cytometry analysis; Signal-receptor interaction

POSTER 33

Functional Drug Screening in High-Grade Serous Ovarian Cancer Reveals Actionable Combinations

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High-grade serous ovarian cancer (HGSOC) is the most common and aggressive subtype of OC. Although standard treatment – cytoreductive surgery followed by Carboplatin and Paclitaxel chemotherapy – initially induces response, ~70% of patients relapse and develop resistance, resulting in poor 5-year survival rate. While genomics driven precision medicine (PM) has introduced targeted therapies, such as PARP inhibitors for patients with homologous recombination deficiencies (HRD), nearly half of these patients develop resistance. Thus, to address this, we applied a functional PM approach using the DET3CT ex vivo drug screening platform. We designed a panel of 15 clinically relevant and investigational drugs, along with two exploratory drug combinations. This panel was used to perform a screen of cells dissociated from tumor or ascites from 62 HGSOC patients, including 35 with matched paired samples. Primary analysis revealed promising findings. The combination of Carboplatin and a Bcl-xL inhibitor A-1331852 showed consistent synergy in significant portion of samples (70%) with an average drug sensitivity score (DSS) of 25. In contrast, clinically relevant Carboplatin and Paclitaxel monotherapy showed limited activity (DSS=2.2 and 1.8, respectively). Flow cytometry-based profiling to assess the proportion of cancer cells and the presence of immune and stromal components in the sample. This analysis added contextual information to help interpret drug response heterogeneity. Interestingly, our results showed a significant correlation between cancer cell presence in the sample and high killing effects of Carboplatin and A-1331852 combination, suggesting cancer cell specific activity. Integration of drug response and clinical data (treatment history, progression-free survival, overall survival, HRD status and BMI) is ongoing. This analysis will aim to evaluate the predictive power of functional drug testing and its potential to guide clinical decision. Together, these findings support the potential of functional PM to identify effective, patient-specific treatment strategies and overcome resistance in HGSOC.

Key words: Ovarian Cancer; Precision Medicine; Drug Screening

POSTER 34

An AI-Powered, Multi-Modal Approach for Therapeutic Targeting and Surgical Stratification in High-Grade Serous Carcinoma

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Background: High-Grade Serous Carcinoma (HGSC) is an aggressive ovarian cancer where extensive surgery does not uniformly improve survival, creating a critical need for better patient stratification. The interplay between surgical trauma, the tumor microenvironment (TME), and residual cancer cells is poorly understood. Malignant ascites, a key component of the TME, is known to promote tumor progression and chemoresistance.

Objective: This study aims to develop and validate a multi-modal, AI-powered model to predict clinical outcomes and improve patient selection for surgical treatment. The model integrates clinical, histopathological, and molecular data to understand the impact of surgical trauma on tumor behavior.

Methods: We performed a comprehensive characterization of the TME using patient-derived solid biopsies (tumor, peritoneum) and liquid biopsies (ascites, blood) collected before and after surgery. Molecular profiles were generated using proteomics, ctDNA analysis, and cytokine assays. Functional responses to surgical stress were assessed in autologous 3D ex vivo explant models. An AI-powered model was developed to integrate these multi-modal datasets, including digital pathology, with clinical variables to identify predictive patterns.

Results: Our data reveal that surgical trauma induces significant changes in the TME. Distinct molecular profiles in both solid and liquid biopsies directly correlate with clinical variables, including surgery duration and time to chemotherapy. Functionally, cancer cell invasion was significantly increased in ex vivo models cultured with post-surgery explants. The integrated AI model successfully identified complex biological signatures that are predictive of patient outcomes and treatment response.

Conclusion: Our findings demonstrate that an AI-driven, multi-modal analysis of the TME provides critical insights into the biological effects of surgical trauma. This approach establishes a powerful predictive tool for personalizing surgical strategies, improving patient stratification, and discovering novel therapeutic targets to combat metastatic disease in HGSC.

Key words: Biomarker; Artificial intelligence; Ovarian Cancer

POSTER 35

Proteome analysis of plasma-isolated extracellular vesicles to understand signaling and treatment response in immune checkpoint inhibitor treated metastatic non-small cell lung cancer patients

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Introduction: Monitoring of immune checkpoint inhibitor (ICI) treatment effect in metastatic non-small cell lung cancer (NSCLC) patients is hampered by the difficulty to biopsy the tumors and their heterogeneity in protein expression and signaling. Thus, liquid biopsies for non-invasive analysis of biomarkers (BMs) are highly needed and extra cellular vesicles (EVs) constitute one way ahead. We have isolated EVs from plasma of NSCLC patients at Karolinska prior to start and after various time points of treatment with the PD-1 inhibitors pembrolizumab or cemiplimab with or without concomitant chemotherapy. Our aims are to identify signaling signatures related to ICI treatment response as well as to PD-L1 and CD73 expressions in EVs.

Methods: EVs were isolated from 1 ml EDTA-plasma of NSCLC patients (n=30) using Izon's qEVoriginal gen 2, 70nm columns on a fraction collector. Fractions 1-5 were pooled and concentrated using Amicon concentrators with 100k cut off. Nanoparticle Tracking Analysis (NTA) was applied to reveal particle size and concentration. Proteins were extracted from the EVs and profiled using proximity extension assay (PEA) on Immuno-Oncology® and Oncology II® panels at the SciLifeLab Affinity Proteomics at Uppsala University. Qlucore® Omics Explorer was used for data analysis and visualization. EV markers were validated using ELISA, FACS and WB.

Results: All samples contained particles of EV size with median sized of around 90 nm and expressed the EV markers CD9, TSG101 and SYND1. PEA protein profiling revealed 127 proteins to be expressed in at least 75% of the samples. Among those were SYND1, PD-L1,

CD73 and other oncogenic and immune signaling proteins, with heterogeneity among the individual samples. Protein signatures that correlated with PD-L1 or CD73 expression level in EVs included among others SYND1, CD40, CD244, EGF, IL18, CEACAM1, TNFRSF4 and LYN. In addition, a preliminary EVs protein signature associated with progression-free survival was revealed that is currently validated in separate cohorts.

Conclusion: We found that EVs isolated from plasma of NSCLC patients have proteins signatures associated with immune-checkpoint targets and response to PD-L1 based therapy.

Key words: Non-small Cell Lung Cancer; Immune Checkpoint Inhibitors; Extracellular Vesicles

POSTER 36

Targeting NCAM1 and FGFR1 in High-Risk and Bone Metastatic Neuroblastoma Cells

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Neuroblastoma (NB) is the most common extracranial solid tumor in infants, characterized by heterogeneous tumor biology and clinical outcome. Despite intensive treatment, ~50 % of high-risk NB patients relapse with poor prognosis. Bone and bone marrow are the most common sites of relapse, where therapy-resistance persists and remains incurable. This highlights the importance of developing new targeted NB treatment strategies. Recently, our single-cell RNA-sequencing data of cell lines, primary human NB samples and bone marrow metastases revealed that NCAM1 is highly expressed by adrenergic tumor cells, while FGFR1 is expressed by resistant mesenchymal cells. We observed that elevated expression of both targets correlates with poorer patient survival. While NCAM1 and FGFR1 have been individually targeted pharmacologically in adult cancers, their combined role in NB has not been explored. Therefore, we aim to evaluate the therapeutic potential of CD56 (NCAM1)-targeting antibody-drug-conjugate Adcitmer® and the FGFR1 inhibitor erdafitinib.

We investigated the impact of NCAM1 and FGFR1 downregulation on NB growth by employing shRNA mediated knockndown. Additionally, we examined the efficacy of Adcitmer® and erdafitinib treatment on NB cell lines and NB bone metastatic tumoroids utilizing cell viability assays and Western blotting. We then applied these treatments to multicellular tumor spheroids (MCTS) and assessed outcome with CellTiter-GloTM 3D cell viability assay as well as immunofluorescence staining.

Our work provides insights that Adcitmer® and erdafitinib treatment effectively reduced NB viability in cell lines. We demonstrate, that FGFR1 inhibition using erdafitinib affected multiple downstream signaling pathways, including the RAS-MAPK, PI3K-AKT, PLCγ, and STAT pathways. Furthermore, Adcitmer® and erdafitinib treatment on MCTS and NB bone metastatic tumoroids resulted in reduced viability and proliferation of NB cells.

In conclusion, we demonstrate novel treatment strategies by effectively targeting NB cells in vitro. This study lays a promising foundation for evaluating the treatment of Adcitmer® and erdafitinib in NB, with potential future clinical application.

Key words: Novel Treatment; Bone/Bone Marrow Metastasis; NCAM1 and FGFR1

POSTER 37

Identification of an IDO-1 inhibitor reducing growth of pancreatic ductal adenocarcinoma cells, but with little effect on surrounding stromal cells.

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) remains an unmanaged disease with only small improvements in overall and median survival times in the past decades. One problem is the small selection of recognized therapies for PDAC and a second is the density of the tumor, making drug delivery more difficult than in well vascularized tumors. Thus, we carried out a high throughput drug screen (HTS) to identify auxiliary treatments for PDAC and applied a 3D cell culture model which incorporates with pancreatic stellate cells/cancer associated fibroblasts a major aspect of the dense stroma prevalent in this disease.

Methods

The HTS was carried out with a 3D cell co-culture model, consisting of Panc1 and human pancreatic stellate cells (hPSC) to form heterospheroids. We developed an automated-image-based digital viability assay, which was further refined to also identify phenotypical changes upon treatment. Additionally, this digital assay showed to be robust against pan-assay interference compounds (PAINS).

Results

We identified an inhibitor of indoleamine 2,3-dioxygenase 1, (iIDO-1) to exert a significant growth inhibitory effect on PDAC cells (Panc1, MiaPaCa-2), while sparing the stromal component of the heterospheroid. It will be interesting to see if this iIDO-1 can synergize with commonly used chemotherapeutics in order to more specifically attck the cancer cells and reduce the systemic toxicity at the same time.

Summary

We used an image-based high throughput screen to identify an IDO-1 inhibitor that has a growth inhibitory effect on pancreatic cancer cells with little effect on surrounding stromal cells.

Key words: Pancreatic ductal adenocarcinoma (PDAC); Novel therapy; 3D cell co-culture model

POSTER 38

Modeling the colon tumor microenvironment: A 3D silk-scaffold in vitro network to investigate the role of estrogen and hypoxia

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Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related death. Men have a higher incidence of CRC than age-matched pre-menopausal women. Notably, estrogen has been shown to have a protective effect against CRC. Estrogen acts via two nuclear receptors, estrogen receptor (ER) alpha and beta, and the transmembrane receptor G-protein coupled receptor (GPER1). It is demonstrated that intestinal-specific ERβ knock-out (ERβKOvil) in the colitis-induced colon tumor mouse model (AOM/DSS) leads to increased tumor numbers in males and increased tumor size in females. ERβ has a protective role against colon tumor development in mice of both sexes, trough both anti-inflammatory and anti-tumorigenic activities. Cell lines are commonly used to model cancer in vitro and to allow mechanistic studies. However, their 2D culture often fails to recapitulate the in vivo tumor and lacks the complexity of the tumor microenvironment (TME), limiting the correlation between in vitro and in vivo data. For this reason, complex 3D culture models have been suggested as a bridge to better mimic the complexity of the in vivo TME by offering improved oxygen- and nutrient gradients, extracellular matrix (ECM) composition, and cell-cell interactions. Among 3D models, recombinant spider silk functionalized with the ECM protein fibronectin (FN-silk) provides a scaffold that facilitates cell growth while upholding cell characteristics.

In this study, we set up a 3D FN-silk-scaffold-based cell culture model for CRC cells and investigated the impact of estrogen signaling and hypoxia on CRC cells. Colon cancer cells lines expressing ERβ or mock were cultured in either FN-silk scaffold or traditional 2D system under different oxygen level conditions. The models were compared, and the impact on key cellular processes such as proliferation and apoptosis was evaluated using immunostainings and gene expression analysis. This work aims to provide insights into the molecular mechanism of action of the estrogen signaling in a more physiologically relevant cell model. Moving forward, the aim is to add complexity to the 3D-model and build up a TME-like model to investigate whether and how estrogen signaling in colonic cells impacts the recruitment and interaction of immune cells such as macrophages and NK-cells.

Key words: Colorectal Cancer; Estrogen; 3D Culture

POSTER 39

Identifying and Targeting the tumorigenic Functions of Oncometabolites in Colon Cancer

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Colorectal cancer (CRC) is one of the deadliest cancers, claiming 9.2% of all cancer-related deaths. Metastatic cases are associated with poor prognosis, and therefore new therapeutic strategies are needed. A key feature of CRC is its dependence on polyamines—essential metabolites that fuel tumor growth and metastasis. Both APC and KRAS, drivers of CRC progression, enhance polyamine synthesis. Despite their critical role, the targets and functions of polyamines in CRC remains unclear.

We investigated the proliferation of CRC cells in the presence and absence of polyamines, using specific inhibitors such as DFMO and 4MCHA. We use PISA (Proteome Integral Stability Alteration) assay, a technique for detection of protein-ligand interactions, to identify the protein targets of polyamines in primary and metastatic CRC cells, including SW480 and SW620.

Using PISA assay, we identified several putative targets that are engaged by polyamines in CRC cell lines. We then shortlisted the top targets based on their expression in CRC tumours vs. healthy tissue, and their association with survival. We aim to validate the binding of polyamines to the top targets using orthogonal techniques. We will also comprehensively validate the downstream effects upon polyamine binding to the targets, e.g., by modulating the expression of the targets in CRC cells.

This project will reveal the mechanisms by which polyamines support CRC progression. Our comprehensive study will identify the protein targets of polyamines in CRC cells and establish their role as druggable targets in cancer. We hope that these findings will pave the way for the development of novel therapeutics targeting polyamine-related pathways in CRC and other cancers.

Key words: Colon Cancer; Polyamines; Cancer Metabolism

POSTER 40

Fighting cancer with the 1-2-punch approach by identifying drug combinations that improve current cancer therapies

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Resistance to therapy has been estimated to contribute to treatment failure in up to 90% of cancer patients and remains one of the fundamental challenges in cancer. Drug tolerant and senescent cells accumulate as a consequence of many cancer therapies and are thought to contribute to therapy resistance. Accordingly, “to develop ways to overcome cancer’s resistance to therapy” was one of the 10 recommendations made from the Blue Ribbon Panel associated to the Cancer Moonshot initiative of the National Cancer Institute. In this regard, one specific idea is to find combinations that can eliminate the cancer cells that resist the initial treatment. This is the basis of the so-called “one-two-punch” strategy for cancer therapy, which aims to maximize the efficacy of the initial treatment and thereby reduce tumor relapse.

To advance this concept, we have developed a high-throughput phenotypic drug screening platform to identify novel one-two punch strategies across various cancer types and in combination with approved therapies. This innovative approach enabled us to discover novel senolytics - drugs that specifically target senescent cells. These senolytics have shown remarkable potential in enhancing the anticancer effects of senescence-promoting drugs, such as the CDK4/6 inhibitor Palbociclib. We are expanding our research by testing the efficacy of our candidate compounds in combination with other therapeutics across multiple cancer cell lines, with a particular focus on breast and lung cancers.

By addressing the critical issue of therapy resistance, we aim to contribute significantly to the advancement of cancer treatment and potentially increase survival rates for patients.

Key words: Cancer Therapy Resistance; Senolytic Drug Discovery; One-two-Punch Strategy

POSTER 41

Exploring Chondroitin Sulfate Proteoglycan 4 and Associated Signaling in Mutant Epidermal Growth Factor Receptor Driven Non-small Cell Lung Cancer

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Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer cases. Of these, 15-30% are driven by mutations in the Epidermal Growth Factor Receptor (EGFR)-gene, offering a precision cancer medicine regimen with tyrosine kinase inhibitors (TKIs), such as erlotinib or Osimertinib [1]. However, resistance to these EGFR-TKIs constitutes a clinical problem and suggests a need for alternative treatment strategies.

We have previously identified the melanoma-associated chondroitin sulphate proteoglycan 4 (CSPG4) protein in EGFR-mutated NSCLC cell lines and in extracellular vesicles (EVs) from plasma of patients with EGFR-driven NSCLC [2]. CSPG4 is a surface proteoglycan known to play a role in cancer cell proliferation, migration, and epithelial-mesenchymal transition (EMT), in melanoma and several other types of cancer. It has recently been recognized as a diagnostic marker and evaluated as a therapeutic target in melanoma and other tumour types [3-4]. Although there are reports of CSPG4 being expressed in lung cancer tissue or cell lines, the role of the protein in mutant EGFR-driven NSCLC remains largely unexplored.

Thus, we are further investigating the role of CSPG4 in NSCLC and in relation to EGFR-TKI resistance. Interestingly, we identified several-fold higher expression levels of CPSG4 in the osimertinib-resistant H1975/OR cells compared to the parental H1975 cells by liquid chromatography with tandem mass-spectrometry (LC-MS-MS). Western blotting and flow cytometry confirmed CSPG4 expression in both cell lines. Treatment of H1975 and H1975/OR cells with an anti-CSPG4 antibody targeting the extracellular region resulted in impaired migration and eventually lead to cell death. Similarly, siRNA against CSPG4 blocked cell growth and induced cell death. To further explore CSPG4 as a novel treatment target in EGFR-driven NSCLC, its interactions with other proteins are being analysed by co-immunoprecipitation on cell lysates from H1975 and H9175/OR cells before and after osimertinib treatment.

In summary, we have been able to demonstrate that CSPG4 is expressed in NSCLC cell lines and might serve as a suitable target to inhibit tumour growth. Furthermore, ongoing studies are being conducted to evaluate if, and if so how, CSPG4 relates to TKI resistance in NSCLC.

Key words: Non-small Cell Lung Cancer; Osimertinib Resistance; CSPG4

POSTER 42

The impact of LAMA4 expression in human mesenchymal stem cells on the chemoresistance of Acute Myeloid Leukemia

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Leukemia cutis or leukemic cell infiltration in skin is one of the commonly observed extramedullary manifestations in acute myeloid leukemia (AML) and signifies a poorer prognosis. However, little is known about its maintenance and impact on AML.

Our group has by using an AML mouse model demonstrated that AML cells infiltrated in the skin co-localize with mesenchymal stem and progenitor cells which share the same immunophenotype as bone marrow (BM) mesenchymal stem cells (MSCs), that are shown to be protective for AML. Interestingly and importantly, the skin MSCs protected AML-initiating stem cells from chemotherapy even to a greater degree than their BM counterparts, accompanied with enhanced mitochondrial transfer from skin MSCs to AML cells compared to that from BM MSCs, suggesting superior chemoprotection of skin MSCs (Sandhow et al., J. Exp. Med, 2023). Furthermore, deletion of extracellular matrix protein LAMA4 in the recipient mice, or in vitro in BM MSCs led to increased residual AML stem cells in the skin (Cai et al., Blood, 2022), indicating a critical role of LAMA4 expression in mouse skin MSCs for AML chemosensitivity. However, the impact of LAMA4 expression in human skin MSCs on human AML remains unexplored.

To investigate this, we have taken advantage of our recently established co-culture system with primary human skin-derived MSCs with or without LAMA4 deletion by either CRISPR-Cas9 or antibody neutralization. Skin MSCs were isolated from healthy volunteers based on their immunophenotype (CD45-CD235A-CD31-CD44+CD146-) and expanded in vitro for usage as a feeder layer in cocultures with human AML cell line THP1. Following cytarabine or vehicle treatment at 24hours after seeding AML cells, the drug responses were evaluated by assessing AML viability using trypan blue.

Similar to our finding in mice, we found that human skin MSCs displayed superior chemoprotection of AML cells than BM MSCs. Notably, our preliminary results indicated that neutralization of LAMA4 protein by pre-treating skin MSCs with monoclonal anti-LAMA4 antibody in the co-culture seemed to further augment this chemoprotective effect. But without inducing clear AML proliferation as observed in cocultures with BM MSCs.

Together, our preliminary data suggest that human skin MSCs possess stronger chemoprotective function for human AML cells than BM MSCs. Mechanistically, LAMA4 expression in human skin MSCs seems to have an impact on its chemoprotective function of

AML cells. Our study introduces the concept of skin as a chemoprotective niche for skin-resident AML cells, pointing to a potential explanation for the adverse prognosis of leukemia cutis. However, more work is required to further elucidate the impact and mechanisms of the chemoprotective function of skin MSCs. Experiments to generate LAMA4 knockout skin MSCs using CRISPR-Cas9 are ongoing.

Key words: Acute Myeloid Leukemia; Skin Mesenchymal Stem Cells; LAMA4

POSTER 44

Understanding the G Protein-Coupled Receptor GPR183 and its Acute Myeloid Leukemia-associated Mutant

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G protein-coupled receptors (GPCRs) are linked to numerous different diseases and are the drug targets for one third of the market drugs today. This makes them an interesting source of investigation when attempting to understand disease and disease-related signaling. GPR183 is a GPCR that mediates the migration of the lymphatic cells and has been connected to hematological cancers as well as other diseases. In this project, a specific acute myeloid (AML)-associated mutation on GPR183, A338V, has been investigated and compared with the wild-type receptor. The aim was to further understand the GPR183 wild-type along with investigating if this mutation causes GPR183 to act differently.

POSTER 45

Reprogramming of tumor-associated-macrophages anti-tumor phenotypes by targeting MNK2

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Introduction: The accumulation of Tumor-Associated Macrophages (TAMs) fuel tumor progression and are associated with poor survival in solid cancers. TAMs exhibit remarkable cellular plasticity, ranging from anti-tumor to pro-tumor phenotypes. These versatile cells regulate vessel functionality, drive tumor growth, and act as gateways for hematogenous dissemination. Our published research has uncovered the crucial role of selective mRNA translation changes as a central hub regulating immunosuppressive functions of macrophages. Mitogen-activated protein kinase (MAPK) interacting protein kinase (MNK)1 and MNK2 selectively modulate mRNA translation by impacting eIF4E phosphorylation, thereby facilitating reshaping of the proteome without altering the abundance of corresponding mRNAs. Here, we aim to determine whether MNK1 and MNK2 have different impact on TAM phenotypes as they both regulate eIF4E phosphorylation.

Methods: The role of MNK1/2 TAMs on immune responses was investigated by performing in vivo co-mingling assay consisting of mixed mammary tumor cells (4T1) with the engineered bone-marrow-derived macrophages (BMDMs).

Results: First, in vitro, we showed that silencing MNK2 gene (Mknk2), rather than the MNK1 gene (Mknk1) in BMDMs, modulates eIF4E activity in these cells. In vivo, this targeted approach resulted in reprogramming of immunosuppressive TAMs, impeding the growth of mammary tumors. Moreover, these reprogrammed TAMs adopt an angiostatic/anti-metastatic phenotype. Firstly, they induce tumor blood vessel normalization, thus facilitating the intratumoral recruitment of cytotoxic T and natural killer (NK) cells while impeding mammary metastatic spreading. Additionally, blocking Mknk2, but not Mknk1, skews immunosuppressive TAMs towards an immunostimulatory phenotype. In contrast, overexpression of Mknk2 but not Mknk1 resulted in a worsening of the 4T1 tumor burden. Reparably, overexpression of Mknk2 led to a decrease of adaptative immune responses in 4T1 tumors.

Conclusions: Overall, our results indicate that MNK2 predominantly controls TAM immunosuppressive phenotype compared to MNK1. Therefore, targeting MNK2 represents a highly efficient strategy to reprogram TAMs into an anti-tumoral phenotype.

Key words: Tumor-Associated Macrophages; Tumor growth adaptive immunity; Metastatic dissemination

POSTER 46

Human skin mesenchymal stem cells provide a unique niche for acute myeloid leukemia-initiating stem cells

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Leukemia cutis/leukemic cell infiltration in skin is one of the common extramedullary manifestations in acute myeloid leukemia (AML) and signifies a poorer prognosis. However, it is not clear how the AML cells are maintained in the skin. We have in mouse models (Sandhow et al., J. Exp. Med, 2023) shown the unique features of skin mesenchymal stem cells (MSCs) and their protective role for mouse AML cells during chemotherapy. However, it remains unexplored whether human skin MSCs exert the same functional impact on human AML cells.

We here have explored the role of human skin MSCs in AML maintenance and survival during chemotherapy by in vitro co-culture of patient-derived AML cells with primary human skin MSCs from healthy donors with a phenotype of CD45-CD235a-CD31-CD44+CD146- in comparison with bone marrow (BM) MSCs. Further flow cytometry analysis indicated that skin MSCs displayed a superior protective effect on human AML cell line THP-1 than BM MSCs during cytarabine/Ara-C treatment in the in vitro co-culture. This result was confirmed by the increased number of cobblestone area-forming cells and colony-forming units within the residual AML cells co-cultured with skin MSCs.

Mechanistically, RNA sequencing revealed high expression of key hematopoietic niche factors including KITLG and LAMA4 and enrichment of genes related to ribosome and fatty acid metabolism in human skin MSCs, supporting its supportive function for AML cells. Single cell metabolism assay (SCENITH) showed higher capacity of fatty acid and amino acid oxidation in the AML cells during Ara-C treatment in co-culture with human skin MSCs than that with BM MSCs, suggesting that skin MSCs might protect AML cells via better metabolic support.

Taken together, our preliminary data indicate human skin MSCs play an important role in protecting AML cells during Ara-C treatment partly through providing better metabolic support. We are further investigating the underlying mechanisms.

Key words: AML; Leukemia cutis; Mesenchymal stem cells

POSTER 47

Spatial metatranscriptomics to define impact of sex and ERβ on the colon

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Colorectal cancer (CRC) is the third most common cancer, and the second leading cause of cancer death worldwide. In recent years, it has been reported that the incidence of early-onset CRC is increasing, and altered diet and lifestyle habits could be major contributors. Lifestyle-related risk factors include a low-fiber, high-fat diet, high meat consumption, alcohol consumption, smoking, lack of physical activity, and obesity. Obesity and a high-fat diet (HFD) can induce an inflammatory state in the colon, which could contribute to the risk. The incidence of CRC is higher among men and post-menopausal women, compared to pre-menopausal women, and estrogen has been shown to have a protective effect. Estrogen exerts its activity through the estrogen receptors (ER) α and β as well as the G-protein coupled receptor GPER1. It has been demonstrated that ERα can improve obesity and the metabolic profile, while ERβ acts anti-inflammatory and anti-tumorigenic in colon of mice. Recently, we have demonstrated that treatment with estrogenic ligands can counteract HFD-induced alterations of the colonic transcriptome and the microbiome and noted sex differences. The link between these pathologies and the specific role of ERβ in the colonic microenvironment under HFD needs further investigation.

In the present study, we investigate the effects of sex and colonic ERβ on the host transcriptome and the microbiome using spatial metatranscriptomics. We use intestinal-specific ERβ knock-out (ERβKOvil) mice fed an HFD (60 kcal% fat) or matched-control diet (CD) for 13 weeks. The body weight gain and different metabolic parameters were measured during the experiment. Samples from colon, liver, and visceral adipose tissue were isolated for histology and transcriptomic analysis. By exploring the interplay between sex, ERβ signaling and microbiome, under HFD, this study can reveal new insights about the colon microenvironment and the mechanisms behind CRC-development.

Key words: Estrogen Signaling; Colorectal Cancer; High Fat Diet

POSTER 48

Germline ALK-R1275Q Mutation Drives a Proliferative, Undifferentiated State and Accelerate Neuroblastoma Initiation

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Background:

Anaplastic Lymphoma Kinase (ALK) alterations—including amplification or activating mutations—are frequent somatic events in neuroblastoma (NB) and correlate with poor prognosis. Rare familial NB cases harbor germline ALK mutations, such as ALK-R1275Q, which phenocopy recurrent somatic variants. While ALK's oncogenic potential is established, its role in NB initiation remains unclear.

Method:

We generated induced pluripotent stem cells (iPSCs) from fibroblasts of NB patients with a germline ALK-R1275Q mutation and from healthy controls. These were differentiated into trunk neural crest cells (NCCs) and sympathoadrenal (SA) lineage cells using a standardized protocol. Bulk and single-cell transcriptomics were performed at multiple stages. To assess tumorigenic potential, MYCN was overexpressed in both control and patient-derived lines, followed by orthotopic adrenal gland injection in mice.

Results and Conclusion:

The ALK-R1275Q mutation did not affect iPSC reprogramming efficiency, pluripotency marker expression, or differentiation into trunk neural crest cells (NCCs). Divergence became evident at the sympathoadrenal (SA) stage: in contrast to control cells, which downregulated ALK expression upon SA commitment, patient-derived cells maintained abnormally high ALK expression. Single-cell RNA sequencing identified a distinct cluster within the SAP (sympathoadrenal progenitor) stage that was predominantly composed of ALK-mutant cells. These cells retained progenitor-like transcriptional features and exhibited elevated proliferative activity.

Trajectory and pseudotime analyses demonstrated a delay in differentiation progression in patient cells. Gene set enrichment analysis revealed suppression of p53 signaling and neurogenic differentiation pathways, alongside enrichment of DNA replication, protein

synthesis, and Fanconi anemia pathways—indicative of a proliferative, undifferentiated state. Pharmacological inhibition of ALK reduced proliferation and partially restored differentiation-associated gene expression at the SAP stage.

In orthotopic xenograft models, ALK-R1275Q alone did not induce tumor formation. However, co-expression of MYCN significantly enhanced tumor initiation and reduced latency compared to MYCN alone. These results indicate that germline ALK activation sustains a progenitor-like population within the SA lineage, creating a transcriptional and proliferative environment permissive to MYCN-driven transformation.

Key words: Neuroblastoma; Developmental Cancer Model; Single-cell Transcriptome

POSTER 49

Patient-derived TXNIP-deficient primary cells exhibit NRF2 activation linked to upregulation of glyoxalase 1 (GLO1)

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Thioredoxin-interacting protein (TXNIP) plays a pivotal role in cellular redox regulation and has been implicated in various pathological conditions, including oxidative stress, inflammation and metabolic disorders including diabetes. In this study, we investigated the metabolic consequences of TXNIP deficiency in patient-derived primary cells, with a particular focus on molecular mechanisms by which TXNIP loss may trigger the NRF2 activation noted in these cells. Studying primary TXNIP deficient myoblasts and fibroblasts, we found that baseline activation of NRF2 was associated with upregulation of glyoxalase 1 (GLO1), a key enzyme responsible for detoxifying the reactive glucose metabolite methylglyoxal (MGO). The upregulation of GLO1 also led to increased production of D-Lactate in TXNIP deficient fibroblasts, a downstream product of MGO detoxification. Moreover, TXNIP-deficient cells exhibited higher glucose uptake and pyruvate accumulation, suggesting an imbalance in glycolytic flux with increased MGO production, in turn activating NRF2. This rewiring of intracellular glucose metabolism provides a mechanistic explanation for how TXNIP deficiency leads to NRF2 activation in absence of detectable oxidative stress. Considering that TXNIP inhibition is proposed as a diabetic or anti-inflammatory treatment modality, MGO-derived NRF2 activation should hence be considered as a potential consequence.

Key words: Thioredoxin-interacting protein; Nuclear factor erythroid-related 2-like 2 activation; Glyoxalase 1

POSTER 50

Distinct Roles of LAMA4 in Chronic Myeloid Leukemia Initiation and Progression

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Tyrosine kinase inhibitors (TKIs) targeting the oncoprotein BCR::ABL1 have dramatically improved the treatment outcomes in patients with chronic myeloid leukemia (CML). However, TKI resistance still occurs in about 19% of patients 12-24 months after TKI therapy start (Lauseker, et al., Leukemia, 2023). It is therefore imperative to find new therapeutic options. Targeting leukemia niche has become one of the focuses in the drug discovery since the niche alterations have been shown to contribute to the protection and resistance of CML-initiating stem cells (LSCs). We have found that the laminin alpha 4 chain (LAMA4), a functional chain of several laminin isoforms, is downregulated in bone marrow (BM) mesenchymal stem cells (MSCs) of patients with CML at diagnosis (Dolinska, et al., Blood, 2023). However, the functional impact of *LAMA4* expression on CML remains to be investigated.

We have used both mouse models and BM aspirates from newly diagnosed CML patients (N=5 patients). The impact of *Lama4* on CML progression was assessed by using the inducible CML mouse model (*SCL×tTA×TRE-BCR::ABL1*) crossed with *Lama4*$^{-/-}$ and *Lama4*$^{+/+}$ mice and by transplanting *BCR::ABL1*-expressing cells into sublethally irradiated *Lama4*$^{-/-}$ and *Lama4*$^{+/+}$ mice. The therapeutic impact of LAMA4 on human CML LSCs was evaluated by treating patient BM CD34^{+}CD38^{-} cells with recombinant LAMA4 peptides in our recent established coculture systems using primary CML patient-derived BM MSCs. These include revised stem cell assays - cobblestone area forming cells (CAFC) followed by colony assay and long-term culture-initiating cell (LTC-IC).

Our data suggested that LAMA4 peptides significantly inhibited CML patient BM-derived CD34^{+}CD38^{-} cell growth in CAFC assay (p=0.001, N=4). Notably, while imatinib did not show any clear inhibition of CD34^{+}CD38^{-} cells (N=2), LAMA4 induced more striking inhibitory effects (about 80% inhibition) in LTC-IC assay. No clear synergy between LAMA4 and imatinib was observed. Further, our pilot test indicated no inhibitory effect of LAMA4 on healthy donor BM CD34^{+}CD38^{-} cells. These data suggest that LAMA4 peptides inhibited human CML stem cell survival. The findings were consistent with a faster CML onset in *Lama4*$^{-/-}$ recipient mice following transplantation of BM cells from mice post induction of CML, reflected in bigger spleen, increased platelet counts and mature myeloid cells (CD11B^{+}GR1^{high}) in the blood of *Lama4*$^{-/-}$ recipient mice compared to that in the *Lama4*$^{+/+}$ recipients. However, in striking contrast to these findings, we observed a delayed CML onset and prolonged survival of *Lama4*$^{-/-}$*×BCR::ABL1* mice, compared to *Lama4*$^{+/+}$*×BCR::ABL1* mice, suggesting that loss of *Lama4* provide an unfavorable niche for CML initiation.

Loss of *Lama4* in the microenvironment seemed to accelerate CML progression after CML is established. Importantly, restoring LAMA4 inhibited human CML LSC growth. However, its loss provided an unfit niche for *BCR::ABL1*-expressing cells during the initiation of CML. Together, our work suggested the opposing roles of *Lama4* in CML initiation and progression. More work is required to understand the mechanisms and to evaluate the therapeutic potential of LAMA4 in vivo.

Key words: Bone marrow microenvironment; Chronic myeloid leukemia

POSTER 51

Ubiquitin-independent proteasomal degradation of TXNL1 by auranofin, an FDA-approved thioredoxin reductase inhibitor

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Recently, we have demonstrated that TXNL1 has dual functions as a thioredoxin-like reductase and also an ATP- and redox-independent chaperone. Here, we found that treatment with auranofin (AF), an FDA-approved thioredoxin reductase inhibitor and the strong activator of transcription factor Nrf2, very rapidly (within hours) downregulates TXNL1 in a time- and dose-dependent manner, while AF had no such effects on thioredoxin 1 (Trx1, TXN1) protein levels. Pre-treatment of A549 cells with proteasome inhibitors (Bortezomib/MG132) reversed the effect of AF on TXNL1 levels, but a ubiquitin activating enzyme inhibitor (TAK-243) did not, suggesting that TXNL1 is degraded via ubiquitin-independent proteasomal manner. Interestingly, CRISPR-Cas9 knockout of TXNL1 in 293T cells resulted in a mild accumulation of poly-ubiquinated proteins and significant downregulation of p62 levels and its monomer compared to WT-cells under non-reducing condition, indicating increased p62 aggregation and/or sequestration in the absence of TXNL1. Moreover, TXNL1 knockout cells showed higher basal activation level of NRF2, although its NRF2-mediated antioxidant response are mildly increased compared to WT–cells treated with different concentrations of AF. Taken together, these results suggest that TXNL1 is involved in regulation of p62 and is a major target in AF-triggered proteasomal degradation, possibly providing a functional link between Nrf2 and the ubiquitin–proteasome system in responses to oxidative stress.

Key words: TXNL1/TRP32; p62; NRF2

POSTER 52

Loss of SMUG1 Sensitizes Cells to PARP Inhibitors via Enhanced PARP1 Trapping and Accelerated Replication

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Poly(ADP-ribose) polymerase (PARP) inhibitors are widely used in the treatment of homologous recombination (HR)-deficient cancers; however, the influence of DNA glycosylases on PARP inhibitor sensitivity remains incompletely understood. Here, we investigate the role of the uracil-DNA glycosylase SMUG1 in modulating the DNA damage response and cellular sensitivity to PARP inhibition. Using live-cell imaging of SMUG1-GFP in U2OS cells, we show that PARP inhibition by Olaparib accelerates SMUG1 recruitment to DNA damage sites while simultaneously reducing its spatial spread, suggesting a more confined but enhanced accumulation at lesion sites. Conversely, inhibition of PARG—the enzyme responsible for PAR removal—also promotes faster SMUG1 recruitment but results in broader spatial distribution, indicating a distinct regulatory role for PAR metabolism in SMUG1 dynamics. Long-term PARP inhibition increases nuclear retention of SMUG1-GFP, accompanied by heightened DNA damage signaling.

CRISPR-Cas9-mediated SMUG1 knockout in HR-proficient cells leads to hypersensitivity to PARP inhibitors Olaparib and Talazoparib, increased γH2AX signaling, and enhanced PARP1 trapping, suggesting a critical role for SMUG1 in buffering replication-associated DNA damage. Notably, this hypersensitivity is not rescued by thymidine supplementation, distinguishing the mechanism from that of uracil-incorporating agents like Floxuridine. While UNG depletion alone causes only mild sensitization to PARP inhibition, combined depletion of SMUG1 and UNG or co-treatment with a dUTPase inhibitor leads to synergistic cytotoxicity and DNA damage, highlighting differential but cooperative roles of uracil-processing enzymes in maintaining genome integrity.

Mechanistically, SMUG1 knockout cells display increased baseline DNA replication speed, further elevated by PARP inhibition, linking replication stress to cytotoxicity. These cells also show increased chromatin-bound PARP1 and elevated PAR synthesis across the cell cycle, supporting a model in which SMUG1 limits PARP1 trapping and modulates replication fork dynamics. Our findings uncover an unexpected role for SMUG1 in regulating PARP activity, chromatin retention, and replication stress, establishing SMUG1 as a key modulator of PARP inhibitor sensitivity with potential implications for biomarker development and combination therapies.

Key words: DNA repair; SMUG1; Genomic Uracil

POSTER 54

Galectin-3 promotes tumorigenesis through primary cilia signaling maintenance in SHH-driven medulloblastoma

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Medulloblastoma (MB) is the most common malignant brain tumor in children. In the sonic hedgehog (SHH) driven MB subgroup, we have previously identified galectin-3, a soluble lectin glycan-binding protein, as a potential tumor-specific target. Galectin-3 overexpression in tumors is often associated with poor survival outcomes due to its contribution to tumor progression by enhancing growth, invasion, and metastasis. Previous experiments have shown that the loss of galectin-3 impairs proliferation, migration, and in vivo tumor formation. Since the mechanisms underlying galectin-3 function in brain tumors have not been investigated yet, we here aimed to elucidate the molecular mechanisms regulated by galectin-3 in SHH-driven MB. Through immunofluorescence staining and image analysis we show that the molecular mechanisms regulated by galectin-3 are mediated through primary cilia maintenance. Analysis of primary cilia in galectin-3 deficient cells revealed that primary cilia are maintained but show a significant structural disruption, which is likely to cause the functional impairs found in vitro and in vivo upon galectin-3 loss. Therefore, our results show that galectin-3 disruption has no effect on cilia formation but rather impairs cilia structure, especially ciliary volume.

To further investigate the role of galectin-3 within the primary cilia-associated SHH signaling pathway, we modulated the SHH pathway by activation with SAG and inhibition with sonidegib in galectin-3 impaired cells. Gene expression analysis of active SHH signaling indicator genes demonstrated that the loss of galectin-3 presents significant effects in both negative SHH regulators as well as positive SHH regulators. The SHH target genes were upregulated upon SAG treatment and downregulation with sonidegib in SHH cell lines. However, we were unable to activate the SHH target genes via SMO activation or inhibit them with sonidegib in galectin-3 impaired cell lines.

Taken our results together, we provide evidence for a novel link between galectin-3 and the canonical SHH signaling pathway mediated through the primary cilia. We demonstrate that galectin-3 loss in SHH-driven MB impairs primary cilia structure, thereby minimizing tumorigenic capabilities. This highlights galectin-3 as a potential therapeutic target for SHH-driven MB.

Key words: Neuro-oncology; Medulloblastoma; Primary cilia signaling

POSTER 55

RNAi therapeutics against childhood cancer

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Childhood cancer survivors show an alarmingly high incidence of health issues such as cardiac toxicity and fertility issues several years after treatment and the overall survival for resistant and relapsed patients is poor. Thus, there is a need for targeted drugs. The Polo-like kinase (PLK) family plays an important role in cell cycle regulation, and we have found PLK1 to be upregulated in pediatric cancer patients. Clinical trials with small molecule drugs against PLK1 in adult cancer patients have shown that specificity is a major issue. RNA interference (RNAi) is known for its catalytic activity and target selectivity and the breakthrough for RNAi therapeutics came in 2018 when the FDA approved the first RNAi-based drug, patisiran. Since then, five more siRNA-based drugs have been approved by the FDA/EMA. Importantly three of them are approved for children. We are utilizing our unique RNAi prodrug technology to knockdown cancer therapy targets, selectively. RNAi prodrugs enter primary peripheral blood and bone marrow mononuclear cells collected from pediatric T- and B-ALL and AML patients and induce mRNA knockdown of an endogenous targets, PLK1, without the use of a transfection reagent. The mRNA knockdown and resulting depletion of the protein, induce cell cycle arrest and apoptosis. Moreover, PLK1 knockdown sensitizes pediatric leukemia cells to chemotherapeutics such as cytarabine, as a combination of RNAi prodrugs and a nontoxic dose of cytarabine increases the number apoptotic cells. We have found PLK1 to be upregulated in several pediatric cancers and that its knockdown results in tumor cell death. Our hope is that PLK1-targeted RNAi prodrugs can be used for treatment of both adult and pediatric cancers and that a combination treatment may lead to a decrease in the concentration of chemotherapeutics. Our goal is to develop a more selective and less toxic therapy against childhood cancer.

Key words: RNAi Therapeutics; Drug Delivery; Polo-like Kinases

POSTER 56

A proteomics pilot study in the PREDIX HER2 breast cancer trial to identify novel biomarkers related to anti-HER2- therapy cardiotoxicity

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Background: Identifying novel biomarkers prognostic of increased risk for anti-HER2 Cancer Therapy Related Cardiac Dysfunction (CTRCD) or for cardiac surveillance during therapy, remain pertinent issues.

Methods: A pilot study was conducted with 28 patients 50 years old in the randomized phase II PREDIX HER2 trial, comparing neoadjuvant trastuzumab, pertuzumab plus docetaxel (THP) to T-DM1. All received adjuvant anthracyclines. Blood samples were collected at three timepoints: i) baseline, ii) prior cycle 4, and iii) 1-year follow-up. Patients with CTRCD during the first year from diagnosis were identified and controls matched for age, BMI and cardiovascular co-morbidities. Biomarker analysis was performed with the OLINK proteomics Target96 Cardiovascular-III panel. Protein levels were curated as normalized protein expression (NPX), followed by log2 transformation and relative comparison. Given the period of the study conduct, CTRCD was defined as LVEF reduction of 10% from baseline or to <50% (ESMO consensus recommendations 2020). The Wilcoxon rank-sum test was used for group comparisons.

Results: Thirteen patients with CTRCD (n=5 THP, n=8 T-DM1) and 15 matched controls (n=10 THP, n=5 T-DM1) were analyzed. Mean age was 58 years. Overall, statically significant changes in inflammatory and vascular endothelial markers were observed at all timepoints. Among others, in patients with CTRCD, an increase in baseline SPON1 (delta [Δ] ΔNPX 0,21, p=0,02), MMP-2 (ΔNPX 0,3, p=0,03), FAS (ΔNPX 0,39, p=0,004) and SELE (ΔNPX 0,48, p=0,03), compared to controls, was observed. Further statistically significant changes will be presented.

Conclusions: Increase in systemic inflammatory markers were observed during (neo)-adjuvant anti-HER2-therapy and were correlated with asymptomatic LVEF reduction. Longitudinal changes will also be presented. Our results were hypothesis generating and further analysis of the entire cohort is ongoing.

Key words: Breast Cancer; HER2; Cardio-toxicity

POSTER 57

Targeted Radiosensitization in High-Risk Neuroblastoma: A High-Throughput Drug Screening Discovery

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Neuroblastoma (NB) is a radiosensitive tumor. Despite this, in clinical practice, the therapeutic potential of radiotherapy is often limited by off-target toxicities. Enhancing tumor sensitivity to radiation by utilizing radiosensitizing compounds could improve clinical response by maximizing therapeutic benefits and minimizing side effects. This project aims to identify radiosensitizing compounds that sensitize patient-derived, high-risk NB (HR-NB) models to radiation-induced damage, with the goal of integrating these radiosensitizers into clinical radiotherapy protocols for HR-NB patients.

We conducted a high-throughput drug screening using the SPECS drug repurposing library to identify compounds that enhance the efficacy of irradiation. Over 5,200 compounds were tested in a primary screen using a patient-derived, HR-NB model from a bone marrow metastasis. Hits demonstrating enhanced cell killing in combination with radiation were advanced to secondary validation and tested on an additional patient-derived HR-NB model, as well as a non-cancer model to evaluate toxicities. Subsequent validation screen of the top 42 compounds was conducted across a broader cohort of patient-derived, HR-NB models and non-cancer models.

The screening identified apoptotic modulators and DNA damage response modulators as potent radiosensitizers for HR-NB. Importantly, these inhibitors demonstrated selective sensitivity towards cancer models in comparison to non-cancer models, suggesting a favorable therapeutic window. To further validate their radiosensitizing properties and toxicity profiles, orthogonal validation assays such as colony formation assay, flow cytometry for apoptotic markers and preclinical evaluation in animal models will be conducted.

This study demonstrates the potent and selective radiosensitization of apoptotic modulators for HR-NB. Integrating these compounds into standard clinical radiotherapy protocols could enhance the benefit of current radiotherapy regimens and contribute to the improvement of outcomes for children with HR-NB.

Key words: Neuroblastoma; High-throughput Drug Screening; Radiotherapy

POSTER 58

Detection of catalase impurity in purification of recombinant peroxiredoxin 2 – elaboration of a sensitive H_2O_2 detection assay

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In our research, we use the overoxidation-sensitive human Peroxiredoxin 2 (hsPrx2) as a model to investigate the sensitivity of selenocysteine to (over)oxidation. Therefore, we expressed and purified various selenocysteine variants of hsPrx2 for comparing their kinetic properties to the wild type (cysteine containing) enzyme. In peroxidase activity assay, when Prx2 was coupled to Trx1/TrxR1/NADPH as a recycling system, we observed a non-stoichiometric stop of the reactions. The reactions were possible to restart with the addition of a bolus H_2O_2, leading to the conclusion that a NADPH-independent reduction of H_2O_2 must have happened.

Our group has previously reported upon the presence of endogenous *E. coli* catalase in recombinant protein preparations, which could interfere with enzymatic assays of redox protein activities. Since catalase reacts with H_2O_2 in a very fast and NADPH-independent manner, its trace presence affects the concentration of H_2O_2 and thus the results of Prx2 kinetic measurements. The unexpected stop in peroxidase activity assay has not been observed in the presence of catalase inhibitor (NaN_3) or using Prx2 isolated from catalase-free *E.coli* MH1 strain.

Since the C321.ΔA strain (suitable for selenoprotein production) contains endogenous catalases, in order to produce catalase-free selenocysteine variants of Prx2, we elaborated a sensitive analytical method to monitor the catalase activity by measuring the NADPH-independent H_2O_2 consumption in the fractions of the purification steps. For this purpose, AmplexRed, an assay system for the determination of H_2O_2 concentration in solutions, was optimized. The method was validated using two approaches: the addition of a catalase inhibitor (sodium azide) to the reaction mixtures, or using Prx2 enzymes isolated from catalase-free *E.coli* strains (MH1, C321.dAEG), then used for monitoring the catalase content of Prx2 fractions obtained during protein purification.

This study contributed to the development of an efficient purification method for catalase-free Prx2 isolation.

Key words: Amplex Red; H_2O_2; Peroxiredoxin 2

POSTER 60

Inhibition of VPS34 in leukemia cells promotes induction of interferon pathway and NK-cell-mediated killing

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Pediatric acute lymphoblastic leukemia (ALL) is the most common childhood malignancy. The curative rate is 80-90%; however, resistance, relapse, and severe adverse effects of the current treatment represent major challenges and, therefore, novel therapeutic strategies are warranted. We have previously shown that one of the major cytotoxic drugs in the current treatment protocol, glucocorticoid dexamethasone (GC Dex), induced profound autophagy prior to apoptosis in ALL cells, and combination of Dex with inhibitors of Vps34, an essential kinase in the initiation of autophagy, had significant synergistic cytotoxic effects on leukemic cells. Inhibition of Vps34 also leads to the activation of cGAS/STING pathway in renal cell carcinoma and melanoma cells, as we have previously showed. The Aim was to determine whether Vps34 inhibitors activate cGAS/STING in ALL cells and whether this can induce NK cell-mediated killing of leukemia cells in vitro. We used Western blotting to study pIRF3 and pSTAT1 induction; RT-PCR with TaqMan probes to study cytokine mRNA induction and FACS analysis to investigate effect of the co-culture of ALL cells with NK cells. We found that inhibition of Vps34 using two different inhibitors leads to a prominent induction of pIRF3 and of pSTAT1 and an increase in pro-inflammatory cytokines' CCL5 and CXCL10 mRNA in ALL cells. Preliminary data showed a stronger induction of IFN-β and CXCL10 mRNAs in a sub-group of pre-B-ALL cells with ETV6-RUNX1 translocation and an upregulated Vps34. Further, using in vitro co-culture of an ALL cell line Sup-B15 with donor-derived IL-2-supported NK cells, we found that Vps34 inhibition by either SAR-405 or PIK-III inhibitors in ALL cells can lead to an increased cell killing by NK cells. Our results provide with the very first evidence that Vps34/ autophagy inhibition is a potential strategy not only for the synergistic cytotoxic action with GC Dex but also to promote a pro-inflammatory cytokine secretion by ALL blasts in the bone marrow – site of their residence – to facilitate the immune cell mediated killing of ALL cells.

Key words: Acute lymphoblastic leukemia; NK cell; Interferon

POSTER 61

Uncovering novel microproteins driving chemoresistance in pancreatic ductal adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease projected to increase mortality by 25.7% across the EU by 2040. Most systemic and targeted therapies developed for PDAC are aimed at known genetic and epigenetic drivers, but they largely fail to control disease progression, due to inherent or acquired chemoresistance. It is therefore critical to identify novel drivers of chemoresistance that may be exploited as drug response predictors or chemoresistance biomarkers in PDAC. Microproteins (MPs), small proteins of <100 codons, have been shown to mediate metastatic phenotypes like cell proliferation, metabolism, and oncoprotein synthesis. Despite growing evidence of their significance in regulating oncogenic mechanisms, their role in PDAC is largely unknown. At the Elsässer lab, we aim to systematically identify and characterize MPs underlying PDAC chemoresistance. We are currently curating a high confidence sORF database, complete with genomic mapping and annotation, subcellular localization and functional predictions, which will serve as a repository for further experiments. We are also performing comparative label-free quantitative proteomics and Ribo-Seq in PDAC resistance models to detect MPs differentially enriched in drug resistant versus sensitive cell populations. Initially, we performed a pilot chemoresistance screen and comparative mass spectrometry analysis in a panel of human drug sensitive/resistant PDAC cell lines with a wide range of chemotherapeutic agents in clinical use. Our results highlight significant enrichment and abundance of MPs differentially enriched in both drug resistant as well as sensitive populations. The abundance of detected MPs in this screen falls within the range of the ‘regular’ proteome, demonstrating that MPs could participate in resistance mechanisms much like ‘regular’ proteins. In addition, we see co-enrichment of ´regular´ proteins canonically associated with drug resistance, which suggests a potential co-regulatory function of these MPs in chemoresistant phenotypes, in conjunction with larger proteins. Further, the MPs identified in this screen have not yet been described as chemoresistance regulators, or characterized in detail, making these novel targets to elucidate, particularly in the context of PDAC. This pilot screen not only proves the feasibility of our comparative MS strategy but also gives us a pool of MP candidates associated with a clear oncogenic phenotype that we can probe in further mechanistic studies. Our future studies include a high-throughput pipeline using PDAC tumour spheroids, and multi-omic strategies/tools developed by the Elsässer lab and collaborators, to identify and elucidate the genetic/proteomic interactome of chemoresistance microproteins. This approach will robustly validate microprotein function and genotype-phenotype relationship, in addition to providing leads for biomarker development and personalized PDAC therapy.

Key words: Chemoresistance; Microproteins; Pancreatic Cancer

POSTER 62

CETSA-Driven Targeted Proteomics for Personalized AML Therapies: Investigating Drug Response Biomarkers and Resistance Mechanisms.

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Cancer drug resistance remains a critical challenge in cancer therapy, necessitating advanced approaches to understand and counteract this phenomenon. Acute myeloid leukemia (AML), an aggressive and heterogeneous cancer, often exhibits resistance to chemotherapy, contributing to high relapse rates. We are employing a Cellular Thermal Shift Assay (CETSA)-driven targeted proteomics workflow to investigate drug response biomarkers and resistance mechanisms, with the ultimate goal of enabling personalized AML therapies. CETSA allows to directly assess a diverse range of biochemical effects in the cells reflecting mechanistic biomarkers induced by cancer drugs.

Initial results from our study reveal distinct CETSA differences between drug treatments and inter-patient variability in CETSA biomarkers, even for the same treatment. For example, while some patients show a robust response to venetoclax, others exhibit limited biomarker changes. Limited biomarker response to venetoclax and no detectable CETSA apoptosis biomarker response to azacitidine were observed in one patient, with azacitidine-specific changes, suggesting potential resistance downstream of these markers. Additionally, some patients appear highly responsive to venetoclax but not to azacitidine, with no additive effect observed when the two drugs are combined. These findings underscore the variability in therapeutic response across patients and emphasize the need for personalized approaches in AML management. By quantifying protein stability levels following treatment with apoptosis-inducing drugs, this targeted approach confirms the relevance of previously identified biomarkers and supports their role in predicting therapeutic efficacy.

Although we do not yet have data from relapsed patients, obtaining such samples is a key goal for the future. These data will allow us to evaluate whether initial treatment responses align with relapse outcomes and may provide critical insights into resistance mechanisms. Understanding CETSA proteomic changes in relapsed patients could uncover key biochemical shifts associated with treatment resistance, ultimately guiding the development of more effective and personalized therapeutic strategies.

While this study is ongoing and not yet influencing clinical practice, the emerging data supports the potential for application of CETSA in personalized biomarker-driven cancer therapy. By measuring CETSA-derived biomarkers with targeted proteomics, we aim to lay the foundation for real-time, precision-guided treatment in AML. Furthermore, these insights may extend to other cancer types, advancing the broader goal of personalized oncology.

Key words: CETSA; Proteomics; Therapy

POSTER 63

Progesterone Receptor Modulator: Novel Avenues in Breast Cancer Prevention

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Women with BRCA1 or BRCA2 gene mutation have an increased risk of developing breast and ovarian cancers. Apart from the direct effect on DNA repair mechanisms, BRCA mutations via non-cell autonomous factors, including progesterone, drive cancer initiation. Our multidisciplinary combined clinical and basic research project aims at developing cancer-preventative strategies via evaluating the potential of using progesterone receptor modulators (PRM) like mifepristone. Two groups of premenopausal women are recruited for this study; The first comprises women undergoing surgery for benign breast reduction mammoplasty. The second consists of women carrying BRCA1 or BRCA2 mutations who are undergoing risk-reducing mastectomy. To investigate and validate our hypothesis, we've developed an advanced high-throughput 3D-organoid culture model using freshly isolated breast tissues. Our findings reveal that PRM effectively reduces the proliferation and growth of cancer precursor cells, encompassing luminal progenitor and basal cells, among both individuals with BRCA mutations and those without. Concurrently, it encourages the differentiation and enrichment of mature luminal cells. Intriguingly, the impact of PRM diminishes as breast cells replicate and age over time. Moreover, we've observed that PRM induces apoptosis in breast cells in a dose-dependent manner. These insights underscore the substantial role of PRM in mitigating the risk of cancer initiation and progression, demonstrating its significance for both normal and BRCA mutation carrier women.

Key words: Breast Cancer Prevention; Progesterone Receptor Modulator; BRCA

POSTER 64

Revisiting Purine Metabolism to Uncover Mechanisms of 6-Thioguanine Resistance and Synergy in AML

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6-Thioguanine (6-TG) is a thiopurine analog approved in 1966 for the treatment of acute myeloid leukemia (AML). While its use has declined with the introduction of newer agents, 6-TG is still employed as a second-line therapy in relapsed or refractory cases, with variable patient responses. 6-TG exerts its cytotoxic effects by incorporating into DNA and RNA, disrupting nucleotide synthesis and causing DNA damage that triggers apoptosis in rapidly dividing cells, however the mechanisms underlying its differential efficacy and resistance remain poorly understood.

To address this, we investigated 6-TG activity to uncover key genes and protein alterations associated with 6-TG response using orthogonal high-throughput strategies, including genome-wide CRISPR knockout screens and proteome-wide proteome integral solubility alteration (PISA) assays. In parallel, we conducted a high-throughput combinatorial drug screen using 528 oncology compounds on top of a 6-TG backbone, followed by a synergy screen with 28 prioritized compounds across multiple doses to uncover actionable co-targeting strategies.

CRISPR screens revealed essential pathways involved in the metabolic activation and incorporation of 6-TG into DNA, which can ultimately lead to intrinsic apoptosis. Core purine metabolism genes such as HPRT1, NT5C2, NUDT15 previously linked to 6-TG metabolism validated the screen, while new candidate genes involved in sensitivity and resistance included NUDT5, BAX, CASP9. The direct link between purine metabolism and these genes is to be investigated. PISA profiling uncovered solubility changes in proteins involved in RNA metabolism and ribosome biogenesis, suggesting RNA-targeted stress actions of 6-TG that have not been well characterized.

The combinatorial screen identified several compounds targeting the intrinsic apoptotic pathway that enhanced 6-TG cytotoxicity, including the AML approved Bcl-2 inhibitor venetoclax and the investigational SMAC mimetic birinapant. These compounds were validated in the synergy screen, confirming cooperative interactions with selected drugs.

Altogether, our findings define critical genetic and proteomic dependencies of 6-TG in AML and highlight combinatorial strategies to enhance its efficacy.

Key words: 6-Thioguanine; Acute Myeloid Leukemia; Precision Medicine

POSTER 65

The SciLifeLab CRISPR Functional Genomics unit

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CRISPR Functional Genomics (CFG) is a specialist infrastructure for CRISPR technology. As a National SciLifeLab facility, we offer the entire range of CRISPR applications, from the creation of knock-out/knock-in cell models to massively parallel, genome-wide or custom perturbation screens in many different modalities. We strive to make the latest technological innovations available to the Swedish research community as quickly as possible. Examples of our toolbox include base- and prime-editing, pooled CRISPR loss- and gain-of-function screens, and coupling pooled screens with single cell transcriptomics. One emerging focus area is target identification and mode-of-action elucidation, which we pursue in collaboration with the Chemical Biology Consortium Sweden (CBCS) and the Chemical Proteomics unit at SciLifeLab.

Key words: CRISPR; Functional Genomics; Genome-wide perturbation

POSTER 66

Control of redox signaling by TXNRD1 studied at single cell level

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The cytosolic selenoprotein Thioredoxin Reductase 1 (TXNRD1) is a central redox regulating enzyme, and a potential therapeutic target for some cancer therapies. Cell-based research on its activities, however, suffers from methodological limitations, such as uncertainty in evaluating diverse cellular conditions and low resolution of assays, relying on bulk analysis. Recent approaches to improving detection methods resulted in a number of novel fluorogenic probes for determination of enzyme activity in live cells. The RX1 probe, which reports selectively on the intracellular activity of TXNRD1, enables high-resolution determination of its roles in signaling at an hitherto unsurpassed level of detail.

By thorough evaluation of the performance of RX1, we found the probe to selectively report on the TXNRD1 enzymatic activity with single cell resolution in immortalized human cancer cells. Due to the heterogeneous nature of cell cultures, and in solid tumors and organs in direct analogy, high resolution in readouts is fundamental to understanding exact mechanisms in redox regulation. By combining RX1 as a highly sensitive reporter for TXNRD1 activity with downstream single cell transcriptomics, the detailed connections between TXNRD1 activity profiles and gene expression patterns can reliably be determined. Such single-cell resolution correlations can form the basis for identification of casual relationships of redox regulation through TXNRD1 with signaling pathways and cellular phenotypes. Redefining the understanding of cell cultures commonly used in fundamental cancer research with single-cell resolution of molecular mechanisms underpinning redox signaling pathways, should have major importance for the understanding of redox processes at large. We believe that this methodological approach will ultimately enable us to better understand how redox processes may be linked to cellular functions, as well as heterogeneous responses of tumor cells to treatment.

Key words: Redox Signaling; Thioredoxin Reductase; Single Cell Transcriptomics

POSTER 68

The pancreatic lobules are a unique microenvironment shaping the phenotype of pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC), the most common form of panreatic cancer, is one of the most lethal cancer forms. The 5-year overall survival of PDAC is curently at 11%. Yet, we lack deep knowledge of how PDAC invades then local tissue, and how it interacts with the tumor microenvironment, which often is characterized by a fibrotic stroma and a state of chronic inflammation. Two main subtypes of PDAC have been established by bulk – and single cell RNA sequencing. Tumors of the basal-like subtype more frequently has an allelic imbalance of mutated and wild-type KRAS, undergo epithelial-to mesenchymal transition and come with the shortest survival. In comparison, the classical subtype usually come with expression of pancreas lineage markers, such as GATA6, and come with a better prognosis. However, classical and basal-like tumor cells can co-exist in one individual tumor at varying proportions.

In the current study, we spatially mapped the individual tumor cells, and quantified their classical – and basal-like related protein expression in a digitalized immunohistochemistry-based QuPath pipeline. Regions of interest, containing only tumor cells, were stratified to tumor in pancreatic lobule, or tumor in desmoplastic stroma. We found that PDAC expression, or subtype state, seem to depend local microenvironment properties at the invasion front.

Notably, basal-like expression was upregulated at stromal invasion, while the classical expressing tumor cells were seen at the parenchymal, lobular invasion front. Both expression patterns were often identified within the same tumor. Hence, we can for the first time elucidate what drives the classical - basal-like expression state dichotomy in PDAC, and bring the previously largely unrecognized lobular invasion into the light.

Key words: Pancreatic cancer; Digital pathology; Intratumoral heterogeneity

POSTER 69

Novel MTHFD1/2 inhibitor TH9619 for treatment of colorectal cancer

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The one-carbon folate metabolic protein methylenetetrahydrofolate dehydrogenase 2 (MTHFD2) has been identified among the most consistently overexpressed protein in cancer compared to normal tissue. The protein regulates mitochondrial formate release and supports thymidine biosynthesis, central to the supply of nucleotides for DNA replication and repair. Given the absence of MTHFD2 in normal tissue, this presents an attractive strategy for developing a cancer-specific therapy. We have developed TH9619, a first-in-class MTHFD1/2 inhibitor that selectively kill cancer cells. Mechanistically, TH9619 prevents thymidine production leading to misincorporation of uracil into DNA, induction of DNA damage and replication stress, as well as death of MTHFD2-expressing cells. Here, we conducted a genome-wide CRISPR-Cas9 drug sensitivity screen in colorectal cancer (CRC) cells and identify the base excision repair DNA glycosylases UNG and SMUG1, responsible for excising misincorporated uracil from DNA, to provide resistance to TH9619. In cell viability assays we show that UNG-KO cells are hypersensitive to TH9619 and Floxuridine, compared to wildtype CRC cells. The toxicity could be rescued through addition of external thymidine and inhibition and/or depletion of dUTPase led to further sensitization to TH9619 and Floxuridine in UNG-KO cells. Excitingly, we find that CRC cells resistant to 5-fluorouracil still respond to TH9619, suggesting diverse mechanism of action and that patients resistant to 5-FU may still respond to TH9619. Moving forward, our studies will focus on detailing the mechanisms underlaying cancers vulnerability to targeting thymidine levels and identification of biomarkers to predict TH9619 response. Overall, TH9619 offers a unique cancer-specific approach to target cancers and has received approval for testing in clinical trials.

Key words: One-carbon metabolism; MTHFD1/2 inhibitor; Cancer treatment

POSTER 70

Functional and molecular drivers of FLT3-inhibitor response

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Acute myeloid leukemia (AML) is characterized by the uncontrolled expansion of immature myeloid cells in the bone marrow. Mutations in the FLT3 gene occur in approximately 25–30% of AML cases and are linked to adverse clinical outcomes. While targeted therapies with FLT3 inhibitors (FLT3i) have become part of standard treatment, a substantial fraction of patients (~40%) fail to achieve durable responses, highlighting the need to better understand underlying resistance mechanisms.

In this study, we present a comprehensive functional systems biology investigation of the drivers behind FLT3i response, integrating high-throughput functional ex vivo drug testing with multi-omics profiling—including proximity extension assays, mass spectrometry-based proteomics, transcriptomics, and genomics —across 73 FLT3-mutant acute myeloid leukemia (AML) patients. To enhance our mechanistic insights, we employ single-cell spatial surface proteomics to characterize the polarization and spatial dynamics of 80 cell-surface markers in response to FLT3i.

Our findings reveal that ex vivo FLT3i responses serve as robust predictors of clinical outcomes, accurately distinguishing remission from relapse. Mechanistically, we identify a resistant subpopulation marked by CD45RA+CD38+ expression. We also observed a functional shift from STAT5 to AKT signaling in response to FLT3i in resistant cells. Notably, combination drug screening showed that the SMAC mimetic birinapant re-sensitizes these resistant cells by reactivating apoptotic pathways. Subsequent validation experiments demonstrated synergistic activity between midostaurin and birinapant, specifically targeting the resistant CD45RA+CD38+ subset.

This study provides a systems-level understanding of FLT3i treatment response in AML, highlighting the dynamic interplay between cellular states, mutations and signaling networks. Furthermore, it emphasizes the translational potential of ex vivo drug testing and leukemic cell phenotyping to refine therapeutic strategies and guide personalized treatment.

Key words: Precision Medicine; Acute Myeloid Leukemia; Drug Resistance

POSTER 71

Swedish TP53 variant p.R181H is associated with a distinct phenotype enabling personalized clinical handling

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Background: Li-Fraumeni Syndrome (LFS) is a severe cancer predisposition syndrome caused by germline variants in the TP53 tumor suppressor gene. Due to the broad tumor spectrum, clinical management and cancer surveillance are challenging. Currently, all LFS patients undergo a standardized surveillance program, including whole-body magnetic resonance imaging (MRI), regardless of their specific TP53 variant. The TP53 variant p.R181H could be a Swedish founder variant, and carriers might benefit from personalized clinical management and surveillance.

Methods: We used haplotype analysis to determine founder status of the variant p.R181H and tumor sequencing to confirm loss of heterozygosity. Genomic and clinical data were retrieved from the newly established Swedish nationwide germline TP53 database (SWEP53), including 189 individuals from 86 families. The database includes all alive and deceased germline TP53 carriers in Sweden.

Results: Haplotype analysis suggested p.R181H could be a founder variant, using in-silico dating we estimated the age at 550 years old. The variant was carried by 22% of all Swedish LFS-families (19 of 86). Cancer incidence was significantly lower compared to other variants ($p < 0.0001$). Adult carriers mainly developed late onset breast cancer, and no children (<18 years) developed any cancers. Furthermore, carriers of p.R181H had a significantly better survival compared to other variants ($p < 0.0001$).

Conclusions: p.R181H could be the first TP53 variant where personalized clinical handling is possible. Carriers of p.R181H could benefit from targeted surveillance with exclusively breast MRI and children should not be tested until adulthood.

Key words: Hereditary Cancer; TP53; Li-Fraumeni Syndrome

POSTER 72

Physical training before and after surgery in patients with esophageal cancer: a prospective non-randomised trial.

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Background:

Standard treatment for locally advanced esophageal cancer includes neoadjuvant chemo(radio)therapy or peri-operative chemotherapy + surgery, or definitive chemoradiotherapy. Patients often have co-morbidities and chemo(radio)therapy can further impair physical fitness, increasing surgical risk. While emerging evidence supports physical training to improve treatment tolerance, evidence on structured prehabilitation and rehabilitation remain limited.

This trial evaluates the feasibility of a supervised and home-based training program during neoadjuvant therapy and after surgery.

Methods:

This prospective, single-center, non-randomized trial included patients receiving neoadjuvant chemoradiotherapy (CROSS) or perioperative chemotherapy (FLOT), followed by esophagectomy.

Patients followed a personalized training program: two weekly supervised sessions (20 min aerobic + 40 min resistance) and three weekly 20-minute home-sessions for 8-10 weeks preoperatively and 8 weeks postoperatively.

Quality of life (QoL) (measured by EORTC QLQ-C30, QLQ-OG25, QLQ-FA12), muscle strength (by handheld dynamometer), and aerobic capacity (estimated VO_2 max, Åstrand test) were assessed at baseline, post-neoadjuvant treatment, post-surgery and one-year follow-up and analyzed using linear mixed-effects models.

Results:

Twenty-five patients with large tumors and significant comorbidities were included. Supervised training attendance was 55% preoperatively and 63% postoperatively. Home-based training was performed by 65% of patients preoperatively and 93% postoperatively.

Lower-limb strength, global health-related QoL and fatigue, and several other QoL domains declined during treatment, but at least partially recovered at one-year. Some domains, including anxiety, improved over time. Upper-limb strength remained reduced. Aerobic capacity remained stable.

Conclusions:

Supervised and home-based physical training during chemo(radio)therapy, before and after esophageal cancer surgery, is safe and feasible. Initial declines in muscle strength and QoL, including fatigue, were at least partially recovered one-year post-surgery.

Key words: Esophageal cancer; Physical training; Multimodal therapy

POSTER 73

Exploring a potential therapeutic option for Blast Crisis Chronic Myeloid Leukemia

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Chronic Myeloid Leukemia (CML) is a myeloproliferative neoplasm characterized by the accumulation of immature myeloid cells due to disrupted differentiation of hematopoietic stem cells. Leukemic stem cells (LSCs) are believed to drive disease initiation, persistence, and progression, particularly through their residence in the bone marrow (BM) niche, where they are protected from therapies and alters the niche to their benefits. Although tyrosine kinase inhibitors (TKIs) have significantly improved outcomes in CML, they fail to eradicate LSCs completely, leading to disease relapse and potential transformation into the advanced phases including blast crisis (BC), linked to a very poor prognosis due to lack of effective treatments.

We have identified CXCL14, a highly conserved chemokine broadly expressed in normal tissues, as the most downregulated cytokine in chronic phase (CP) CML. Restoring CXCL14, by adding recombinant CXCL14 protein, inhibited LSC proliferation in vitro and reduced leukemic engraftment in vivo in CP CML (Dolinska et al., Blood, 2023). However, its effects on BC-CML remain to be explored.

We have here explored the therapeutic impact of CXCL14 on BC-CML by treating CML cell line K562 in a co-culture system with CML patient BM mesenchymal stem cells (MSCs), a key niche component known to be altered in patients with CML and promote CML stem cell proliferation, creating an in vitro system with high fidelity to the in vivo niche in patients. The potential synergistic effects of CXCL14 with 2nd and 3rd generation of TKI (Nilotinib (Nil), Ponatinib (Pon), respectively) were also determined. The reduced expression of CXCL14 in the CML MSCs compared to healthy BM MSCs was confirmed by quantitative PCR (qPCR).

We have observed a suppressive effect of CXCL14 monotherapy on K562 cells at a dose of 10 ng/mL (1nM). Combination of CXCL14 with Nil or Pon appeared to induce a further inhibition ($p<0.001$ by one-way ANOVA), indicating a synergy between CXCL14 and the TKIs in suppressing the growth of K562 cells.

Taken together, our data suggest CXCL14 could suppress the growth of BC-CML cell line K562 cells and potentiate the inhibition of Nil and Pon. Further work includes long term culture-initiating cells (LTC-IC) and xenograft transplantation using immunodeficient mice mouse model.

Key words: Blast Crisis Chronic Myeloid Leukemia (BC-CML); CXCL14; Bone marrow niche

POSTER 74

Investigating NK Cell Dysfunction in Cutaneous T-Cell Lymphoma

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Cutaneous T-cell lymphomas (CTCL) are clonal T-cell malignancies that primarily affect the skin. While often indolent, advanced-stage disease is associated with poor prognosis. We recently identified natural killer (NK) cells to be increased in the skin of CTCL patients; however, they exhibited reduced cytotoxicity and a less active phenotype. To investigate the underlying mechanisms, we are pursuing two complementary approaches examining how the CTCL tumor microenvironment alters NK cell function. Based on findings in solid tumors showing that tumor interactions can disrupt NK cell metabolic activity and compromise their effector functions, we hypothesized that interactions with CTCL cells similarly impair NK cell metabolism, leading to dysfunction. To explore if this is the case, we treated human primary NK cells with conditioned media from CTCL cell lines and quantified the expression levels of metabolic marker CD98 (amino acid transporter) and CD71 (transferrin receptor) via flow cytometry. Preliminary results show a trend of decreased NK cell metabolism following exposure to soluble factors from CTCL cells in a time dependent manner, evident at 24, 48, and 72 hours of incubation. We aim to evaluate this further, by investigating changes in key signalling pathways relevant to immune cell metabolism (e.g. mTOR), with immunoblotting. In parallel, we have developed an in vitro multicellular tumor spheroid model, composed of dermal fibroblasts and tumor cells, that mimics the CTCL microenvironment. This model enables us to assess NK cell infiltration into CTCL tumors using confocal microscopy and investigate strategies to enhance this infiltration, for example by IL-15 pretreatment of NK cells. In conclusion, our ongoing research seeks to reveal the underlying mechanisms of NK cell dysfunction in CTCL to develop targeted strategies that restore their metabolism, enhance tumor infiltration, and improve immunotherapy. These strategies aim to offer new treatment options for advanced-stage patients who currently lack effective treatments.

Key words: Cutaneous t-cell lymphoma; Tumor immunology; NK cells

POSTER 75

Novel drug combinations exploit metabolic vulnerabilities in neuroblastoma

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Background:

Neuroblastomas frequently harbor mutations in genes associated with neuritogenesis and Rho/Rac signaling and we have previously established Rho/ROCK signaling as a promising therapeutic target. This project aims to evaluate the therapeutic potential of the ROCK2-specific inhibitor KD025 (Belumosudil, Rezurock™) and potential combination treatments for refractory disease.

Methods:

Drug combination screening was performed using KD025 together with a cancer drug library containing 528 drugs. Cell proliferation and cell death were assessed in neuroblastoma monolayer cultures and tumor spheroids using IncuCyte® LiveCell analysis, while viability was measured with the the CellTiter-Glo® assay. Additionally, the SynergyFinder web application was applied to analyze the drug combination efficacy. To investigate metabolic changes, we employed Seahorse XF extracellular flux analyzer, nutrient deprivation experiments, and glucose uptake assays. In vivo efficacy was evaluated using 9464D allografts and homozygous TH-MYCN mice. RNA-sequencing and gene set enrichment analysis were applied to study transcriptomics.

Results:

Monotherapy with KD025 impaired growth of neuroblastoma cell lines, 9464D allografts, and tumors in homozygous TH-MYCN mice but did not achieve complete tumor regression. Notably, RNA-sequencing of KD025-treated tumors demonstrated downregulation of genes associated with metabolic processes. A drug combination screening revealed several combination partners for KD025 with known metabolic effects, including TIC10 and NMS-873, and synergistic effects were confirmed in various neuroblastoma models. We observed that TIC10 and NMS-873 inhibited oxidative phosphorylation (OXPHOS) and depleted the glycolytic reserve. However, addition of KD025 reduced glucose uptake and prevented the glycolytic shift induced by OXPHOS inhibition.

Given the increased interest in drug repurposing as a cost- and time effective approach to discover new drugs for cancer therapy, we have also evaluated the combination of KD025 and

metformin, a commonly used drug to treat diabetes known to inhibit OXPHOS. This combination induced synergistic effects in a panel of neuroblastoma monolayer cultures and tumor spheroids.

Conclusion:

Combining KD025 with OXPHOS-targeting agents provides a promising therapeutic approach for neuroblastoma. These combinations effectively disrupt the neuroblastoma cell metabolism, resulting in robust synergistic anti-tumor effects.

Key words: Pediatric Cancer; Cancer Metabolism; Drug Combinations

POSTER 76

Visualizing DNA replication nanostructures and quantifying DNA synthesis kinetics using 3D-SPARK

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DNA replication occurs within a complex topological and epigenetic landscape, requiring precise spatiotemporal regulation to ensure high-fidelity genome duplication. Conventional methods like DNA fiber assays provide valuable insights into replication dynamics but lack spatial resolution and are limited to bulk cell populations, obscuring single-cell spatial constraints. To address this, we developed the 3D Spatial Assay for Replication Kinetics (3D-SPARK), a novel antibody-free approach combining two independent DNA replication labeling methods with super-resolution microscopy. This technique enables in situ measurement of replication dynamics and nanoscale visualization of DNA synthesis events within their native nuclear context. 3D-SPARK is compatible with immunostaining, facilitating the study of replication dynamics in relation to nuclear bodies, DNA damage markers, and subnuclear domains. Optimized for structured illumination microscopy and 3D expansion microscopy, we classified basic replication nanostructures in unperturbed and perturbed human cells. Notably, hydroxyurea-induced replication stalling reduced the size of ongoing synthesis events while increasing initiation frequency, revealing cellular strategies to ensure replication completion. Moreover, RIF1 deficiency altered the spatiotemporal organization of replication foci and initiation event geometry within the 3D nucleus. Our findings demonstrate that 3D-SPARK offers unparalleled insights into both numerical and structural perturbations in DNA replication dynamics at nanoscale resolution. We look forward to engaging with the scientific community to discuss these advancements and their implications for understanding genome maintenance in health and disease.

Key words: DNA replication; Genome organization; Super-resolution microscopy

POSTER 77

NUDT5 regulates the global efficacy of nucleoside analog drugs by coordinating purine biosynthesis

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Previous genetic studies have implied that NUDT5 is a mediator of thiopurine (6TG) metabolism and toxicity, a mainstay nucleoside analog (NA) drug for acute lymphoblastic leukemia (ALL). We could reproduce these findings using NUDT5-targeted shRNA but not potent NUDT5 inhibitors, suggesting a potential non-enzymatic function is responsible. To address this, we have identified NUDT5 proteolysis-targeting chimeras (PROTACs) using a scalable, dual reporter target degradation platform. The lead molecule, DDD2, is a selective, VHL-dependent NUDT5 degrader (DC50 up to 20 nM and Dmax up to 90%). DDD2-mediated degradation phenocopies RNAi by desensitizing cells to 6TG by up to 10-fold with a dependency on NUDT5 abundance, which can be reverted by competition with NUDT5 inhibitor, TH5427. A broader screen of ~160 NAs revealed that NUDT5 degradation selectively desensitizes cells to nearly all cytostatic/cytotoxic NAs tested and extends to leukemia-directed drugs in ALL models. Mechanistically, ineffective rescue of methotrexate toxicity by nucleotide precursors in DDD2-treated cells suggests nucleotide salvage is compromised, while metabolomics data indicates that de novo purine biosynthesis (DNPB) is constitutively active in NUDT5-depleted cells. PPAT, the first and committal enzyme of DNPB, was identified as NUDT5 interactor by co-IP/MS and can be modelled into a high-confidence complex using AlphaFold3, suggesting that NUDT5 residues 70-80 are key in mediating the interaction. Our data identifies that a scaffolding function of NUDT5 coordinates the balance of de novo synthesis and salvage of purines by putatively sequestering PPAT, thereby regulating the global efficacy of anti-cancer NA drugs. Therefore, NUDT5 abundance could be a prognostic biomarker for NA drug activity and its control by targeted protein degradation could be leveraged for new therapeutic avenues in cancer.

Key words: Nucleotide Metabolism; NUDT5; Proteolysis-targeting chimeras (PROTACs)

POSTER 78

A new IL-8 humanized mouse model to dissect the role of IL-8 in immunotherapy

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High systemic IL-8 levels are linked to cancer progression and poor responses to immune checkpoint blockade. However, the lack of IL-8 in mice has made it challenging to understand the role of IL-8 in vivo. We therefore generated bacterial artificial chromosome (BAC) transgenic mice for both IL-8 (CXCL8) and its receptors (CXCR1 and CXCR2). In contrast to existing transgenic IL-8 mouse models, our new model (Hum-IL8) better captures the full spectrum of IL-8 responses. Our aim is to use Hum-IL8 mice to understand the role of IL-8 in immune checkpoint blockade treatment and CAR-T cell responses against solid tumors. Hum-IL8 mice were generated by pronuclear injection of BACs containing human CXCL8, CXCR1 and CXCR2 genes into C57BL/6J zygotes, followed by validation of IL-8 production and expression of CXCR1/2 by ELISA and flow cytometry. Immune cells from Hum-IL8 mice indeed produced IL-8 in response to TLR agonists, and Hum-IL8 neutrophils expressed human CXCR1 and CXCR2. We engineered mouse MC38 cancer cells to express human IL-8, and LL/2 cancer cell to express human CD19 and human IL-8. To test the role of IL-8 in immune checkpoint blockade, we injected MC38-IL8 cells subcutaneously into Hum-IL-8 and WT, followed by treatment with anti-PD1 or isotype control antibody. To test the role of IL-8 in CAR-T cell responses against solid tumors, we injected LL/2-hCD19-IL8 cells subcutaneously into Hum-IL-8 and WT, followed by treatment with m1928z CAR-T cells. Preliminary results indicate that anti-PD1 treatment of MC38-IL8 tumors was more effective in wild-type mice compared to Hum-IL8 mice ($p<0.05$). In addition, while CAR-T treatment of wild-type mice with LL/2-hCD19-IL8 tumors led to a significant transient tumor delay in tumor growth ($p<0.05$), that effect was not seen in Hum-IL8 mice. Our results indicate that IL-8 negatively affect both immune checkpoint blockade and CAR-T cell treatment of solid tumors.

POSTER 79

Cold-Inducible RNA-Binding Protein RMB3 as a Key Multifunctional Regulator of Liver Cancer Progression

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RNA binding motif protein 3 (RMB3) is a cold-inducible RNA-binding protein involved in multiple steps of RNA metabolism. Recent evidence suggests that RMB3 not only participates in stress adaptation but may also contribute to tumor progression. In this study, we investigated the effects of RMB3 on cell proliferation, motility, and cell death regulation in cancer cells. Functional analyses revealed that elevated RMB3 expression markedly accelerated cell proliferation, delayed cell death, and conferred resistance to apoptosis. Wound healing and transwell migration/invasion assays demonstrated that high RMB3 expression enhanced cell motility and invasive capacity. Mechanistically, the RNA-binding activity of RMB3 stabilized and promoted the translation of mRNAs associated with proliferation and motility, while its cold-inducible nature provided a survival advantage under metabolic and environmental stress conditions. Importantly, analysis of tumor tissues revealed that RMB3 expression is elevated at both mRNA and protein levels compared to adjacent non-tumor tissues, and high RMB3 expression correlates with poor overall survival. Collectively, these findings suggest that RMB3 acts as a multifunctional oncogenic regulator by promoting cell proliferation, enhancing motility, and suppressing apoptotic processes. RMB3 therefore emerges as a potential biomarker and therapeutic target in cancer.

Key words: RMB3; Cell cycle regulatory; Liver cancer

POSTER 80

Tumor encapsulation in time and space: Optimisation of diphtheria toxin-based tumor cell ablation in a mouse model of colorectal and pancreatic cancer liver metastases

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Partly explained by the anatomical location, the liver is the main target organ for colorectal and pancreatic cancer metastasis, which is the leading cause of death from these malignancies. Aggressive replacement of the normal liver tissue is the main mode of tumor invasion. In some cases, benign fibrosis-like liver injury reaction can result in the development of a stromal capsule separating the tumor cells from the liver tissue. This type of response is associated with superior outcomes. Encapsulation can be induced by successful chemotherapy, implying impaired tumor cell fitness in the initiation of a liver injury cascade to suppress tumor invasion.

In this ongoing project, we aim to mimic encapsulation by impairing tumor cell fitness to understand the liver's reaction to tumor growth and capsule formation. In mouse liver metastases, diphtheria toxin (DT) mediated impairment of tumor cells engineered to express primate diphtheria toxin receptor (DTR) leads to histologically similar tumor encapsulation as seen in human colorectal and pancreatic cancer liver metastases. This model allows us to study the transition from replacement type growth to encapsulation, aiming to spatial characterisation of tumor encapsulation in vivo at high temporal resolution.

Our goal is to understand the changes in molecular mechanisms and immune responses between the growth patterns to find therapeutical targets that shift the aggressive mode of tumor invasion into more stable form by inducing the mechanisms of reparative injury response of liver to stimulate tumor encapsulation.

Key words: Liver metastasis encapsulation; Colorectal cancer; Pancreatic cancer

POSTER 82

A microbiome-derived metabolite that kills cancer

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Background

Colorectal cancer (CRC), a leading cause of cancer-related mortality worldwide is closely associated with the gut microbiota, which plays a critical role in the tumor microenvironment. Gut microbes can directly or indirectly affect host cells, and disruption of the normal microbial community leads to dysbiosis, which can impact cancer progression. Recent studies have identified specific microbiome profiles associated with colorectal cancer. Currently, studies are examining the impact of metabolites produced by the gut microbiome on the progression of colorectal cancer.

Results

Colon cancer primary and metastatic cell lines surprisingly did not exhibit altered viability when infected with pathogens that have been associated with colorectal cancer. However, treatment with a specific microbial supernatant (hereafter referred to as ""bacteria X"" or ""Bx"") led to a significant decrease in the viability of SW480 and SW620 cells. By testing over 20 cancer cell lines from various origins, we found that this phenomenon occurred specifically in colon and medulloblastoma cell lines. Importantly, normal cells remained unaffected. Furthermore, supernatants from different Bx strains demonstrated differential killing potential, while commensal bacteria had no effect. Finally, using supernatant fractionation, we identified the active component responsible for the tumor-killing potential as a metabolite(s). Moreover, thanks to protein expression data from Cancer Cell Line Encyclopedia, we found that the metabolite(s) potentially downregulate expression of SNATX transporter. Finally, we identified key altered proteins by the Bx metabolite.

Conclusions

By discovering novel metabolic vulnerabilities, this study highlights the importance of microbial metabolites as a potential source of therapeutic anticancer agents and opens new perspectives in drug discovery.

Key words: Colon Cancer; Gut Microbiome; Bacterial Metabolites

POSTER 83

High-throughput Selection of DNA Nanostructures for Cellular Uptake in Neuroblastoma

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Neuroblastoma is a pediatric solid tumor arising from the sympathetic nervous system, characterized by poor prognosis in high-risk cases and limited options for targeted delivery of therapeutics. We present a modular DNA nanostructure platform designed to identify topologies with enhanced uptake into neuroblastoma cells, aiming to support the development of tumor-specific delivery systems for functional nucleic acids or drugs.

Our approach uses a combinatorial library of circular DNA structures built from modular single-stranded fragments—including linear, branched, bent, and loop-forming oligos—with controlled characteristics. A subset of the library includes a loop presenting the GD2-specific aptamer DB67, targeting the disialoganglioside GD2, which is overexpressed on neuroblastoma cell membranes. Other loop types, referred to as conjugation loops, were included to accommodate future attachment of cell-penetrating peptides or cholesterol, which are intended to enhance cellular uptake, but were left unconjugated in the present experiment.

The initial DNA library was incubated with $GD2^+$ IMR-32 neuroblastoma cells, followed by separation into three post-incubation conditions: whole cell lysate, DNase-treated intact cells (to eliminate surface-bound structures), and isolated nuclei. DNA was recovered after each round using AMPure purification, amplified, and re-circularized for the next selection cycle. After five rounds, all conditions and time points were pooled and sequenced using Oxford Nanopore MinION. A custom mapping algorithm was applied to deconvolute individual reads into their constituent fragments, enabling structure-by-structure analysis.

Results show consistent and strong enrichment of GD2 aptamer-containing structures across all conditions, increasing from ~45% in the crude library to >90% by round 5 in the whole-cell and DNase-treated fractions. This indicates successful selection for internalizing aptamer-targeted nanostructures. Conjugation loops were progressively depleted, consistent with the absence of conjugated ligands and thus no selection pressure to retain them. Additionally, structural selection favored linear fragments of 16–18 nucleotides, while highly branched motifs (e.g., 4-arm junctions) were disfavored.

This platform offers a powerful method for selecting DNA nanostructures optimized for neuroblastoma-specific cellular uptake. Future work will expand to $GD2^-$ control lines, test conjugated delivery enhancements, and arm the top-performing structures with therapeutic cargo such as intercalating drugs, siRNAs, or gene constructs. Together, these efforts aim to advance programmable, tumor-specific delivery vehicles for high-risk neuroblastoma.

Key words: Neuroblastoma; DNA nanostructures; Aptamer-mediated delivery

POSTER 84

The Swedish Promoting Resilience in Stress Management targeting adolescent and young adults newly diagnosed with cancer – A feasibility study

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Background: Psychological distress is prevalent among adolescents and young adults (AYAs) with cancer yet, there exist no standardized psychosocial support program for this group of patients in Sweden.

Objectives: To describe the feasibility of the second Swedish version of the Promoting Resilience in Stress Management (PRISM) program.

Methods: PRISM is a short manualized program designed to strengthen AYAs individual resources for managing stress by promoting resilience skills: stress-management, goal-setting, cognitive reframing and meaning-making. It is delivered 1:1 by an interventionist via physical- or video-visits. PRISM was developed and tested in the US and has been adapted and feasibility tested by our research group. Based on the findings, improvements were made: 1) making PRISM more person-centered and 2) creating a care model where all AYAs are reached by a youth coordinator and offered psychosocial support, including the PRISM program. A second feasibility study has now been conducted with participants recruited from the Karolinska Comprehensive Cancer Center in Sweden. Inclusion period: nov 2023-dec 2024. Participants were newly diagnosed with cancer in the age of 16-30 years and able to read and speak Swedish. Recruitment-, completion-rates and pre- and post- exploratory psychosocial outcome measures (resilience, emotional functioning and global health status), calculated with the Wilcoxon signed rank test are presented.

Results: Of the 56 AYAs, identified by the youth coordinator, 47 agreed to meet with the youth coordinator and were subsequently offered participation in PRISM. Among those 47, 27 (57%) agreed to participate and 23 (85%) of them completed four modules in PRISM. Participants were in median 25 years of age (range 18-29 years of age), 63% were female and had the following cancer diagnoses: 40% hematological, 26% sarcoma, 19% thyroid and 15% other. Twenty-one of the participants completed the pre-and post- survey. Resilience improved significantly from pre- to post PRISM (median 28.0 vs 32.0, z=-2.70, p=0.007) as well as emotional functioning (median 58.3 vs 66.7, z=-1.99, p=0.046). The global health displayed no significant improvement median 58.3 vs 66.7, z=-1.07, p=0.286)

Conclusions and clinical implications: The second version of the Swedish PRISM was linked to improved recruitment- and completion rates compared to the first version. Additionally, findings indicate improved psychosocial outcomes following participation in PRISM, suggesting that this program may benefit AYAs newly diagnosed with cancer.

Key words: Adolescent and Young Adults; Psychosocial Program; Resilience

POSTER 85

Revealing Sequential Epigenetic and Genetic Changes in Medulloblastoma Pathogenesis Using 1 PTCH1-Mutant Neural Stem Cell Models

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Cancer development is a complex evolutionary process, beginning with a normal cell acquiring few genetic or epigenetic changes and giving rise to a clone of neoplastic cells. Over time, in a stepwise process, these neoplastic cells accumulate additional genetic/epigenetic changes and eventually transform into cancer cells. Understanding these dynamic changes in tumor development is crucial for developing more effective diagnostic and treatment approaches. However, owing to the difficulty in longitudinal sampling during tumor progression in patients, our current knowledge of cancer evolution is mostly derived from retrospective inferences of genomic characterization of patient tumor samples at a single time point.

This limitation is also evident in medulloblastoma (MB), the most common malignant pediatric brain tumor. Although large-scale studies have characterized the genomic landscape of MB, they provide only static snapshots, making it difficult to reconstruct the sequential molecular events that underlie tumor initiation and progression. In particular, the Sonic Hedgehog (SHH) subgroup, which accounts for ~30% of cases and is frequently driven by germline or somatic PTCH1 mutations, remains poorly understood. To address this, we used our previously established iPSC-derived tumor model, in which human neural stem cells carrying a heterozygous PTCH1 mutation, along with nine derived SHH medulloblastoma cell models representing different stages of tumor development. Using whole-genome sequencing and genome-wide DNA methylation profiling, we aim to（1)Identify epigenetic and genetic changes in the patient's iPS-derived neural stem cell model. (2)Experimental validation of identified key targets. (3) Elucidate how epigenetic and genetic alterations contribute to the initiation and progression of SHH-MB.

Result: Human neural stem cell-derived SHH medulloblastoma exhibited a stepwise acquisition of novel genetic variants during tumor evolution. At early stages, we identified frameshift mutations in SLC30A4 and CASKIN2, as well as a missense mutation in CSMD1. In later stages, the cells displayed a recurrent chromosomal alteration characterized by the gain of chromosome 1q. In parallel, DNA methylation profiling revealed dynamic epigenetic remodeling. Early during tumorigenesis, epigenetic alterations predominantly affected pathways related to neurogenesis and neural differentiation, including regulation of the Rho-GTPase signaling pathway.

Key words: SHH medulloblastoma; Genetic and epigenetic alterations; iPSC-derived tumor model

POSTER 86

Transcriptomic Differences Between Metastases and Primary Tumors in Merkel Cell Carcinoma

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Merkel cell carcinoma (MCC) is an aggressive skin cancer with high metastatic potential and poor prognosis. However, the molecular mechanisms distinguishing primary tumors from metastatic lesions remain poorly understood. To address this, we utilized bulk and single-cell RNA sequencing (RNA-seq) data from treatment-naïve, immunocompetent MCC patients. Bulk RNA-seq analysis of the publicly available GSE235092 dataset (comprising 39 primary tumors and 23 metastases) identified 708 differentially expressed genes (DEGs; fold change ≥ 2, adjusted $p < 0.05$). Gene set enrichment analysis (GSEA) revealed enrichment of immune- and progression-associated pathways, such as IFN-γ response, IFN-α response, KRAS signaling, epithelial-mesenchymal transition (EMT), allograft rejection and complement, in primary tumors. To reduce confounding effects from non-tumor cells, we performed pseudobulk analysis of tumor cells using single-cell RNA-seq (scRNA-seq) data from GSE235090 (7 primary tumors and 5 metastases). The analysis identified 165 DEGs and confirmed the enrichment of immune and EMT-related pathways in primary tumor cells. Single-sample GSEA (ssGSEA) further supported increased IFN-γ response and EMT activity in primary tumors, with ssGSEA scores associating with early-stage tumors. Collectively, these findings suggest that primary MCC tumors exhibit a more immunologically active and mesenchymal transcriptional profile, which declines with progression to metastasis. Our study provides transcriptomic insights distinguishing MCC primary tumors from metastases and identifies potential biomarkers and therapeutic targets within immune and EMT pathways.

Key words: Merkel cell carcinoma; Transcriptomic profiles; Metastasis

POSTER 87

N-Acetylcysteine Amide Attenuates Acute Graft-versus-Host Disease by Regulating Neutrophil Infiltration

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Graft-versus-host disease (GVHD) is the major complication post allogeneic hematopoietic stem cell transplantation (HSCT), which remains the leading cause of non-relapse death in transplanted patients. While the allogeneic activation of donor-derived T cells is recognized as the main contributor in the onset and development of acute GVHD, recent studies have highlighted neutrophils, the most abundant type of leukocytes, play a role in the pathogenesis of intestinal GVHD. Neutrophils cleave chemokines and produce reactive oxygen species in response to the invaded bacteria, thereby promoting T cell activation and amplifying tissue damage caused by the conditioning regimen. Therefore, we proposed a novel antioxidant, n-acetylcysteine amide (NACA), as a potential prophylactic strategy against the acute GVHD by decreasing intestinal neutrophil activation and protecting small intestine against irradiation-induced damage.

Our previous data showed that NACA as a prophylactic treatment can significantly minimize the severity of GVHD by using a major-mismatched C57BL/6 to BALB/c transplant GVHD model. To investigate the underlying mechanism, we also analyzed the effect of NACA on intestinal neutrophil recruitment and migration. With the 7-day pretreatment, neutrophil infiltration was reduced in duodenum, jejunum and ileum. Additionally, the neutrophil proportion in mesenteric lymph node (mLN) was also lower in NACA treatment mice, indicating the reduced neutrophil trafficking into the secondary lymphoid tissue. Considering the antigen-presenting capacity of neutrophil in the mLN, we further evaluated the expression of antigen-presenting markers in migrated neutrophils. NACA treatment resulted in the decreased expression of the antigen-presenting marker, suggesting the diminished neutrophil-mediated T cell priming. Overall, our findings demonstrate the effect of NACA on neutrophil infiltration in the intestine and cellular communication in the mLN, thereby attenuating T cell expansion and GVHD severity.

Key words: Graft-versus-Host Disease (GVHD); Neutrophil; N-Acetylcysteine Amide

POSTER 88

Clonal evolution analysis of paediatric lymphoma reveals a highly diversified cellular status and potential targets for precision medicine

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Lymphoma is the third most common paediatric cancer (10-15%) worldwide. Current treatment for paediatric lymphoma is associated with severe long-term side-eOects including secondary malignancies. Moreover, relapsed lymphoma is associated with drug resistance and poor survival. These issues post an urgent need for precision medicine that specifically target lymphomagenesis and spare normal cells to the largest extend.

To reveal mechanisms underlying lymphomagenesis, we combined single cell RNA sequencing (scRNAseq) and immune receptor sequencing (VDJseq) of paediatric samples from non-cancerous reactive lymph nodes (n=7) and the three major types of paediatric lymphoma; Burkitt (BL, n=3), Hodgkin (HL, n=5), and T lymphoblastic lymphoma (TLBL, n=5). VDJ clonal expansion and copy number variation (CNV) were used for cancer cell annotation for BL and TLBL. To investigate the tumour microenvironment (TME) in HL, we integrated scRNAseq with spatial transcriptomics (Visium, n=4).

Among BL and TLBL samples, we identified a highly proliferative programme normally used by DZGCB cells is also important for lymphomagenesis. VDJseq showed hyperexpanded VDJ clone in all BL and most TLBL samples, whereas CNV inference revealed lymphoma subclones partially or completely absent of immune receptor expression, suggesting a likely origin of/reversion to premature lymphoid developmental stage during lympomagenesis. Transcriptomically, lymphoma cells distributed across many cellular status, suggesting lymphomagenesis is a continuum. Trajectory analysis showed a diverse transcription profile towards the proliferative cluster, indicating the mechanism of this transition. Using a predictive treatment score, we observed heterogeneity in gene expression of the lymphoma clones highlighting the eOicacy and short-coming of current treatment. Finally, spatial resolved transcriptomics revealed HL TME had exhausted T cells infiltration and was enriched in several exhausion marker expression, including CTLA4 and LAG3 but not PD-1.

Taken together, our data reveals that paediatric lymphomagenesis is characterized by its diversified transcriptome which is valuable information to guide novel therapeutics development.

Key words: Pediatric lymphoma; scRNAseq; Novel therapeutics identification

POSTER 89

Novel integrated tumor micro-/macro-environment (TMME) subtypes for prediction of treatment response to neoadjuvant anti-HER2 therapy

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Introduction: The crosstalk between tumor microenvironment and systemic immune responses during neoadjuvant treatment for HER2-positive breast cancer (BC) has not been fully characterized. In this study, we conducted a longitudinal assessment of tumor-systemic immune profiles within the PREDIX HER2 randomized phase II clinical trial (NCT02568839), comparing neoadjuvant trastuzumab emtansine (T-DM1) versus dual HER2 blockade and chemotherapy (DHP).

Method: Fresh frozen biopsies were collected at baseline and analyzed by RNA sequencing (RNA-seq). Serial plasma samples were collected at baseline and on-treatment, at cycle 2 for Immuno-Oncology multiplexed plasma proteomics analysis, using the Olink Proximity Extension Assay (Uppsala, Sweden). Pre- and on-treatment FFPE tissue biopsies were also used for tumor-infiltrating lymphocytes (TILs) enumeration and multiplex immunofluorescence (mIF) for an immune antibody panel.

Unsupervised clustering using a joint latent variable model was applied. Logistic regression model was used for correlation with pathologic complete response (pCR).

Results: Matched tumor and plasma samples were available for 108 patients. By integrating immune gene signatures and plasma proteomic data, we generated three novel distinct tumor micro-/macro-environment (TMME) subtypes (i.e. excluded, quiet, infiltrated). The immune-infiltrated subtype demonstrated enriched systemic and local immune profile, higher TILs levels (CD8+ and CD4+ T-cells) and lower monocyte to lymphocyte ratio. Patients with TMME-excluded subtype demonstrated worse treatment response, especially those treated with T-DM1 (OR=5.71, 95% CI=1.53-28.8, p=0.02). Adding longitudinal plasma markers expression (ΔIL33, ΔNOS3) and estrogen receptor to TMME subtypes improved the prediction of treatment response (AUC=0.79 for pCR).

Conclusions: We developed novel integrated TMME subtypes with distinct clinical outcomes in patients with early HER2-positive BC, highlighting the importance of evaluating tumor macro- and microenvironment for therapy response to antibody-drug conjugates.

POSTER 92

Celastrol targets the BACH1 to regulating HMOX1-induced ferroptosis and apoptosis in TNBC

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Background: Celastrol has a very wide range of pharmacological activities, especially in the anti-tumor area. However, the specific target and mechanism of celastrol anti-TNBC action remain poorly understood.

Purpose: This study aimed to untangle the mechanism of celastrol in anti-TNBC and evaluate its potential benefits as an anti-tumor agent.

Methods: The effects of celastrol on TNBC cells were evaluated using MTT assays, xCELLigence real-time cell analysis, high-content imaging, Transwell migration assays, and apoptosis analysis. Integrated proteomics and metabolomics were employed to investigate the underlying mechanisms. Molecular docking and cellular thermal shift assays (CETSA) were conducted to identify celastrol's molecular target. Additionally, the anti-tumor efficacy of celastrol in vivo was assessed in BALB/c nu/nu mice bearing orthotopic MDA-MB-231 xenografts.

Results: Our results showed that Celastrol significantly suppressed TNBC cell viability, proliferation, and migration while inducing apoptosis in vitro. And celastrol binds to BACH1 to up-regulation HMOX1 and induce ferroptosis. Mechanistically, celastrol directly bound to BACH1, downregulating its expression and inhibiting its nuclear translocation, thereby upregulating HMOX1. This cascade led to intracellular Fe2+ accumulation, lipid peroxidation, and subsequent ferroptosis and apoptosis. In vivo experiments confirmed that celastrol effectively inhibited the growth of MDA-MB-231 xenografts.

Conclusion: Our findings demonstrate that celastrol targets BACH1 to induce ferroptosis and apoptosis in TNBC cells via HMOX1-mediated ferroptosis and oxidative stress. This study provides novel insights into the therapeutic potential of celastrol for TNBC treatment.

Key words: Celastrol; Triple Negative Breast Cancer (TNBC); Ferroptosis

POSTER 93

Embryonic cell state transitions define tumor heterogeneity in paraganglioma and neuroblastoma

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Intratumor heterogeneity and high plasticity contribute to therapy resistance and poor clinical outcomes in neuroblastoma and paraganglioma patients. The underlying causes of plasticity and cell state transitions during tumor progression remain poorly understood. Here we profile single-cell transcriptomes of genetically engineered mouse sympatho-adrenal tumors at various stages, from embryonic pre-neoplastic hyperplasia to aged tumors. We deleted Kif1bβ and Nf1 in the embryonic sympatho-adrenal lineage and observed Paraganglioma (PPGL), Neuroblastoma (NB), and composite tumors arising in aged mice. Deep single-cell RNA sequencing, combined with immunohistochemistry and RNAscope, revealed that embryonic neuroblast-to-chromaffin cell state transitions are hijacked during tumor progression, contributing to tumor plasticity. Cancer cells progressively adopt a neuroblast lineage identity, computationally predicted to be mediated through a common embryonic chromaffin-neuroblast transitional, high-plasticity cell state.

Next, we analyzed transcriptomes from human paraganglioma and neuroblastoma using deep single-cell sequencing and performed comparative analysis with a normal human adrenal gland reference atlas, which established by integrating normal embryonic and postnatal adrenal gland. We identified a significant enrichment of cells in PPGLs representing medullary ""Embryonic Cycling Neuroblast"", as well as the enrichment of cells in high-risk NB representing medullary ""Embryonic Early Chromaffin Cell"".

We provide evidence that embryonic neuroblast-to-chromaffin cell state transitions are present in both, human PPGL and NB. To address their prognostic relevance, we deconvoluted a large bulk transcriptome of metastatic PPGL (Bruna Calsina, et al. Nat. Comm. 2023). We observed that metastatic PPGLs exhibit significantly higher enrichment of cell representing both the ""Embryonic Cycling Neuroblast"" and ""Embryonic Early Chromaffin Cell"", while non metastatic PPGL showing a higher proportion of cell representing ""Postnatal Chromaffin Cell"". These findings suggest that chromaffin to neuroblast state transitions are prognostically relevant in metastatic PPGL.

Spatial transcriptomic sequencing of human neuroblastoma and pheochromocytoma revealed similar patterns. We observed tumor cell clusters from high-risk neuroblastoma showed a relatively higher proportion of ""Postnatal Chromaffin"" cells, and clusters from pheochromocytoma were enriched in ""Embryonic Late Neuroblast"" cells.

Key words: Neuroblastoma; Paraganglioma; Cell state transition

POSTER 94

Liquid biopsy for circulating cell-free HPV-DNA before, during, and after treatment in HPV-positive head and neck cancer, according to data from a follow-up in the Stockholm Gotland Region, Sweden

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Background/aim: The incidence of human papillomavirus-positive (HPV+) head and neck cancer (HNC), particularly oropharyngeal squamous cell carcinoma (OPSCC), is rising globally. Although radio- or chemo-radiotherapy yields better outcomes for patients with HPV+ tumors compared to those with HPV-negative tumors, these treatments do not cure all cases and are associated with significant side effects. This underscores the need for more personalized therapy. To help individualize treatment, we evaluated the presence of cell-free HPV DNA (cfHPV-DNA) in plasma collected before, during, and after therapy in patients with HPV+ HNC and correlated cfHPV-DNA levels with clinical characteristics, HPV genotype, and treatment response.

Material and Methods: 267 longitudinal plasma samples were collected from 83 patients with HPV+ HNC/cancer of unknown primary, at diagnosis/recurrence, during treatment and follow-ups, and tested for cfHPV-DNA with droplet digital PCR assaying for HPV16, 18, 33 or 35.39

Results: 76/81 (93.8%) eligible diagnostic or recurrence samples tested positive for cfHPV-DNA, while a diagnostic sample was unavailable/or had inadequate cfDNA in 2/83 cases. cfHPV-DNA levels declined in most patients by 3–4 weeks post-radiotherapy initiation and became undetectable (fast responders) in approximately 30% of cases. By 3–6 months post-treatment, most patients had no detectable cfHPV-DNA. To date, no new recurrences have been documented, although two patients were unresponsive to therapy.

Conclusion: Most HPV+ OPSCC patients were cfHPV-DNA-positive at diagnosis and cleared their cfHPV-DNA upon treatment, but the speed of clearance varied depending on tumor sub-site, patient characteristics and treatment. The data suggest that follow-up with cfHPV-DNA is a promising clinical approach and should be expanded to include more patients and longer time periods.

Key words: Head and neck cancer; Cell-free HPV DNA; Digital droplet PCR

POSTER 95

Patient collaboration - in research, development, and implementation

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Background

Patient involvement in research is increasingly recognized as an ethical and democratic imperative, as well as a driver of innovation and improved studies. Including patient perspective could contribute to that research questions, study designs, and outcomes reflect patient needs and priorities. Despite this recognition, patient–researcher collaboration is still rarely implemented in practice.

Method

This poster summarizes ongoing initiatives to strengthen patient-researcher collaboration throughout all phases of a research project. Approaches include to identify meaningful collaboration in early project stages, i.e., co-design of protocols and patient information, participation in advisory boards, review of applications and publications, and contributions to national health assessment processes. Importantly, patient organizations can also act as independent partners in research projects and bring collective knowledge on the patient perspective to the project. In addition, standardized reimbursement for patient involvement is important. We also suggest practical enablers and invite you to a workshop on patient-researcher collaboration.

Result

Experiences show that structured patient-research collaboration brings multiple benefits, i.e., recruitment in studies improve and the patient perspective is better reflected, enabling tailored patient-reported outcomes such as quality-of-life. Collaboration also promotes mutual learning and motivation for both patients and researchers, and may strengthen the policy impact of research. Several formats for researcher–patient collaboration have been suggested to be

effective, including informal meetings, targeted workshops, and collaborative advisory structures.

Conclusion

We believe that incorporating patient-researcher collaboration as a natural component in research projects may provide several advantages. The next steps include promoting integration of patient-researcher collaboration by developing sustainable platforms for interaction and acknowledging to need of standardized recognition and reimbursement.

Keywords: Patient-researcher collaboration; patient-perspective; patient-involment in research

POSTER 96

Interplay between metabolic reprogramming and the tumor microenvironment for development of precision medicine strategies for neuroblastoma

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Background

Neuroblastoma (NB) tumors exhibit significant metabolic alterations crucial for malignant progression. Central to this metabolic adaptation are lipid droplets (LDs), involved in energy storage, signaling, and stress protection, often associated with therapy resistance and aggressiveness. However, our previous research linked LD formation to differentiation and MYCN inhibition in NB. Despite their critical roles, the precise mechanisms of LD formation and their interactions with the tumor microenvironment (TME) remain poorly understood.

Aims

This study aims to investigate the molecular mechanisms driving LD accumulation in NB, exploring their influence on differentiation, TME modulation, and therapy response to uncover novel therapeutic opportunities.

Methods

For MYCN downregulation, SK-N-BE(2) cells were treated with inhibitors 10058-F4, 10074-G5, and JQ1 or transduced with shMYCN lentivirus. Lipid droplet accumulation was assessed with LD-BTD1, while neurite outgrowth was analyzed using phalloidin and quantified with Incucyte live-cell imaging. Proteomic analysis was performed using Reactome, and metabolic analyses using Seahorse extracellular flux analyzer and nutrient deprivation assays. To investigate cell-to-cell interactions, we either cultured tumor microenvironment (TME) cells in SK-N-BE(2) conditioned media or co-cultured them to form spheroids.

Results

Proteomic analysis of SK-N-BE(2) cells upon MYCN inhibition revealed downregulation of most enzymes in the fatty acid oxidation (FAO) pathway. Metabolic profiling confirmed that FAO is impaired following MYCN downregulation, and reducing lipid availability in the media prevents LD formation. We further found that inhibition of triglyceride (TG) synthesis not only reduces LD accumulation but also decreases proliferation of NB cells, highlighting a potential metabolic vulnerability. Moreover, when culturing TME cells in conditioned media from SK-N-BE(2) cells, or when co-cultured in a spheroid model, these cells accumulated LDs, suggesting that factor released from neuroblastoma cells can induce lipid remodeling. To investigate the underlying mechanisms, we analyzed significantly upregulated proteins related to LD biology and identified caveolin-1 (CAV1) as a potential mediator of lipid exchange between NB cells

and the TME. Future work will explore the roles of these targets in differentiation, LD accumulation, and lipid dynamics within NB and the TME.

Conclusions

MYCN downregulation in NB promotes LD accumulation and differentiation through disrupted FAO and upregulation of key lipid metabolism and differentiation genes, providing insights into lipid toxicity protection and therapeutic opportunities within the TME.

Key words: Metabolic reprogramming; MYCN; Lipid droplets; Tumor microenvironment; Targeted therapy

POSTER 97

Induction of reprogramming of lipid metabolism by EZH2 inhibition in Sonic Hedgehog medulloblastoma

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Medulloblastoma (MB) is one of the most common malignant brain tumors of childhood. Notably, pediatric brain tumors harbor fewer genetic mutations compared to their adult counterparts, with some displaying epigenetic alterations. This suggests a critical role for epigenetic modulation in development and progression of childhood cancer. The role of the master epigenetic regulator Enhancer of Zeste Homolog 2 (EZH2) in MB is still unknown. Here, we show that EZH2 inhibition leads to robust induction of lipid droplet (LD) accumulation upon treatment of cells from the sonic hedgehog (SHH) MB subgroup. Our integrated transcriptomic and lipidomic analyses revealed a significant upregulation of lipid metabolism pathways, accompanied by elevated levels of triglycerides (TGs) and cholesteryl esters (CEs). In addition, the genes encoding the two key rate limiting enzymes of lipogenesis, fatty acid synthase (FASN) and 3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMGCR), were strongly upregulated. Pharmacological inhibition of FASN upon treatment with UB006 or Orlistat and HMGCR following Simvastatin incubation prevented lipid accumulation caused by EZH2 suppression. We further demonstrated that culturing cells in media with low lipid content led to reduced LD accumulation. Notably, the suppression of EZH2 led to an increased dependency on fatty acids as fuel for energy production compared to untreated cells. Together, our findings suggest that combined targeting of EZH2 and lipid synthesis may provide an attractive therapeutic approach for patients with SHH MB.