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ABSTRACT BOOKLET



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P 01

Biologically informed data augmentation improves the generalization of deep learning models for prediction of single-cell CRISPR perturbation responses

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Abstract

CRISPR-based single-cell perturbation screens with scRNA-seq readouts are transforming functional genomics. These datasets are also increasingly used to train deep learning models to predict perturbation responses across different cell states. Such in-silico CRISPR screens, if reliable, could accelerate discovery of disease mechanisms and prioritize new perturbation experiments. However, single-cell perturbation datasets often have strong cell-state heterogeneity, limited perturbation coverage, and few cells per perturbation. This leads predictive models to frequently fail when applied beyond the cell states and experimental contexts observed during training.

To address this challenge, we introduce a biologically informed augmentation framework for CRISPR single-cell perturbation prediction. The method generates biologically constrained synthetic training examples from baseline cell state and perturbation identity, combining empirical priors from public CRISPR scRNA-seq screens with regulatory and pathway information through an evidence-gated routing rule. These augmentations are introduced during training of a downstream deep learning perturbation prediction model, improving generalization to held-out datasets.

Across a 9-screen leave-one-dataset-out panel of public CRISPR perturbation screens, augmentation improved held-out perturbation prediction over a real-only baseline trained with matched real-data exposure in all 9 screens. It increased the Pearson correlation for predicted differential expression effects from 0.006 to 0.153, averaged across all 9 screens and 3 seeds. The augmented approach also showed improved metrics for differential expression ranking, perturbation effect direction, and perturbation specificity metrics.

These results support prior-informed synthetic augmentation as a practical strategy for improving data efficiency in single-cell perturbation prediction. Rather than requiring dense measurements in every new context, the approach helps downstream models make better use of sparse CRISPR screens by adding biologically constrained training signal during model training.

Keywords

Single-cell CRISPR screens, Perturbation response prediction, Synthetic data augmentation

P 02

Improving neuroblastoma differentiation therapy using combinatorial single-cell CRISPR screens

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Abstract

Despite the routine use of retinoic acid derivatives to induce differentiation of residual cancer cells in children with high-risk neuroblastoma, the mechanisms governing this process remain poorly understood and fatal relapses remain common. Thus, identification of more effective differentiation targets holds promise for improving survival outcomes in these patients. This can be systematically done through pooled CRISPR KO screens with single-cell RNA sequencing (scRNA-seq) readouts, however, current technologies lack the capacity for combinatorial KOs needed to study the multi-gene interactions that likely drive the complex differentiation process.

To this end, we are developing a CROP-seq inspired Cas12a-based platform for pooled CRISPR screens enabling KO of multiple genes within the same cell, with a readout that is directly compatible with standard scRNA-seq setups. Using this platform, we aim to identify novel therapeutic targets that can be leveraged to achieve more efficient and stable differentiation in neuroblastoma.

In a proof-of-concept study of this technology, we generated a Cas12a-expressing SK-N-BE(2) cell line with doxycycline-inducible activity to enable temporal control of the gene editing. These cells were transduced with a dual-guide KO library targeting 60 differentiation-associated genes and were processed with a 10X genomics 3' scRNA-seq workflow. Subsequent analysis confirmed efficient capture of guide RNAs (gRNAs) with stable library representation and minimal recombination, enabling confident assignment of transcriptional profiles to individual KOs. To experimentally validate gene editing at the intended target sites, long-read sequencing of the cDNA from the same cells with Oxford Nanopore is currently ongoing. Similarly, a repeat of the experiment with Parse SPLIT-seq based processing is being analyzed for technological benchmarking.

Future applications of this platform include assessment of neuroblastoma differentiation in multiple patient-derived tumoroids, enabling identification and validation of targets across different genetic contexts. Using a machine-learning model developed in the lab, these data will be used to predict optimal combinations of perturbations to achieve stable differentiation, which can then be experimentally validated with iterative screenings. Taken together, this approach provides a scalable strategy for identification of targets for stable neuroblastoma differentiation with promise for high translational capacity – with the potential for similar applications in many other complex phenotypes.

Keywords

Neuroblastoma, Differentiation therapy, Pooled CRISPR screens

P 03

The perceptions of a home-based blood flow restriction training program during and after surgery for hepatopancreatobiliary cancer

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Abstract

Background

Blood-flow restricted resistance training (BFR-T), which relies on low training loads, may support faster muscle recovery by minimizing the mechanical stress typically linked to heavy-load resistance exercise. When implemented in a home-based setting, it could improve exercise accessibility for cancer survivors; however, research exploring their perspectives remains scarce. This study aims to qualitatively investigate participant's perspectives of a home-based BFR-T training program, specifically identifying barriers and facilitators to participation and adherence, as well as experiences related to the use of an activity tracker and protein supplementation during the intervention.

Methodology

Nine participants were interviewed between October and November 2024. All had taken part in a two-arm randomized controlled trial examining whether BFR-T could mitigate muscle loss and improve functional capacity and mental health in patients with pancreatic, biliary tract, and liver cancer. Interviews were transcribed verbatim and analyzed using inductive reflexive thematic analysis.

Results

We developed three main themes from the focus groups – From uncertainty to understanding BFR-T (Instructions for BFR-T, Adherence to BFR-T, Expectations vs Experience, Recommending BFT-R, Pressure cuffs), Feeling the Change—In Body and Beyond (sub-themes: Witnessing the effects of BFT-R, Physical effects of BFTR, Effects on Continuing to exercise), Beyond the cuffs - Additional components that support or challenge engagement in BFT-R (Training from home, Fitness tracker, Protein supplement).

Conclusion

Participants moved from initial uncertainty to clearer understanding and acceptance of home-based BFR-T, supported by clear instructions, growing familiarity, and perceived benefits. Early challenges included cuff discomfort and skepticism, while the activity tracker, protein supplement, and home-based format played mixed but generally supportive roles. Effective implementation should prioritize clear guidance, comfortable equipment, and flexible use of supplementary elements. Overall, with adequate support, home-based BFR-T appears practical and acceptable, with potential to promote sustained exercise participation.

Conflict of interest

No conflict of interest to declare



Keywords

Blood-flow restricted resistance training, Reflexive thematic analysis, Qualitative

P 04

Greatly increased catalytic efficiency and resistance towards hyperoxidation of cysteine-to-selenocysteine variants of human peroxiredoxin 2

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Abstract

Human Peroxiredoxin 2 (Prx2, PRDX2) is a major antioxidant enzyme playing key role in cytosolic H_2O_2 reduction and redox regulated signaling pathways, with context dependent functions in both tumor suppression and tumor promotion. Prx2 exists mostly in the cytosol as a homodimer, but also readily forms larger oligomers. Prx2 belongs to the typical 2-Cys peroxiredoxin family having two active site cysteines (Cys, S) in the peroxidatic (C_P) and resolving (C_R) position. In the catalytic cycle of Prx2, the C_P-SH is oxidized to sulfenic acid (C_P-SOH). The sulfenic acid is prone to further oxidation, resulting in the formation of sulfinic (C_P-SO_2H) and sulfonic acid (C_P-SO_3H) derivatives in the presence of higher concentrations of H_2O_2. The sulfinic acid derivative of Prx2 is catalytically inactive, but can be reduced back to sulfenic acid by Sulfiredoxin in an ATP dependent reaction, while the sulfonic acid form is irreversibly overoxidized. In the resolution step, the sulfenic acid, C_P-SOH forms a disulfide bond with C_R-SH of another subunit, which is then reduced, mostly by Trx in the final catalytic recycling step.

In some bacterial peroxiredoxins, the peroxidatic cysteine is naturally replaced by selenocysteine (Sec, U). In general, Sec exhibits increased reactivity compared to Cys due to the higher nucleophilicity and lower pKa of its selenol group, making it more reactive in biological systems. Thus, we here wished to investigate if or how a C_P-to-U_P substitution in Prx2 would alter its enzymatic properties, which could shed further light upon the evolutionary or functional constraints that govern the choice of Cys over Sec in nature.

We here found that when the C_P residue of Prx2 is replaced with Sec, the enzyme becomes two orders of magnitude more active as a peroxidase compared to wild-type Prx2. We also determined the second order rate constant between H_2O_2 and the U_P variant of Prx2 using HRP competition assay in stopped-flow, which was three times higher than the wild type enzyme. The two orders of magnitude higher peroxidase activity of U_P variant compared to three times higher second order rate constant, suggest that the Sec variant is better substrate of Trx1 than the wild type enzyme. When testing the susceptibility of the U_P variant to hyperoxidation, we found that it remained resistant even in the presence of 5 mM H_2O_2 during the six-minute treatment, whereas the wild-type lost its activity under the same conditions.

The explanation of why the less active cysteine variant in human peroxiredoxin evolved over time is possibly related to the role of the enzyme in redox regulation.

Keywords

selenocysteine, peroxiredoxin, trx system

P 05

Immune phenotyping of molecular subtypes derived from early multiomic blood profiling for prognostic stratification in NSCLC

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Abstract

Background: Immune checkpoint inhibitors (ICIs) have transformed the treatment landscape for non–small-cell lung cancer (NSCLC). However, primary resistance occurs in approximately 70% of patients, representing a critical clinical challenge. Consequently, there is an urgent need for predictive, minimally invasive biomarkers that not only accurately anticipate clinical outcomes but also elucidate the systemic biological mechanisms driving the anti-tumour immune response.

Methods: Using blood samples collected at the second cycle of ICI therapy, we integrated circulating cell-free DNA (cfDNA) methylation and extracellular vesicleassociated microRNA (EV-miRNA) signatures via MOFA2 to define post-treatment molecular subtypes (MDCs; MOFA2-derived clusters). This approach identified three distinct molecular clusters (MDC1, MDC2, and MDC3). To characterise the immunological context associated with these MDCs, we analysed matched flow cytometry data and evaluated bulk RNA-seq profiles from peripheral blood mononuclear cells (PBMCs) representative of the three clusters. Transcriptomic profiles were deconvoluted using CIBERSORT to estimate immune cell composition, and these findings were subsequently correlated with both MDC assignments and the flow cytometry data.

Results: The MDCs robustly stratified patients into clinically distinct risk categories. MDC1 and MDC2 formed a low-survival state associated with poor outcomes, demonstrating median progression-free survival (PFS) of 4.66 and 7.71 months, and median overall survival (OS) of 7.78 and 9.90 months, respectively. Conversely, MDC3 patients exhibited a highly favourable profile with markedly prolonged survival (median PFS: 28.89 months; median OS: 40.79 months), achieving statistical separation between risk groups (log-rank $p < 0.001$). Furthermore, integration with circulating immune cell subsets highlighted key immunological correlates: MDC1 and MDC2 were associated with immunosuppressive or dysfunctional profiles, whereas MDC3 strongly correlated with signatures of active anti-tumour immunity. The correlation among MDC assignments, CIBERSORT-derived cellular composition, and flow cytometry confirmed that these molecular subtypes capture systemic immune differences at an early on-treatment time point.

Conclusion: This integrative multiomic analysis of cfDNA methylation and EVmiRNAs establishes MDCs as clinically relevant, minimally invasive biomarkers of early treatment adaptation. The correlation of MDCs with CIBERSORT and flow cytometry data supports the utility of integrated liquid biopsy approaches to refine NSCLC patient stratification and to uncover the systemic mechanisms mediating immunotherapy efficacy



Keywords

Methylation , EVs miRNA, Predictors

P 06

Uncovering neuron-tumor crosstalk controlling vascular dynamics and cellular plasticity in pediatric brain tumors

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Abstract

Medulloblastoma (MB) is the most common malignant pediatric brain tumor. Despite aggressive treatment, relapse and long-term toxicity remain major challenges. Angiogenesis and blood–brain barrier (BBB) dysfunction are central to MB growth and metastasis, yet anti-angiogenic strategies have limited efficacy, suggesting alternative vascular mechanisms are involved [1, 2]. This points to alternative vascular control mechanisms that are not being targeted. Neurons are increasingly recognized as active regulators of the tumor microenvironment [3], but their role in MB vascular remodeling and metastasis remains poorly understood. **We hypothesize that progressive loss of astrocytic and neuronal regulation of blood vessels results in BBB breakdown, promoting invasion and leptomeningeal spread.** Our preliminary in vivo and in vitro data support this by showing (i) progressive vascular abnormalities and loss of astrocytic vessel coverage across increasing tumor malignancy; and (ii) aggressive tumor NES (tNES) cells secrete factors that enhance vascular complexity, further amplified by neuron–tumor co-culture.

We aim to define how a neuron–tumor–vasculature signaling axis shape an aggressive niche that drive BBB dysfunction, vascular instability, and metastasis in MB and to identify targets with direct translational potential. Specific aims include **(1)** to define how vasculature and BBB remodeling correlate with aggressiveness in models and patients and **(2)** to identify and test neuron–tumor–endothelial signals that drive vascular instability and tumor progression.

Keywords

Medulloblastoma, Tumor microenvironment, Brain cancer

P 07

KD025-mediated ROCK2 inhibition attenuates metastatic progression and hypoxic tumour growth in neuroblastoma

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Abstract

Background:

Neuroblastoma, the most common cancer in infants, is characterized by a high rate of metastases, frequently present at diagnosis. This underscores the urgent need for therapeutic agents capable of simultaneously targeting primary tumour growth and metastatic spread. The Rho/ROCK-signalling axis has emerged as an attractive novel therapeutic target in neuroblastoma, given its well established roles in key processes that drive metastatic progression, including cytoskeletal remodelling and actin dynamics.

Aim:

This study investigates the anti-metastatic potential of KD025 (Belumosudil), an FDA and EMA-approved ROCK2-specific inhibitor, in neuroblastoma.

Methods:

In vitro migration and invasion assays were performed in a panel of neuroblastoma cell lines grown as monolayers and tumour spheroids, using the Incucyte® Live-Cell Analysis System. The effects of KD025 were also assessed under hypoxia in comparison to chemotherapeutics used in standard neuroblastoma treatment protocols.

Results:

KD025 inhibited neuroblastoma growth more potently under hypoxic conditions than normoxia, in contrast to standard cytostatic drugs. In monolayer cultures, KD025 reduced neuroblastoma cell migration and blocked cell invasion. Additionally, in the spheroid model, KD025 suppressed 3D tumour cell invasion into a Matrigel matrix in a dose-dependent manner. Collectively, our findings provide a preclinical rationale for investigating the effects of KD025 on neuroblastoma metastasis *in vivo*.

Conclusion:

High-risk neuroblastoma is frequently associated with metastatic disease, and hypoxia is an important microenvironmental driver of metastatic progression. Our findings suggest that inhibition of ROCK2 can target both tumour growth and invasive capacity in neuroblastoma, with enhanced efficacy under hypoxic conditions. Thus, inhibition of Rho/ROCK signalling may offer a promising therapeutic strategy to combat metastatic neuroblastoma.

Keywords

Neuroblastoma, ROCK2, Drug targeting

P 08

Analysis of Thioredoxin System–Regulated Transcription Factor Networks

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Abstract

Reactive oxygen species (ROS) and redox-regulated signaling pathways play fundamental roles in tumor progression, therapy response, and immune regulation in cancer. To maintain redox homeostasis under elevated oxidative stress, cancer cells rely heavily on antioxidant systems, particularly the thioredoxin and glutathione pathways. Among these, thioredoxin reductase 1 (TrxR1/TXNRD1), a cytosolic NADPH-dependent selenoprotein reductase, has emerged as a key regulator of redox-sensitive signaling mechanisms and a promising therapeutic target in cancer.

Recent studies have demonstrated that TrxR1 influences receptor tyrosine kinase (RTK)-associated signaling pathways and transcription factor activation, including the STAT3 pathway. Inhibition of TrxR1 using selective inhibitors such as TRi-1 induces oxidative stress and alters phosphorylation-dependent signaling, leading to modulation of STAT3 activity alongside NRF2 and NF-κB responses. Since STAT3 is a major regulator of tumor cell survival, proliferation, immune evasion, and PD-L1 expression, thioredoxin system–dependent regulation of STAT3 signaling may directly influence the immunological phenotype of cancer cells and responsiveness to immunotherapies.

To investigate the interconnected regulation of redox-sensitive transcription factor networks, we developed a multiplex fluorescent reporter system, pTRAF, enabling simultaneous single-cell resolution monitoring of NRF2, NF-κB, and STAT3 activity by flow cytometry. Combined with complementary fluorescent H_2O_2 reporter tools, this platform allows dynamic analysis of signaling responses following perturbation of the thioredoxin system. Using these approaches, we further examined the role of TXNL1, a redox-active thioredoxin-like chaperone protein implicated in proteostasis and oxidative stress adaptation, in shaping transcription factor activity under oxidative conditions.

Our results demonstrate substantial heterogeneity in redox signaling responses and reveal tight functional coupling between the thioredoxin system and transcription factor regulation. The combined application of pTRAF-derived methods and live-cell redox reporters provides a powerful platform for dissecting how thioredoxin system perturbation reshapes signaling pathways linked to cancer progression and immune regulation. These findings may support the development of biomarker-guided therapeutic strategies targeting TrxR1 in combination with RTK inhibitors or immunotherapies.

Keywords

TrxR1, STAT3, Nrf2

P 09

A spatial AI-based biomarker with independent prognostic value for risk-stratification of early low- and intermediate- risk breast cancer

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Abstract

Introduction: In the low and intermediate ER+/HER2- subgroup of breast cancer patients, clinicopathological factors alone cannot accurately risk-stratify patients sufficiently, and additional methods such as molecular assays or AI-based risk stratification are typically needed. However, bulk-level molecular assays and current generation of AI-based medical devices for whole slide image (WSI) analyses disregard spatial localisation and spatial distribution of the molecular or digital biomarkers. We hypothesize that the spatial distribution and localisation of a previously reported AI-based marker provides independent prognostic value beyond its global score.

Methods: This multicentre cohort study includes training data from two sites in Stockholm (Sweden N=3077) and independent external test data from three sites in Skåne (Sweden, N=3028), in total 6,105 patients with primary invasive breast cancer. H&E WSIs were pre-processed, and tile-level DeepGrade predictions (ViT_DG, a vision-transformer adaptation of the original model). The presence of spatial clusters of high-risk tiles localised at the invasive tumour front was defined as a binary biomarker. Primary analyses were pre-specified in the clinically challenging ER-positive/HER2-negative/lymph-node-negative, NHG1-2 subgroup. Prognostic associations with breast-cancer recurrence-free interval (BCRFI) and distant metastasis (DRFI) were assessed using multivariable Cox proportional hazards models.

Results: In the primary subgroup (n=1,535 training; n=1,403 test), tumour front clusters were present in 37% and 39% of patients respectively. Cluster-positive status was associated with a 2.34-fold increase in the risk of recurrence in the training cohort and 2.43-fold in the external test. Comparing to standard clinical risk groups, cluster-negative patients achieved a 10-year BCRFI of 95.3% equivalent to NHG1 patients and better than the NHG2 groups (92.4%), whereas the cluster-positive patients (10-year BCRFI 90.2%) did worse than the NHG3 reference, approaching the recurrence rate of the lymph-node positive patients. This retained independent prognostic value within the subgroup of patients classified as low-risk by ViT_DG (test set multivariable HR 2.85) demonstrates that the spatial biomarker identifies high-risk patients not captured by the global score.

Conclusions: The absence of high-risk morphology clusters in the tumour front constitutes an independent prognostic indicator, identifying a low-risk group of patients irrespective of the initial marker status. More broadly, preserving the spatial distribution of AI-derived predictions within histology slides enables prognostic stratification beyond slide-level aggregation and merits further investigation.

Keywords

Breast Cancer, AI-based biomarker, Spatial Intratumour Heterogeneity

P 10

Molecular and Cellular Parallels Between Glioblastoma and a Clinical HCMV Isolate: A Model to Decipher Non-Canonical HCMV Signatures?

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Abstract

Introduction
HCMV-associated proteins and nucleic acids have been reported in several human malignancies, yet their molecular characteristics and biological relevance remain poorly understood. In particular, some tumor-associated HCMV signals differ from those expected during canonical HCMV infection. In our analyses, HCMV IE protein was detected in 90% of primary glioblastoma specimens and 6 of 7 patient-derived glioblastoma cultures, supporting a strong association between HCMV-associated signatures and glioblastoma. During the characterization of a panel of 140 clinical HCMV isolates, we identified a single isolate, C26, exhibiting unusual molecular and cellular features that closely resembled those observed in human tumors. We therefore investigated whether C26 could provide an experimental model for characterizing these non-canonical HCMV-associated signatures in glioblastoma.

Materials and Methods
Primary glioblastoma tissues and patient-derived cultures were analyzed for HCMV immediate-early (IE) protein expression by IHC, flow cytometry, and WB. Cancer patient plasma was used as primary antibody in WB to assess recognition of non-canonical proteins in glioblastoma cells. Viral genomic features were investigated by PCR, while FISH assessed IE-associated nucleic acid localization. Features identified in glioblastoma samples were compared with those observed in C26-infected cells.

Results
Glioblastoma-derived cells exhibited predominantly cytoplasmic HCMV IE-related signals, contrasting with the typical nuclear localization during HCMV infection. WB analysis of glioblastoma tissues and patient-derived cultures revealed smaller IE-reactive proteins rather than canonical IE72 and IE86 proteins. Nested PCR and TaqMan analyses identified an intron 2-deficient IE sequence in 96.9% of glioblastoma samples. Importantly, plasma from cancer patients recognized proteins of corresponding molecular weights in primary glioblastoma cell cultures expressing cytoplasmic IE proteins, indicating the presence of circulating antibodies reactive against these non-canonical protein species. C26-infected cells reproduced several features observed in glioblastoma, including a similar pattern of non-canonical IE-reactive proteins and predominantly cytoplasmic IE-associated signals. FISH analysis further revealed predominantly extranuclear localization of IE-associated nucleic acid signals.

Conclusions
Glioblastoma displays a distinct pattern of non-canonical HCMV-associated features characterized by altered IE protein expression and cellular localization. The recognition of corresponding proteins by antibodies present in cancer patient plasma provides an additional link between these tumor-associated molecular signatures and the host immune response. The ability of the C26 clinical isolate to recapitulate several of these features provides an experimental model for investigating their molecular origin and biological significance in glioblastoma.

Keywords

Glioblastoma, HCMV virus, Virus-cancer interactions

P 11

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

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Abstract

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[1-3]. 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 500 HEK293T derived clones[4]. 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&Tag[5] 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.

Keywords

Epigenomics, Epidrugs, Transcription factors

P 12

Single-cell Multi-omics Analysis Reveals Transcriptional and Epigenetic Landscape in Irradiated Human Skin

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Abstract

Adjuvant radiotherapy is essential for breast cancer management but frequently causes persistent skin complications that can last for years after treatment. However, the mechanisms that maintain skin cells in this dysfunctional state remain poorly understood. We performed single-cell multiome sequencing, including paired scRNA-seq and scATAC-seq, on four pairs of skin biopsies from breast cancer patients, comparing irradiated chest skin (RT+) with matched non-irradiated abdominal skin (RT−). Two additional paired samples were profiled using single-cell Nano-CUT&Tag for H3K27ac and H3K27me3. At the cellular composition level, RT+ skin showed an overall reduction in epidermal cells, with a marked loss of spinous keratinocytes, particularly the spinous-III subcluster. In the dermis, endothelial populations were remodeled after radiotherapy, with relative increases in both lymphatic and vascular endothelial cells. Keratinocytes exhibited the most profound radiation-associated molecular alterations. GSEA revealed significant enrichment of TNFα/NF-κB signaling and epithelial–mesenchymal transition (EMT) programs in RT+ keratinocytes. Motif enrichment analysis of RT+-accessible chromatin regions further revealed strong activation of AP-1 family transcription factors, including FOS, JUN and JUNB, indicating an AP-1-dominated inflammatory regulatory program in irradiated epidermis. Gene regulatory network analysis identified 570 domains of regulatory chromatin (DORCs), 141 of which were more accessible in irradiated skin and converged on NF-κB-, AP-1-, and EMT-associated transcription factors. SCENIC+ further identified 39 eRegulons that were upregulated at both the transcriptomic and chromatin accessibility levels, highlighting a coordinated NF-κB–AP-1–EMT regulatory axis. CellChat analysis revealed enhanced collagen-mediated signaling between keratinocytes and fibroblasts, suggesting a self-reinforcing fibrogenic loop. By integrating transcriptomic, chromatin accessibility, and histone modification profiles at single-cell resolution, our study demonstrates that radiotherapy establishes durable epigenetic memory in keratinocytes driven by NF-κB, AP-1, and EMT programs. These findings identify potential regulatory targets for mitigating radiation-induced skin complications.

Keywords

Single-cell multiomics, Nano-CUT&Tag, Radiation-induced skin injury

P 13

High-dimensional immunophenotyping reveals changes associated with adverse events and response to anti-PD1 therapy in lung cancer patients

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Abstract

Non-small cell lung cancer (NSCLC) is one of the leading causes of cancer-related death. Late diagnosis and tumor microenvironment heterogeneity are among the causes of therapy failure and disease progression, ultimately contributing to the observed high mortality. Although commonly used to measure response to immunotherapy and predict survival, evaluation of T cell expansion and activation may not be enough to predict clinical responses to immunotherapy. In this study, immunotherapy responses (anti-PD1) were evaluated in longitudinal peripheral blood samples from NSCLC patients. High-dimensional full spectrum flow cytometry panels and protein interactome analysis revealed several changes in immune cell frequencies. For instance, poor responders have elevated CD8 T cells at baseline, while good responders show a higher frequency of non-classical monocytes, TEMRA CD4 T cells, and NK cells subpopulations. Additionally, the activation and frequency of myeloid-derived suppressor cells influenced disease progression and treatment-related toxicities. Overall, our findings support the need for a holistic approach in predictive biomarker discovery, considering detailed analysis of immune populations and their crosstalk abilities.

Keywords

Lung cancer, Natural killer cells, Immunophenotyping

P 14

Unravelling the role of microglia in macrophage recruitment in DMG

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Abstract

Diffuse midline gliomas (DMG) are highly aggresive pediatric high-grade gliomas (pHGG) frequently driven by the H3K27M mutation. Accumulating evidence indicates that DMG tumors present a significant infiltration of tissue-resident microglia as well as peripherally-recruited bone marrow-derived macrophages (BMDM). Analysis of publicly available transcriptomic datasets (Kids First, TCGA and GTEx) revealed the expression of BMDM-associated markers (ITGA4, CD44, SIGLEC1) together with microglial genes linked to myeloid cell recruitment, suggesting an active role for microglia in shaping the immune landscape of DMG. Building on our previous findings showing a reactive, pro-tumoral microglial phenotype in H3K27M-mutant tumors, we aim to investigate their contribution to BMDM recruitment. To this end, we will combine *in vivo* characterization of immune infiltrates in murine DIPG models with *in vitro* co-culture systems involving BV2 microglia, H3K27M-mutant DMG cells and H3-wt pHGG cells, coupled with macrophage migration assays. Ongoing experiments suggest enhanced macrophage migration towards DMG cells combined with BV2 microglia. Our findings will contribute to a better understanding of myeloid cell dynamics within the DMG microenvironment and may uncover novel therapeutic opportunities targeting tumor-supportive immune interactions.

Keywords

Diffuse midline glioma, Microglia, Tumor microenvironment

P 15

Combined EZH2 inhibition and irradiation enhances T cell-mediated killing in Soft tissue Sarcoma

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Abstract

Soft tissue sarcomas (STS) are rare and heterogeneous malignancies with limited treatment options, particularly in advanced or metastatic disease. Radiotherapy (RT) is widely used in STS management, yet clinical responses are limited, highlighting the need for predictive biomarkers and radiosensitizing drugs. Here, we identified that Enhancer of Zeste Homolog 2 (EZH2), an epigenetic regulator implicated in tumor progression, is associated with lower survival among patients receiving RT and with reduced immune-related pathway activity, suggesting that epigenetic repression contributes to immune-mediated treatment resistance. Using patient-derived *ex vivo* sarcoma spheroids and autologous tumor-infiltrating lymphocytes (TILs), we show that pharmacologic EZH2 inhibition enhances T cell-mediated tumor killing and potentiates the effects of low-dose RT. Immune profiling revealed increased CD8+ T cell infiltration and enrichment of tissue-resident memory-associated phenotypes with combinatorial treatment. Mechanistically, EZH2 inhibition in tumor cells upregulated the adhesion molecule ICAM-1. Collectively, these findings identify EZH2 as a regulator of radioresistance and suggest that epigenetic modulation can enhance RT responses by strengthening tumor-immune engagement in STS.

Keywords

Soft tissue sarcoma, Epigenetics, T cells

P 16

Peripheral lysosome levels dictate mTORC1 inactivation even when catabolically impaired

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Abstract

The mechanistic target of rapamycin complex 1 (mTORC1) is a central driver of cell growth that is frequently hyperactivated in cancer. While mTORC1 is activated at the lysosomal surface in response to growth factors and amino acids, the processes governing its inactivation remain incompletely understood.

Here, we report that sustained mTORC1 suppression during leucine or arginine starvation requires the translocation of peripheral lysosomes to the perinuclear region. Our data suggest that a pool of mTORC1 remains active at peripheral lysosomes during starvation and that increased spatial separation between lysosomes and the plasma membrane attenuates PI3K/Akt signaling, thereby reducing inputs that maintain mTORC1 activity.

Preventing lysosome translocation sustains mTORC1 signaling during prolonged starvation in a PI3K/Akt-dependent manner independently of autophagy. Notably, mTORC1 activity persists even when lysosomal catabolism is impaired, indicating that peripheral lysosomes can support nutrient signaling independently of their degradative function.

Together, these findings identify peripheral lysosome levels as a critical determinant of mTORC1 inactivation during nutrient stress and reveal a spatial mechanism that may contribute to aberrant mTORC1 signaling in cancer.

Keywords

lysosome positioning, mTORC1, amino acid starvation

P 17

Adipocyte-induced metabolic and molecular remodeling in colorectal cancer cells: an in vitro co-culture approach

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Abstract

Introduction

Obesity is a major modifiable risk factor for colorectal cancer and is associated with adipose tissue dysfunction, chronic low-grade inflammation and altered lipid metabolism. In this context, adipocytes may act as active components of the tumor microenvironment by releasing soluble factors, lipids and metabolites capable of modulating tumor cell behavior. However, the mechanisms by which mature and hypertrophic adipocytes affect colorectal cancer cell metabolism remain incompletely understood. This study aimed to characterize the metabolic and molecular effects of adipocytes on colorectal cancer cells using an *in vitro* co-culture model.

Materials and Methods

An *in vitro* co-culture model was optimized using human SGBS preadipocytes differentiated into mature or hypertrophic adipocytes and HCT-116 colorectal cancer cells. Tumor cell lysates, and culture supernatants were collected for untargeted LC–MS metabolomic analysis. Data were processed using MetaboAnalyst 6.0, RStudio and a customized Python pipeline. Multivariate analyses, including PCA and PLS-DA, together with univariate statistical analysis, were used to identify differential metabolites and altered metabolic pathways. Selected molecular markers related to lipid metabolism, tumor-associated signaling and extracellular matrix remodeling were evaluated by western blot.

Results / Discussion

PCA analysis showed a clear separation between control HCT-116 cells and HCT-116 cells co-cultured with mature or hypertrophic adipocytes, especially in tumor cell lysates and culture supernatants. Differential metabolite analysis of tumor cell lysates revealed extensive metabolic remodeling in both co-culture conditions compared with HCT-116 control cells, with approximately 90–95 significantly altered metabolites. The direct comparison between hypertrophic and mature adipocyte co-culture conditions showed fewer differences, suggesting that the main metabolic shift was driven by the presence of adipocytes. The altered metabolites were mainly associated with glycolysis, TCA cycle-related intermediates, glutaminolysis, redox metabolism and acyl-glycine conjugates.

Culture supernatants also showed marked differences between control and co-culture conditions, supporting the existence of metabolic communication between adipocytes and tumor cells. In parallel, western blot analysis showed increased expression of proteins related to lipid metabolism, including CPT1A and FASN, activation of AKT/mTOR signaling, and modulation of extracellular matrix-associated markers such as fibronectin and integrin α5.

Keywords

Metabolomics , Tumor microenvironment , Adipocytes

P 18

Tumour Infiltrating Lymphocytes and the Long-term Risk of Distant Metastasis in ER-positive Premenopausal Breast Cancer Patients: Insights From the STO-5 trials with 20 years Follow-up

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Abstract

Introduction: Oestrogen receptor (ER)-positive breast cancer is associated with a persistent long-term risk of distant metastasis that can extend up to at least 25 years after diagnosis. Young premenopausal patients, in particular, exhibit a distinct tumour biological profile and have an elevated long-term risk of developing distant metastasis compared to older postmenopausal patients, highlighting the importance to study these patients further. While tumour-infiltrating lymphocytes (TILs), their underlying biology, and spatial distribution have been extensively studied in triple-negative and HER2-positive breast cancer, this is not true for ER-positive breast cancer. The biological and clinical relevance of TILs for ER-positive breast cancer is not well understood and little is known about the influence on long-term metastatic risk and endocrine treatment benefit as well as potential differences in premenopausal breast cancer. Improved understanding of TIL-associated tumour microenvironment may be important to further understand risk of late metastasis and enable treatment stratification for this patient group.

Methods: Using the Stockholm Tamoxifen premenopausal trial (STO-5) including 576 ER-positive premenopausal breast cancer patients with newly annotated clinical markers, scanned H&E whole tumour images, and gene expression data. The STO-5 trial included both high- and low-risk patients randomised to receive either endocrine therapy or no endocrine therapy and have now reached at least 20 years follow-up. A deep learning–based image analysis approach was implemented, integrated with pathologist annotations, to identify TILs in accordance with the clinical guidelines for TILs assessment. Levels of TILs and interacting TILs (defined as TILs having cytoplasmic physical contact) were assessed to evaluate their long-term prognostic value (risk of distant metastasis) and potential impact on endocrine treatment benefit.

Conventional survival analyses with univariate Kaplan–Meier analysis and multivariable Cox proportional hazards regression, as well as multivariable time-varying analysis using flexible parametric analysis will be performed. Analyses will be done in all ER-positive patients, as well as subgroup analyses by tumour aggressiveness and subtype. Immune related gene expression markers (obtained from microarray gene expression data from the primary breast cancer tumours) will be coupled with the TILs level data to evaluate the prognostic value in addition to the TIL levels.

Results: We have finalized running the deep learning model and recently obtained the data identifying TILs within the pathologist-annotated regions. Currently, extraction of the resulting spatial and quantitative data is ongoing and will be analysed and finalized before the CRKI retreat.

Keywords

ER-positvie breast cancer, Long-term metastatic risk, Tumour infiltrating lymphocytes

P 19

CRISPR Recovery Screens to Expose Drug-Withdrawal Vulnerabilities

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Abstract

Drug resistance remains a major obstacle in cancer treatment. Cancer cells can evolve drug resistance through various mechanisms, such as mutations in drug targets, bypass of signaling pathways, and epigenetic reprogramming. This is particularly evident in BRAF V600-mutant melanoma, where initial responses to BRAF/MEK inhibitors are dramatic but short-lived. To overcome this, traditional combinatorial therapies seek to improve and prolong responses by blocking the coping mechanisms cancer cells activate during therapy.

Here, we propose an alternative approach of exploiting transient vulnerabilities that emerge while cancer cells recover from drug withdrawal. The aim is to develop and use a novel CRISPR "recovery" screen platform that captures gene dependencies specifically during the drug withdrawal recovery phase. We propose that BRAF/MEKi drug removal re-engages oncogenic MAPK signaling, creating a distinct recovery phase characterized by metabolic demand, translational stress, and synchronized cell cycle re-entry. These transient dependencies might represent therapeutic opportunities invisible under continuous dosing and could be exploited through time-staggered, pulse-based drug combinations. Pulse-based dosing may also reduce the selective pressure driving resistance, while lowering total drug exposure and side effects.

To complement our own screening efforts, we have developed a cancer cell CRISPR screen analysis tool (https://correlate.cmm.se, based on data from DepMap and other resources) and our preliminary analyses across 55 human melanoma cell lines support this concept, revealing that BRAF-mutant melanomas exhibit distinct vulnerabilities compared to BRAF wild-type lines, including increased dependence on the MAPK-buffering phosphatase DUSP4 and on nucleotide biosynthesis genes such as GMPS (1). These dependencies may be particularly relevant during recovery, when rapid MAPK reactivation demands tight signaling control and increased biosynthetic capacity.

Candidate hits from the recovery screens will be validated across multiple cell lines and patient-derived metastatic melanoma material. If successful, this platform is extendable to other oncogene-driven cancers, providing a generalized approach to identify pulse-based combination strategies that extend the durability of targeted therapies.

1. Soham Deolankar, and Fredrik Wermeling. Correlate: A Web Application for Analyzing Gene Sets and Exploring Gene Dependencies Using CRISPR Screen Data. BioRxiv, 2026, https://doi.org/10.64898/2026.04.02.716070 (preprint)

Keywords

Cancer drug resistance, CRISPR screen, Targeted drug therapy

P 20

Input Resolution Drives Performance in Deep Learning-Based Mammographic Prediction of Breast Cancer Clinical Subtypes

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Abstract

Purpose: Breast cancer subtype may vary due to spatial and temporal tumor heterogeneity, potentially necessitating renewed needle biopsy. Our aim was to optimize an interpretable deep learning framework for clinical subtype prediction (Luminal vs. Non-Luminal) applicable to sequential mammography images ('virtual biopsy'), by systematically evaluating network architecture and input resolution. **Methods:** This retrospective study included 9,057 breast cancer patients (22,256 mammograms) diagnosed between 2007 and 2021, with clinical subtypes assigned from the Swedish National Breast Cancer Register. Data were split at patient level into a development set (training: 5,323 patients; validation: 1,315 patients) and an independent held-out test set (2,419 patients) that remained unused during model development and hyperparameter selection. We benchmarked ResNet18, ResNet50, and EfficientNetV2-Small using mammograms resized to 224×224, 384×384, and 512×512 pixels. Models were trained on single-view inputs and evaluated at study level by fusing CC and MLO predictions via max selection. Performance was measured using Area Under the Curve (AUC) and Matthews Correlation Coefficient (MCC), with 95% confidence intervals estimated via stratified study-level bootstrapping (1000 iterations). Model behavior was examined using Integrated Gradients attribution maps. **Results:**The optimal configuration was ResNet50 at 512×512 achieved MCC of 0.258 (95% CI: 0.216–0.295) and the highest AUC of 0.726 (95% CI: 0.700–0.752). Across all architectures, increasing input resolution from 224×224 to 512×512 consistently improved discrimination. Attribution maps confirmed focus on clinically relevant tumor regions.**Conclusion:** In this study we demonstrate that increasing input image size significantly improve mammography-based clinical subtype prediction. Interpretability analyses support the presence of resolution-dependent breast cancer tumor phenotypes.

Keywords

AI, Breast Cancer, Clinical Subtypes

P 21

Allosteric activators control SAMHD1 hydrolase activity towards nucleoside analogue metabolites

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Abstract

Objectives: Our lab recently identified the deoxynucleoside triphosphohydrolase (dNTPase) SAMHD1 as a resistance factor to Cytarabine therapy in adult and childhood leukaemia [1]. SAMHD1 confers resistance by hydrolysing the active metabolite of Cytarabine, ara-CTP. Cells can be re-sensitised to ara-C by inhibiting ribonucleotide reductase (RNR) [2], which is currently being evaluated clinically in adult AML [3]. However, a complete understanding of the molecular mechanism of this sensitisation is still lacking. SAMHD1 is allosterically activated by (deoxy)nucleotides, which triggers the formation of the enzymatically active homotetramer. We hypothesise that the changes in dNTP pool composition that are observed upon RNR inhibition indirectly influence SAMHD1 activity by affecting its allosteric activation. We therefore aim to assess the effect of different dNTP activators on SAMHD1's ability to hydrolyse nucleoside analogue metabolites.

Methods: We assessed the effect of different allosteric activators on SAMHD1 tetramer stability using chemical crosslinking, mass photometry quantification and functional enzyme-coupled activity readouts. Enzyme activity assays were also used to compare the effect of allosteric activators on the hydrolysis of nucleotide analogues. Finally, the response of SAMHD1 wild-type and knockout cell lines to a combination of RNR inhibitors and nucleoside analogue drugs was determined with cell viability synergy experiments.

Results: Tetramer stability measurements show reduced tetramer lifetime when SAMHD1 is allosterically activated with pyrimidine vs purine dNTPs. Enzyme-coupled activity measurements show that, contrary to other endogenous dNTP activators, dCTP-activated SAMHD1 does not hydrolyse the triphosphate metabolites of arabinose-based nucleoside analogues, such as Cytarabine, Fludarabine or Nelarabine while ribose-based nucleoside analogue metabolites are not affected.

Conclusions: Upon co-treatment with RNR inhibitors, chemotherapy-resistant cell- and animal models can be resensitised to Cytarabine. We hypothesise that this is due to changes in intracellular dNTP compositions, specifically the increased pyrimidine-to-purine ratio. We show that stability of the enzymatically active SAMHD1 tetramer is decreased when pyrimidine dNTPs are bound to its allosteric sites when compared to purines. In addition, hydrolysis of arabinose-based nucleotide analogues is impaired when SAMHD1 is activated with the pyrimidine deoxynucleotide dCTP. Together, this study improves our understanding of the molecular mechanism behind the sensitisation of cells to Cytarabine therapy through RNR inhibition, a strategy already being evaluated in the clinic [3], as well as allowing us to make predictions about other possible treatment combinations.

1. Herold et al. (2017), Nature Medicine. DOI: 10.1038/nm.4265
2. Rudd et al. (2020), EMBO Mol Med. DOI:10.15252/emmm.201910419
3. Jädersten et al. (2022), J Intern Med. DOI: 10.1111/joim.13553

Keywords

SAMHD1, nucleoside analogues, chemotherapy resistance

P 22

Implementation of Enhanced Recovery After Surgery (ERAS) in Renal and Testicular Cancer Surgery

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Abstract

Background
Enhanced Recovery After Surgery (ERAS), originally developed within colorectal surgery, is now an established perioperative care concept across multiple surgical specialties. Within urology, well-developed ERAS protocols exist for cystectomy; however, corresponding structured programs for renal and testicular cancer surgery remain limited.

Objective
To evaluate changes in length of stay (LOS) following implementation of an ERAS protocol in urological oncological surgery.

Methods
The ERAS protocol was based on current colorectal surgery guidelines and adapted to urological practice. The program was implemented for open and laparoscopic partial and total nephrectomies, retroperitoneal lymph node dissection for testicular cancer (open and laparoscopic), as well as laparoscopic pyeloplasty. A total of 164 patients managed according to the ERAS protocol (October 2025–March 2026) were compared with 50 consecutive patients operated on prior to implementation (December 2024–February 2025).

Results
Median LOS decreased from 4.9 to 3.3 days. The rate of postoperative infections was reduced from 18% to 5%. Postoperative pain scores decreased from NRS 7 to 4.

Discussion
The observed effect may be underestimated, as several ERAS components were already applied preoperatively and initial protocol compliance was moderate (60.7%).

Conclusion
Implementation of an ERAS-based perioperative care pathway in renal and testicular cancer surgery was associated with shorter hospital stay, fewer postoperative infections, and lower postoperative pain levels, with further potential for improvement through increased protocol adherence.

Keywords

ERAS, Renal cancer, Testicular cancer

P 23

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

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Abstract

Neuroblastoma (NB) is a common pediatric extracranial solid tumor originating from the sympathetic nervous system. Due to its great genetic heterogeneity and acquisition of resistance mechanisms in relapsed and refractory disease, it remains a major therapeutic challenge. Current multimodal treatment strategies often require high drug doses, resulting in long-term toxicity. DNA-based drug carriers offer a promising alternative, enabling tumor-specific delivery with improved bioavailability and reduced off-target effects. We present a method for generating, screening, and selecting DNA nanostructures with enhanced cellular uptake in NB, supporting the development of tumor-specific drug delivery systems.

Combinatorial libraries of DNA nanostructures were assembled from structural fragments—linear segments, bulged motifs, junctions, and hairpins—via random ligation, generating diverse 3D structures, amplified by PCR and characterized by Nanopore sequencing. Incorporation of the GD2-specific DB67 aptamer in a subset of hairpins introduced specificity against the membrane-bound disialoganglioside GD2, overexpressed in NB cells. The inclusion of conjugation loop hairpins allows future functionalization with cell-penetrating peptides or GD2-specific antibodies.

Selection was performed in GD2+ IMR-32 NB cells across 3 conditions: whole lysate, DNase I-treated lysate for removal of membrane-bound structures, and isolated nuclei. Recovered structures underwent AMPure purification, re-amplification, and refolding before reapplication to IMR-32 cells. This was repeated for 8 rounds, facilitating iterative enrichment of structures with advantageous internalization characteristics. Samples from all rounds and conditions were subsequently analyzed by Nanopore sequencing.

Sequencing analysis revealed clear structural preferences. GD2 aptamer-containing structures were favored over conjugation-loop-only structures, consistent with the aptamer aiding specificity and uptake, while unconjugated loops add no positive selection pressure. Larger, more complex structures were disfavored, with a shift toward shorter 16–18 nucleotide linear fragments and away from 3- or 4-branched fragments.

This method offers a faster, high-throughput approach for the discovery and selection of DNA nanostructures with optimized cellular uptake in NB. Further work will focus on enhancing cellular uptake via cell-penetrating peptides, as well as isolating and evaluating structural candidates for potential delivery of therapeutic cargo.

Keywords

DNA Nanotechnology, Neuroblastoma, Drug Delivery

P 24

Persister states and relapse in childhood leukemia

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Abstract

In pediatric B-ALL, relapse is the main cause of treatment failure. However, the mechanisms through which B-ALL cells evade treatment remain largely unexplained and it is not known to what extent differences in treatment response between cells are genetically encoded.

Here, we tracked clonal evolution in 30 children with ALL from diagnosis through treatment, relapse and chemo-refractory states. We profiled >30,000 individual cancer cells using joint single-cell DNA/RNA sequencing (DNTR-seq), which simultaneously captures both genomic clone identity and gene expression in each cell. This allowed us to detail the phenotypic variation within and between closely related leukemic clones as cells survived the pressure of standardized chemotherapy.

We found that treatment persistence and genetic evolution operate through distinct, temporally separated mechanisms. We observed minimal clonal selection during intensive induction chemotherapy — despite 100-1000 fold reduction in leukemic burden. Instead, surviving cells adopt a predictable cell state characterized by maturation markers, independent of any pre-existing genetic features or stem-like properties. In contrast, relapsed disease consistently showed evidence of clonal evolution with new genetic alterations. This suggests that selection for resistant clones is more likely to happen during or after low-intensity treatment, and not during the initial high-intensity phase.

Keywords

B-cell precursor acute lymphoblastic leukemia, Treatment persistance, Single-cell multiomics

P 25

Harness the innate immune system for improved cancer immunotherapy

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Abstract

Background and objective: Natural killer (NK) cells and CD8$^+$ T cells are the principal cytotoxic effectors of antitumor immunity. NK cells have been shown to support CD8$^+$ T cells by enhancing their activation, proliferation, and recruitment. Moreover, NK cells can compensate when CD8$^+$ T cells fail to eliminate tumors cells that lack MHC class I expression. Despite this, only a limited number of studies have explored whether CD8$^+$ T cells can modulate NK cell function. In this study, we investigate the impact of CD8$^+$ T cells on NK cell activation.

Methods: Using peripheral blood mononuclear cells (PBMC) from healthy donors, we assessed the effects of CD8$^+$ T cells on NK cell function.

Results: Our findings demonstrate that activated CD8$^+$ T cells can modulate NK cells activation, proliferation, and metabolic activity. Notably, NK cell activation positively correlated with the activation status of CD8$^+$ T cells. Furthermore, CD8$^+$ T cell-mediated NK cell activation was maintained under conditions that mimic the tumor microenvironment, including hypoxia and glucose deprivation.

Keywords

Cancer, Immunotherapy, NK cells

P 26

Deciphering the Molecular Mechanism of Action of Nelarabine for Improved Clinical Efficacy in the Treatment of T-cell lymphoblastic leukemia

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Abstract

Nelarabine, a deoxyguanosine analogue, is an antimetabolic drug found to selectively target T-cell lymphoblastic malignancies (e.g., T-ALL), which has led to its wider adoption in clinical protocols for refractory T-ALL. Recent data from the Rudd and Herold groups have identified the dNTP triphosphohydrolase SAMHD1 as a novel vulnerability dictating cellular sensitivity to nucleoside analogues such as nelarabine. SAMHD1 activity is more highly correlated to responses to nelarabine relative to other nucleoside analogs. However, this does not fully explain the variation in patient responses nor account for the cellular phenotypes observed upon treatment *in vitro*.

We have integrated transcriptomics, proteomics and CRISPR knockout screening from patient biobank material as well as cell line models, which has allowed us to identify novel regulators of nelarabine sensitivity. Our preliminary functional validations have led to a comprehensive time-resolved picture of the effects of nelarabine on intercompartmental cellular redox flux and the consequential disruption of proteostasis.

These latest findings provide a novel perspective on the mechanism of action of nelarabine and will serve as a starting point for better stratification of responders and predictive molecular biomarkers, as well as constitute a blueprint for studying other clinically relevant drugs of this class.

Keywords

T-cell lymphoblastic leukemia, nucleotide metabolism, drug mechanism of action

P 27

Hepatocyte plasticity at the tumor-liver interface shapes metastatic invasion

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Abstract

Colorectal cancer liver metastases (CRLM) are associated with poor prognosis and frequent relapse after resection, and the hepatic microenvironment is increasingly recognized as a key determinant of metastatic behavior and clinical outcome. Histopathological growth patterns (HGPs) at the metastasis margin can be distinguished into two major classes — encapsulated and replacement-type — which reflect distinct modes of tumor-host interaction and carry prognostic significance.

In areas of replacement-type HGPs, metastases expand by direct contact between tumor cells and hepatocytes. Work by our group has shown that this triggers a **multi-stage regenerative injury response** comprising JAK/STAT3-driven acute-phase activation and JAG1/NOTCH2/SOX9-mediated hepatocyte dedifferentiation. In contrast, in areas of encapsulation the tumor is surrounded by a zonated stroma separating tumor cells from the liver parenchyma. Large fractions of metastases encapsulation confer improved survival outcomes in patients with CRLM and, similarily, in patients with pancreatic cancer liver metastases (PCLM).

Using intrahepatic-injection based mouse models, we could show that pharmacological inhibition of JAK/STAT signaling suppresses acute-phase responses and hepatocyte dedifferentiation in vivo. Additionally, genetic modification of *Jagged1*in tumor cells interfers with hepatocyte dedifferentiation and induces metastasis encapsulation in vivo. To study the impact of cellular regeneration in the metastasis-surrounding hepatic microenvironment, we investigated further how portal vein embolization (PVE) — a preoperative strategy used to induce hypertrophy of the future liver remnant before multilobal CRLM resection — modulates CRLM invasion. Extensive HGP scoring revealed that metastases growing in embolized, atrophic liver lobes showed increased replacement-type growth. In contrast, a hypertrophic environment was associated with a tendency towards metastasis encapsulation.

In summary, our data demonstrate that **hepatocyte plasticity in the metastasis-surrounding liver shapes the mode of invasion** in CRLM and PCLM. Using well defined clinical cohorts in combination

with functional assessment in mouse models we identify **tumor–hepatocyte interactions as potential modulators of metastasis aggressiveness**.

Keywords

liver metastases, tumor microenvironment, pancreatic and colorectal cancer

P 28

Ascites viscosity promotes peritoneal dissemination in ovarian cancer

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Abstract

Physical stimuli such as stiffness, fluid shear stress, and hydrostatic pressure regulate tumour behaviour. While viscous fluids drive cell migration and metastasis in vitro and in animal models, the role of viscosity in human cancer-relevant fluids remains unexplored. Ovarian high-grade serous carcinoma (HGSC) typically spreads in the peritoneal cavity through the ascites fluid and invades adipose-rich peritoneal tissues. Here, we show how the viscosity of malignant ascites fluid is associated with poor survival in HGSC patients. Through synthetic biomimetic platforms that recapitulate the ascites microenvironment and peritoneal tissues, we reveal that increased viscosity enhances both tissue adhesion and invasive capacity. At the molecular level, viscosity cooperates with biochemical cues, including extracellular matrix ligands and TGF-β, to potentiate invasion via the mechanically activated calcium channel TRPV4. Validation using patient-derived ascites and peritoneal tissue explants confirms the clinical relevance of these findings. Collectively, our work identifies ascites viscosity as a previously unrecognised biophysical determinant of tumour aggressiveness.

Keywords

Ovarian Cancer, Mechanobiology, Tumour Microenvironemnt

P 29

Evaluation of responses to immune-checkpoint inhibitor treatment in non-small cell lung cancer patients by analyzing extracellular vesicle protein cargo

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Abstract

Background: Lung cancer patients have poor survival with a 5-year survival of only 15% when diagnosed at late stage. A substantial proportion of non-oncogenic driven non-small cell lung cancer (NSCLC) patients are given immune checkpoint inhibitor (ICI) treatment, yet just a fraction of the patients has clinical benefit illustrating a need for biomarkers to predict response. The location of tumor(s), their heterogenous oncogenic drivers and the frequently observed metastatic spread already at diagnosis sometimes makes molecular analyses of tissue biopsies challenging. One way is to analyze tumor- and immune-cell derived extracellular vesicles (EVs) secreted into the patient´s blood. They contain nucleic acids, lipids and proteins from their cell of origin and express several immune checkpoint targets, including PD-L1. Here we explored the protein cargo of EVs from plasma of metastatic NSCLC patients at diagnosis and related them to patient-response to ICIs with the aim to reveal future treatment selecting biomarkers.

Material and Methods: EVs were isolated from 0.5 ml EDTA plasma of the NSCLC patients (n=31), prior ICI therapy with the PD1 inhibitors pembrolizumab or Cemiplimab, alone or in combination with chemotherapy, and also longitudinally. Size exclusion chromatography (Izon, 70nm columns) was used for isolation, and the EV particle concentration and size were examined using Nanoparticle Tracking Analysis (NTA). Lysed EVs were protein profiled using proximity extension assay (PEA), on the Immuno-Oncology® and Oncology II® panels. Data was analyzed and visualized by the Qlucore® Omics Explorer software and EV markers and proteins associated with ICI response validated using western blotting and ELISA.

Results: The median size of the particles was around 90 nm and they expressed the EV markers CD9, TSG101 and SYND1, confirming isolation of EVs. PEA protein profiling identified 66 out of 166 oncogenic and immune signaling proteins to be expressed over limit of detection (LOD) in 75% of the EV samples. PD-L1 was detected in EVs from around 60% of the plasma samples, while the immunosuppressive protein CD73/NT5E was expressed in all EV samples. Nine proteins correlated with PFS with a cutoff at 1 year. They do most likely origin from different cell types and need to be further evaluated. A set of proteins were found to have similar expression patterns in the EV sample cohort as PD-L1 and CD73/NT5E, these were among other CD244, CSF-1, PDGFB, SYND1. Their signaling interactions and the cellular origin of the EVs will be further analyzed.

Conclusion: We found that affinity-based protein profiling of EVs isolated from EDTA-plasma of metastatic NSCLC patients prior ICI treatment reveals a protein signature with a potential to predict

clinical benefit of ICI regimens. Validation of these protein markers in external cohorts is needed to set out their clinical usefulness.

Keywords

Extracellular vesicles, non-small cell lung cancer, biomarkers

P 30

Chemotherapy rewires neuroblastoma into a targetable NCAM1–FGFR1 state

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Abstract

Neuroblastoma (NB) is the most common extracranial solid tumour in infants and is characterised by substantial heterogeneity in tumour biology, treatment response and clinical outcome. Despite intensive multimodal therapy, approximately half of the patients with high-risk disease relapse due to therapy resistance and face poor survival, underscoring the need for new targeted treatment strategies. Longitudinal patient-derived xenograft (PDX) models were used to recapitulate clinical treatment trajectories and relapse-associated disease states, enabling dissection of chemotherapy-induced remodelling of neoplastic and microenvironmental cell states and their spatial organisation.

Single-cell transcriptomic profiling of NB patient samples and a chemoresistant PDX NB tumour model revealed chemotherapy-driven remodelling of neoplastic and microenvironmental cell states and identified NCAM1 and FGFR1 as candidate therapeutic targets linked to an acquired relapse-associated state driven by the tumor niche. Ligand–receptor inference analysis highlighted NCAM1–FGFR1 as a prominent autocrine and paracrine signalling axis, and spatial analyses demonstrated that NCAM1–FGFR1-positive cells assemble into treatment-induced microniches in tumours following chemotherapy. Furthermore, elevated NCAM1/FGFR1 co-expression in patient cohorts correlated with adverse clinical features and poor overall survival

Therapeutic targeting of the axis was assessed using the CD56 (NCAM1)-directed antibody–drug conjugate Adcitmer® and the FGFR1 inhibitor erdafitinib in NB cell lines and multicellular tumour spheroids. Pharmacological inhibition impaired tumour cell viability. Moreover, FGFR1 blockade affected multiple downstream pathways, including RAS-MAPK, PI3K-AKT, PLCγ and STAT signalling. Together, these findings identify the NCAM1-FGFR1 axis as a spatially organised, therapeutically actionable target in relapsed NB and provide a rationale for further preclinical and clinical evaluation of Adcitmer® and erdafitinib.

Keywords

Neuroblastoma, Microniche, Chemoresistant

P 31

Time-Resolved, Multi-Channel Live-Cell Imaging and AI-Driven Phenotypic Profiling of Drug Responses in Ovarian Cancer

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Abstract

Current preclinical drug discovery platforms often fail to capture the complex and evolving treatment responses observed in solid tumors, contributing to the high failure rate of oncological drug candidates. A key limitation of conventional drug screening approaches is their reliance on static, endpoint-based measurements, which provide only a snapshot of tumor behavior and fail to capture the dynamic cellular adaptations that drive therapeutic resistance and disease progression. This shortcoming is especially evident in ovarian cancer, a disease characterized by extensive intratumoral heterogeneity and the rapid emergence of treatment resistance, making it a particularly challenging context for predicting therapeutic response.

Here, we present a time-resolved, AI-enabled phenotypic profiling framework for the analysis of ovarian cancer drug responses. Developed within the TIMED (Time-resolved Imaging and Multi-channel Evaluation of Cellular Dynamics) consortium, the platform will combine high-throughput multiplexed live-cell imaging with advanced AI and machine learning–based image analysis to quantify dynamic cellular state transitions in response to drug perturbations.

As the first component of this platform, we are developing a washout-based morphological profiling assay. Built on a high-throughput imaging platform, the workflow integrates automated drug handling and image acquisition, supporting temporal imaging at intervals as short as one hour. Multiplexed staining of distinct subcellular compartments generates rich morphological information, enabling detailed profiling of cellular state transitions over time. By capturing phenotypic changes following treatment, the assay facilitates longitudinal monitoring of the emergence and evolution of resistant cell states, providing deeper insight into mechanisms of therapeutic adaptation and resistance. The workflow is currently being optimized in ovarian cancer cell lines and will ultimately be applied to primary patient-derived samples.

This work will support the efforts of the TIMED consortium to establish a drug screening platform that more accurately captures the dynamic and temporal effects of treatment, enabling more informed therapeutic development. Although initially developed in ovarian cancer, the framework is readily transferable to other cancer types and disease contexts where dynamic cellular adaptation plays a critical role in treatment response.

Keywords

Phenotypic profiling, Ovarian cancer, High-content imaging

P 32

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

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Abstract

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. It is demonstrated that intestinal-specific estrogen receptor beta (ERβ) knock-out (ERβKO^{vil}) in colitis-induced colon tumor mouse model (AOM/DSS) leads to increased tumor numbers in males and increased tumor size in females. This indicates of ERβ protective role against colon tumor development in mice of both sexes, through 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 not 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 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.

Keywords

Colorectal cancer, Estrogen, 3D culture

P 33

Mapping the target landscape of polyamines in colorectal cancer with versatile proteomics tools

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Abstract

Colorectal cancer (CRC) remains a leading cause of cancer-related mortality, with metastatic progression significantly worsening prognosis. A hallmark of CRC is a pathological dependence on polyamines—essential metabolites that fuel cancer cell proliferation and migration. While the Vogelstein drivers (APC and KRAS) are known to upregulate polyamine synthesis, the specific protein targets through which polyamines potentially exert their tumorigenic effects have remained elusive.

In this study, we utilized an isogenic model of CRC progression (SW480 primary and SW620 metastatic lines) to map the polyamine-protein interactome. We initially employed the Proteome Integral Stability Alteration (PISA) assay to identify putative protein targets engaged by polyamines. These candidates were prioritized based on their differential expression in clinical CRC cohorts and their correlation with poor patient survival.

To map the polyamine binding regions at the proteome-wide scale, we utilized the peptide-centric local stability (PELSA) assay as an orthogonal approach. PELSA allowed us to move beyond global protein stability and confirm specific peptide-level binding sites, providing structural evidence of polyamine-target interactions. Additionally, predictions from AlphaFold were used to support and contextualize the identified binding sites, strengthening confidence in their structural plausibility. We have recombinantly expressed several metabolic and non-metabolic targets to validate the observed interactions, facilitating our follow-up functional experiments.

Our findings reveal that polyamines engage several oncogenic proteins modulating their stability and supporting cancer cell proliferation. By integrating PISA and PELSA, we have potentially identified and validated novel druggable nodes within the polyamine pathway. We believe this work will set a roadmap for the development of next-generation therapeutics aimed at disrupting the oncometabolic dependencies of CRC.

Keywords

Colorectal cancer, Polyamine, Metabolism

P 34

Fighting Cancer with a 1-2-Punch: Dual-Targeting Strategies to Improve Clinical Outcome of Lung and Breast Cancer

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Abstract

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 the candidate compounds in combination with other therapeutics across multiple cancer cell lines, in particular 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.

Keywords

Cancer therapy resistance, Senolytic drug discovery, One-two-punch strategy

P 35

Decoding Immune-Tumor Interactions: Cell-Surface Mapping in Merkel Cell Carcinoma under Immune Checkpoint Inhibition

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Abstract

Immune-checkpoint inhibition (ICI) has revolutionized the treatment of many cancers, including Merkel cell carcinoma (MCC), a highly aggressive skin cancer. Disruption of the PD-1/PD-L1 immunosuppression axis results in durable responses in approximately half of patients, whereas the other half exhibit primary or acquired resistance – despite the cancer's immunogenicity and frequent immune cell infiltration. However, the mechanisms responsible for treatment resistance remain incompletely understood. Since immune-tumor interactions occur through cell surfaces, we employed Molecular Pixelation to characterize the cell-surface phenotypes of immune cells in the tumor microenvironment (TME) of patients with varying responses to ICI. This technology enables the mapping of the abundance, distribution, and colocalization of 76 immune-related proteins on the surface of single cells. Using this method, we identified the predominant cell types within dissociated tumor samples, while assessing the potential impact of enzymatic dissociation. Within cell types, cell-surface markers formed distinct spatial patterns that suggest different functional states. Through our investigation of inter-patient tumor-cell heterogeneity, we found significant differences in the expression level and spatial distribution of CD44 that may impact signaling and patient survival. In an on-treatment sample series from an ICI-responding patient, we demonstrated the reactivation of a subset of CD38+ HLA-DR+ terminally exhausted tissue-resident cytotoxic T cells. We also characterized the changes in other cell types, thus providing insights into the impact of ICI on the TME. By providing a detailed analysis of cell-surface signatures that shape tumor-immune interactions, we elucidate a novel layer of biological complexity with mechanistic implications. Additionally, we identified several potential therapeutic targets on tumor cells that may eventually expand the treatment options for MCC patients.

Keywords

Immune checkpoint inhibition, Proteomics, Single-cell technologies

P 36

Protocol to culture, recover and dissociate patient-derived 3D ovarian cancer tumor microspheroids for single-cell analysis

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Abstract

Cellular heterogeneity is known to shape disease progression and treatment response in cancer, yet preserving the native microenvironment and cellular composition for ex vivo studies remains challenging. We present a scalable, flexible, and cost-efficient protocol for generating hundreds to thousands of 3D microspheroids from patient-derived tumor cells, including recovery and dissociation into single-cell suspensions for molecular analysis. The system employs recyclable molds and common reagents, allowing straightforward integration into existing workflows. Using patient-derived ovarian tumor cells, we show that the method yields spheroids with patient-specific morphology while largely maintaining the cellular composition of the original material, including rare populations. The dissociation protocol preserves single-cell integrity regarding viability and surface marker expression. Furthermore, we demonstrate applicability for assessing compound mechanisms of action at single-cell resolution. While validated with ovarian tumor cells, the protocol can be adapted to other tumor entities or tissues, providing a broadly useful platform for functional precision medicine.

Keywords

3D microspheroids, Patient-derived ex vivo models , Single-cell functional analysis

P 37

BET inhibition induces differentiation and metabolic rewiring in medulloblastoma cells

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Abstract

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Medulloblastoma (MB) is the most common malignant brain tumor in children and comprises four major subgroups: wingless (WNT), sonic hedgehog (SHH), Group 3, and Group 4 with Group 3 showing the worst prognosis. Despite multimodal therapy, most patients suffer severe side effects, highlighting the need for novel therapies. Amplification of one of the *MYC* oncogenes correlate with high-risk and represent promising therapeutic targets. Bromodomain and extraterminal inhibitors (BETis) efficiently downregulate *MYC* expression via epigenetic modulation. Here we analyzed the therapeutic potential of BETis in non-*MYC*-amplified SHH and *MYC*-amplified Group 3 MB cell lines.

Our data shows that cells from both subgroups had reduced proliferation following BETi treatment. Notably, we observed both morphological and biochemical modifications of UW228-3 SHH cells upon BETi incubation characterized by neurite outgrowth and expression of the neuronal markers β3-tubulin, microtubule-associated protein 2 (MAP2), and neurogenic differentiation factor 1 (NeuroD1). In contrast, SHH DAOY cells did not differentiate but underwent senescence as shown by X-Gal staining and the characteristic fried egg-like cell morphology.

MYC-amplified Group 3 D458 cells showed metabolic rewiring upon BET inhibition as demonstrated by reduced glycolysis, while UW228-3 SHH cells remained unaffected. Using bulk RNA sequencing of D458 cells treated with the BETis we identified the gene encoding the thioredoxin-interacting protein (TXNIP), a regulator of glucose transporter (GLUT1-4) localization and glucose uptake, as the top upregulated hit. We confirmed the increase on TXNIP protein level. Given the reported suppression of TXNIP by MYC via *miR*-17, we investigated this axis and found that *miR*-17 inhibition indeed increased *TXNIP* mRNA levels, whereas BET inhibition reduced *miR*-17 levels. We next interrogated whether the glucose-responsive MAX-like protein (MLX)–MLX-interacting protein (MLXIP) could contribute to the metabolic rewiring. Proximity ligation assays demonstrated increased MLX–MLXIP interaction after BET inhibition, indicating enhanced transcriptional activation at the *TXNIP* promoter, in line with *TXNIP* being a known MLX/MLXIP target gene.

Together, our results indicate that BETis may offer subtype-specific therapeutic opportunities for children with MB. While these molecules enable differentiation or senescence-based therapy approaches for SHH MB cases, their potential benefit for Group 3 MB patients may rely on their ability to induce metabolic rewiring. We are currently conducting additional studies in cell-based and zebrafish models to further characterize this mechanism.

Keywords

Medulloblastoma, BET inhibition, Differentiation

P 38

Aberrant ribosome biogenesis offers an actionable vulnerability in acute myeloid leukemia

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Abstract

Acute Myeloid Leukemia (AML) remains one of the most challenging cancer types to treat, with limited effective therapies and poor survival outcomes. Ribosome biogenesis (RiBi) is a fundamental homeostatic process that plays a crucial role in the progression and treatment of cancer. Using RNA-Seq data, we identified a 31-gene RiBi signature with prognostic and predictive value in AML. Higher RiBi scores were associated with poorer survival outcomes, reduced differentiation, and enrichment of immature progenitor cells. By integrating RiBi score with chemical screen data, we mapped drug classes with therapeutic potential across various RiBi levels. We further showed that AML primary cells with elevated RiBi scores exhibited heightened sensitivity to Actinomycin D (ActD), a RiBi inhibitor that induced ribosomal stress and activated the tumor suppressor p53 through the Impaired Ribosome Biogenesis Checkpoint. Furthermore, RiBi inhibition relieved the differentiation block in AML, promoting the maturation of progenitor-like cells. ActD also enhanced the efficacy of the BCL-2 inhibitor Venetoclax, by altering the expression of pro/anti apoptotic genes, particularly in high RiBi AML cell models. These findings highlight the potential of targeting RiBi to induce both apoptosis and differentiation, offering a promising strategy to improve personalized treatment outcomes in AML.

Keywords

Stockholm, Stockholm, Stockholm

P 39

Activities of TXNL1 may affect mitochondrial function through reductive stress

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Abstract

The thioredoxin-fold protein TXNL1 (also named TRP32) supports thioredoxin reductase 1 (TrxR1, TXNRD1)-driven redox activities in disulfide reduction reactions, has chaperone activity, and furthermore interacts with the proteasome. It was observed that in wild-type (WT) HEK-293T cells specific chymotrypsin activity of the proteasome was higher than in CRISPR/Cas9-mediated knock-out TXNL1 cells (TXNL1 KO) suggesting that among other proteins degradation of NRF2 maybe modulated by the lack of TXNL-1.

Auranofin (AF), an FDA-approved anti-inflammatory drug, is a TrxR1 inhibitor that downregulates TXNL1 and disrupts mitochondrial redox homeostasis. AF was here found to increase NRF2 levels more in TXNL1 KO cells than in WT cells both cytosol and nucleus of the cells. Intriguingly, the absence of TXNL1 resulted decreased basal and AF, RSL3, cumene H2O2 induced NQO1 levels and vulnerability to lipid peroxidation. However, a crosstalk between TXNL1 and NRF2 may enhance the activity of pentose phosphate pathway (PPP) and mitochondrial function, either directly or indirectly, suggesting that TXNL1 can be a hitherto unknown regulator of mitochondrial redox status although it is a cytosolic protein.

Keywords

TXNL1, NRF2, NQO1

P 40

Effects of a 12-Week Live-Remote Exercise Intervention for Cancer Survivors: Findings from a Randomised Controlled Trial

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Abstract

Purpose

Exercise offers important benefits for cancer survivors; however, participation remains low due to barriers such as time constraints, distance, and limited availability of suitable services. Supervised live-remote delivery may overcome these barriers while retaining professional support. This trial evaluated the effects of a 12-week live-remote supervised exercise intervention on health-related quality of life (HRQoL) and other patient-reported and physiological outcomes in survivors of breast, prostate, and colorectal cancer.

Methods

Between January 2022 and December 2023, 200 participants (mean age = 58.5 ± 10.4 years; 62% women) were randomised to either a live-remote exercise intervention or usual care. Half had been treated for breast cancer, 23% for prostate, and 26% for colorectal cancer; 52% had received chemotherapy, and 41% were receiving endocrine therapy. The intervention comprised twice-weekly, 60-minute virtual group sessions (≤8 participants) led by upskilled personal trainers for 12 weeks. Outcomes were assessed at baseline, post-intervention (3 months), and 6 months. The primary outcome was overall HRQoL (EORTC QLQ-C30). Secondary outcomes included physical functioning, cardiorespiratory fitness (VO_2max), strength, and physical activity. Exploratory analyses examined moderators including sex, endocrine therapy, age, cancer type, and baseline health status.

Results

Median session attendance was 75%, with no serious adverse events. No between-group difference was found for overall HRQoL. However, compared with controls, the intervention group showed greater improvements at 3 months in self-reported physical functioning (ES = 0.31, $p \leq 0.001$), sit-to-stand performance (ES = 0.22, $p = 0.003$), VO_2max (ES = 0.12, $p = 0.045$), upper body strength (ES = 0.17, $p = 0.010$), and physical activity (aerobic ES = 0.57, $p < 0.001$; resistance ES = 1.03, $p < 0.001$). Most effects were not maintained at 6 months. Endocrine therapy and sex significantly moderated several outcomes, with women and participants receiving endocrine therapy showing greater gains in HRQoL, fatigue, and strength. Participants with lower baseline HRQoL or fitness derived greater benefit.

Conclusions

Supervised live-remote exercise was associated with improved physical activity, physical function, fitness, and strength among cancer survivors, with meaningful gains in physical functioning but no significant change in overall HRQoL. Benefits were most evident post-intervention and greater among participants with lower baseline health, women, and those receiving endocrine therapy.

Keywords

Exercise, Digital, Quality of life

P 41

A novel copper ionophore links proteasome and autophagy impairment to copper-mediated cell death

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Abstract

Protein homeostasis maintains cellular functions through coordinated protein synthesis, folding, and degradation. To cope with increased protein synthesis and turnover, cancer cells frequently exploit multiple protein quality control mechanisms, including the ubiquitin-proteasome system (UPS) and autophagy, two major degradation pathways. Consequently, targeting the UPS or autophagy represents a promising therapeutic strategy in cancer. In a previous high-content screen for novel UPS inhibitors, we identified CBK79, a compound that impairs both the UPS and autophagy, leading to cell death independently of apoptotic signaling. Transcriptomic profiling revealed that CBK79 upregulates genes involved in metal homeostasis, and structural analysis confirmed its ability to bind copper. Copper is an essential trace element involved in diverse cellular processes, including mitochondrial respiration, and its intracellular levels are tightly regulated to prevent oxidative damage and disruption of protein homeostasis. Recent studies have shown that excessive copper accumulation drives the aggregation of lipoylated mitochondrial proteins and proteotoxic stress, eventually leading to cytotoxicity through a process termed cuproptosis. Here, we find that CBK79 is a copper ionophore promoting both UPS and mitochondrial dysfunction, ultimately leading to cell death. Furthermore, we demonstrate that CBK79 induces oligomerization of the mitochondrial protein DLAT, a hallmark of cuproptosis. Surprisingly, depletion of FDX1, required for the induction of cuproptosis, fails to restore UPS functionality or rescue cell viability. Taken together, these findings reveal that CBK79 induces an FDX1-independent, copper-mediated cell death associated with UPS impairment.

Keywords

Protein homeostasis, Copper, Cuproptosis

P 42

The tumor–pancreas interface in pancreatic neuroendocrine tumors: linking vascular remodeling and peritumoral atrophy to modes of tumor invasion

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Abstract

Background
Tumor-cell infiltration into the host tissue is a marker of aggressiveness. Yet not all tumors grow invasively but instead are surrounded by a fibrotic capsule that separates the tumor from the adjacent parenchyma. Pancreatic neuroendocrine tumors (panNETs) provide a bona fide example of this phenomenon.
PanNETs arise from the endocrine cells of the pancreas and represent the second most common primary pancreatic malignancy. The growth patterns of these tumors range from encapsulation to aggressive invasion of tumor cells into the pancreas. Previous studies on panNET growth patterns have linked a high degree of encapsulation to a more indolent clinical behavior. Yet, it remains unclear which mechanisms drive panNET encapsulation and invasion, respectively.
Little is known about how panNET growth shapes the adjacent pancreas. We hypothesized that tumor-associated remodeling of the peritumoral pancreas may play a crucial role in capsule formation, and that the state of the peritumoral pancreas differs between areas of encapsulation and invasion.

Methods
This ongoing study aims to characterize the panNET–pancreas interface to understand the key mechanisms involved in encapsulation and invasion. Using a detailed manual histopathological annotation approach, we analyzed 49 consecutive panNETs resected at Karolinska University Hospital between 2010 and 2015. Across 238 whole-slide images comprising 62,674 individual annotations, we mapped the tumor invasion front, distinguishing areas of encapsulation from areas of invasion. In the peritumoral pancreas, we assessed the morphology of the vasculature and the degree of pancreatic atrophy.

Results
A high proportion of invasion along the tumor–pancreas interface correlated positively with the Ki-67 index ($p = 0.041$). Predominantly encapsulated panNETs trended toward improved recurrence-free survival ($p = 0.054$).
The morphology of the peritumoral pancreas differed markedly between predominantly encapsulated and predominantly invasive panNETs.
Encapsulation correlated with severe atrophy of the peritumoral pancreas ($p = 0.002$) and a high density of peritumoral vessels ($p < 0.001$). Peritumoral atrophy and vessel density were independently correlated ($p = 0.043$). Conversely, predominantly invasive panNETs were surrounded by less atrophic pancreas and displayed the lowest density of peritumoral vessels.

Discussion
Based on these findings, we propose a model of panNET encapsulation in which vascular and

atrophic remodeling of the surrounding pancreas contribute to capsule formation. A high density of peritumoral vessels may reflect tumor-associated remodeling of the host vasculature, potentially redirecting blood flow toward the tumor and promoting pancreatic atrophy. The fibrotic capsule may represent an advanced stage of this atrophic remodeling.
Future work will test this morphology-based model mechanistically and explore whether capsule formation can be promoted in areas of invasive tumor growth.

Keywords

pancreatic neuroendocrine tumors, invasion, tumor-host interaction

P 44

Single-cell multiomic profiling reveals aging-driven alterations in human skin wound healing

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Abstract

Aging is associated with delayed and impaired skin repair, yet the cellular and gene-regulatory mechanisms underlying age-related wound-healing decline remain incompletely understood. Here, we investigated how aging shapes human skin wound responses using a well-controlled wound-healing model in young donors (28–32 years) and older donors (60–61 years). Skin and wound samples were collected at intact skin and post-wounding time points (days 1, 7, and 30) and profiled using single-cell RNA sequencing, single-cell ATAC sequencing, paired multiome analysis, and spatial transcriptomics.

Transcriptomic profiling resolved 37 fine cell states across eight major cell types, while chromatin accessibility analysis identified 29 fine cell states. Cell-state abundance analysis revealed age-associated remodeling across keratinocyte, vascular, immune, and fibroblast compartments. Among these, fibroblasts exhibited particularly pronounced age-dependent alterations at both the transcriptional and chromatin accessibility levels. Compared with young donors, older donors displayed reduced wound-induced fibroblast activation, suggesting impaired engagement of repair-associated cellular programs.

Trajectory analysis further identified wound-specific fibroblast state transitions, including inflammatory/proliferative and scar-associated myofibroblast trajectories. These trajectories expanded more robustly in young wounds, whereas older wounds showed attenuated progression through repair-associated states. Driver gene analysis linked these fibroblast trajectories to TGF-β signaling, mesenchymal development, chemotaxis regulation, and extracellular matrix organization, highlighting core programs involved in inflammation, tissue remodeling, and scar formation.

To identify upstream regulatory mechanisms, we integrated chromatin accessibility and gene expression using SCENIC+, chromVAR, and ChromBPNet. These analyses identified candidate enhancer-driven regulatory networks and transcription factor motifs associated with wound-responsive fibroblast states, including TWIST1, CEBP, KLF, and NFE family factors.

Together, this multimodal single-cell atlas demonstrates that aging reshapes human skin wound healing through coordinated alterations in cell-state composition, fibroblast activation trajectories, and chromatin-based regulatory programs. These findings nominate fibroblast state transitions and their upstream regulatory circuits as potential therapeutic targets for improving tissue repair in aged skin.

Keywords

Wound healing, Multiomics, Spatial transcriptomics

P 45

Transcriptomic profiling of human sympathoadrenal development reveals mechanisms of ALK-driven neuroblastoma initiation

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Abstract

Neuroblastoma (NB) is a severe pediatric malignancy originating from the sympathoadrenal (SA) lineage of the neural crest. While the germline ALK R1275Q mutation is a known predisposing factor for familial NB, the precise early developmental events driving tumor initiation remain unclear. Here, we established a directed differentiation model using patient-derived induced pluripotent stem cells (iPSCs) to map the SA lineage commitment trajectory at single-cell resolution. We demonstrate that the ALK R1275Q mutation acts as a lineage-modifying "priming" factor. Rather than acting as a standalone oncogenic switch, the mutation induces a developmental arrest, driving the abnormal expansion of a Schwann cell precursor (SCP)-like progenitor population. In vivo modeling further confirms that this ALK-driven permissive state requires a secondary oncogenic hit, such as MYCN amplification, for full malignant transformation. Beyond standard gene expression changes, we are also exploring the role of alternative splicing dynamics during this critical developmental window. By profiling early isoform switching across our differentiation time-course, we aim to uncover additional layers of RNA regulation that may influence normal neural crest commitment and ALK-associated developmental arrest. Together, these findings provide new mechanistic insights into the cellular origins of ALK-driven neuroblastoma and highlight the broader transcriptional complexity underlying early tumor initiation.

Keywords

Neuroblastoma, Development disease model, Inititiation

P 46

The impact of Il4ra signaling in the host microenvironment on acute myeloid leukemia

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Abstract

The impact of Il4ra signaling in the host microenvironment on acute myeloid leukemia

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Acute myeloid leukemia (AML) is a genetically heterogeneous hematologic malignancy with an overall poor survival rate. Drug resistance and relapse remain the main treatment challenges and are attributed to the protection by leukemia microenvironment in bone marrow (BM) and tissues outside BM, such as skin. Leukemia cell infiltration in skin in AML patients signifies even a poorer prognosis and we have shown in mice that skin mesenchymal niche can protect AML stem cells, even to a greater degree than their BM counterparts, which is accompanied by enhanced inflammatory signaling and a high expression of Il4ra (Sandhow et al, JEM, 2023). However, the functional impact of Il4ra expression in the niches on AML remains unexplored. We here by using *MLL-AF9* AML transplantation demonstrated *Il4ra*$^{-/-}$ host niche promoted AML development. Specifically, *Il4ra*$^{-/-}$ immunocompetent recipient mice (without prior irradiation) displayed an earlier symptomatic AML onset, reduced survival, and increased AML burden in the peripheral blood compared to *Il4ra*$^{+/+}$ mice. Such a difference was not observed in the lethally irradiated *Il4ra*$^{-/-}$ recipients where hematopoietic cells were eliminated. Consistent with the accelerated AML progression, we detected a significant impairment of NK cell maturation in the *Il4ra*$^{-/-}$ recipient blood on day 14 and 21 after AML transplantation. We did not observe any dramatic difference in phenotypically defined niche cell populations including mesenchymal stem cells (MSCs, CD45^{-}TER119^{-}CD44^{-}CD51^{+}SCA1^{+}) and endothelial cells (CD31^{+}) by flow cytometry in the BM and skin. However, whether these stromal cells have altered secretory profile and they contribute to the NK cell maturation impairment remains to be revealed by RNA sequencing and functional evaluation. Together, our data so far indicate that *Il4ra* expression in the host niche appears to suppress AML progression, associated with impaired NK cell maturation. Our future work will focus on the underlying mechanisms and the specific impact of *Il4ra* expression in MSCs and immune cells on AML maintenance during steady state and chemotherapy as well as AML infiltration into the skin.

Keywords

Acute myeloid leukemia, Il4ra, Leukemia niche

P 47

A “one-two-punch” strategy against KRAS-driven lung cancer

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Abstract

Recent advances in targeting the previously “undruggable” KRAS oncogene have transformed lung cancer treatment, yet resistance almost invariably emerges, limiting durable responses. Growing evidence highlights the role of non-genetic adaptations and cellular plasticity in enabling a rapid and reversible drug-tolerant state in response to KRAS inhibition (KRASi). This transient state allows cancer cells to evade initial therapy, facilitating the emergence of resistant clones and ultimately driving patient relapse. Targeting this reduced pool of KRASi-tolerant cells could thus help to achieve long-term responses in KRAS-mutant lung cancer patients.

A recent strategy oriented towards the eradication of drug-tolerant cells is that of the “one-two-punch”, whereby the sequential administration of two drugs maximizes treatment efficacy by targeting the cells that endured the initial drug with a second “punch”. Building on this concept, we have now implemented a high-throughput phenotypic screening workflow and have identified compounds that can selectively kill lung cancer cells that have been able to tolerate KRASi treatment. Altogether, this project aims to understand and exploit therapeutic vulnerabilities in KRASi-tolerant lung cancer cells to improve long-term treatment responses.

Keywords

phenotypic screening, drug tolerance, lung cancer

P 48

TXNL1 acts as a redox sensor that bridges NRF2 and the Ubiquitin-Proteasome System

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Abstract

TXNL1 is a multifunctional protein that acts as both a thioredoxin-like reductase and an ATP- and redox-independent chaperone; however, its role in cellular oxidative stress responses remains poorly understood. Here, we demonstrate 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 undergoes ubiquitin-independent proteasomal degradation. Disruption of the Trx1 or GSH/Grx antioxidant systems using TRi-1 or BSO, respectively, as well as oxidative stress induced by H_2O_2, further accelerated AF-mediated TXNL1 degradation within minutes, without additional accumulation of global ubiquitin-conjugated proteins or oxidized proteins. Interestingly, CRISPR/Cas9-mediated knockout of TXNL1 in 293T cells resulted in a mild accumulation of polyubiquitinated proteins and increased HO-1 protein expression compared with WT cells, indicating an adaptive cellular response to mild oxidative and/or proteotoxic stress in the absence of TXNL1. Although AF IC_{50} values in cell viabilty assay were similar between WT and TXNL1 KO cells, TXNL1 deficiency markedly enhanced NRF2 activation and antioxidant responses, including increased expression of HO-1, GCLM, and GCLC, but not NQO1. In addition, TXNL1 KO cells displayed elevated BACH1 levels under basal conditions, which were further enhanced following AF treatment compared with WT-treated cells.Taken together, our findings suggest that TXNL1 functions as a redox-sensitive target of AF-induced ubiquitin-independent proteasomal degradation, possibly providing a functional link between NRF2 and the ubiquitin–proteasome system (UPS) during cellular responses to oxidative stress.

Keywords

TXNL1/TRP32, NRF2, Ubiquitin–Proteasome System (UPS) udapest

P 49

Antibody-Displaying Extracellular Vesicles for Precision B-Cell Targeting

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Abstract

Introduction: Extracellular vesicles (EVs) are naturally secreted nanocarriers that mediate intercellular communication by transferring proteins, nucleic acids, and metabolites. Their intrinsic biocompatibility and stability make them promising candidates for therapeutic delivery. However, a key limitation in their clinical translation is the lack of cell-type specificity, which restricts targeted delivery to relevant immune cell populations. To address this challenge, we engineered Fc-binding extracellular vesicles (Fc-EVs), designed to display antibodies on their surface, enabling selective and efficient targeting of B cells.

Methodology: Fc-EVs were generated by incorporating Fc-binding domains into the EV membrane as we previously generated, creating docking sites for antibody fragments without requiring chemical conjugation or genetic modification of the antibody. B-cell–specific antibodies were attached to Fc-EVs, and vesicle characterization was performed using nanoparticle tracking analysis, imaging flow cytometry, and electron microscopy to confirm structural integrity. Targeting efficiency was evaluated by flow cytometry and confocal imaging, while functional cargo delivery was assessed using fluorescent reporters and activity-based assays. Non-targeted EVs served as controls.

Results: Fc-EVs displayed antibodies stably on their surface while maintaining vesicle morphology and size distribution. Compared to unmodified EVs, Fc-EVs showed significantly enhanced binding and uptake by B cells, with minimal off-target internalization by T cells and other immune subsets. Functional assays demonstrated that Fc-EVs effectively delivered encapsulated cargo to B cells, leading to robust reporter activation and improved delivery efficiency. Importantly, the platform retained versatility, as different antibody fragments could be exchanged to retarget Fc-EVs toward alternative immune populations.

Conclusion: This study establishes Fc-EVs as a modular and adaptable system for antibody-guided targeting of B cells. By combining the natural stability of EVs with antibody-mediated specificity, Fc-EVs provide a novel strategy for precision delivery of therapeutic molecules. The platform holds significant potential for applications in B-cell malignancies, offering a foundation for next-generation EV-based immunotherapies.

Key words: EVs, Fc-EVs, Antibody display, B-cell targeting, Cargo delivery, Immunotherapy, Precision medicine

Keywords

Fc-EVs, Targeting, Cancers

P 50

Uncovering the role of SAMHD1 for immunotherapy response in melanoma

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Abstract

Cutaneous melanoma (CM) is one of the most aggressive and deadly skin cancers, often associated with elevated tumor mutational burden and high immunogenicity. Even though treatment with immune checkpoint inhibitors (ICIs) has improved the survival of CM patients, nearly half do not achieve lasting benefits. Therefore, new combination therapies are needed to overcome immune resistance. SAMHD1, a deoxynucleoside triphosphate (dNTP) phosphohydrolase, plays a complex role in cancer development. We hypothesize that SAMHD1 loss in CM hyperactivates the cGAS–STING–TBK1 pathway, leading to upregulated expression of immunomodulatory molecules and induction of anergy. This highlights the therapeutic potential of STING pathway inhibition to enhance the anti-tumor response to ICIs in SAMHD1-negative CM and frames the project's aim to investigate how SAMHD1 modulates immune activity in melanoma. Our data support the association between SAMHD1 loss and STING pathway hyperactivation in SAMHD1 wild-type (wt) and knockout (KO) melanoma cell lines. Specifically, SAMHD1-KO cells showed increased expression of downstream target genes (ISG15, IFI16, CXCL10) and the immunomodulatory molecule PD-L1, which decreased upon STING inhibition. Furthermore, we demonstrated that SAMHD1-wt cells elicit stronger NK-mediated killing in two- and three-dimensional cocultures. However, NK activation against SAMHD1-KO cells can be enhanced upon STING inhibition. Finally, we investigated the impact of SAMHD1 expression and STING pathway blockade on tumor progression and antitumor immune responses *in vivo*. Overall, these findings highlight the role of SAMHD1 in CM and its potential to guide novel therapeutic strategies.

Keywords

Melanoma, SAMHD1, Immune response

P 51

Towards Real-Time Functional Precision Medicine for Pediatric Cancer

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Abstract

High-risk pediatric cancer patients suffer from poor prognosis, as the overall survival (OS) and 2-year progression-free survival (PFS) rates are 10% and 20%, respectively, and the estimated probability of cure is lower than 30%. Despite recent medical advances, 1 in 4 children who initially respond to standard of care will experience a relapse. Current precision medicine strategies leverage molecular profiling to identify actionable targets, however only 10% of these patients carry high-evidence mutations, leaving the majority without personalized treatment opportunities.

Emerging studies suggest that functional precision medicine (*f*PM) approaches can potentially bridge the treatment gap for a broader range of patients. In *f*PM, a patient's tumor cells are directly screened against a panel of clinically relevant drugs *ex vivo*, allowing treatment recommendations to be based on observed drug responses rather than genomic predictions alone. We aim to implement this pipeline in Sweden, building on the lessons from Genomics Medicine Sweden (GMS) and ongoing clinical studies.

In this project, we will adapt our Drug Efficacy Testing in 3D Cultures (DET3Ct) platform, which uses high-content, live-cell imaging to quantify drug responses in short-term 3D tumor models, to the pediatric setting. We will characterize distinct cell populations within patient tumoroids using immunofluorescence and evaluate the drug effects on the tumor and its microenvironment. In parallel, we aim to optimize sample handling logistics for pediatric tumors across clinical sites, which is a critical and often overlooked barrier to successful *f*PM implementation nationwide.

We are currently starting a first prospective observational clinical trial: FORCE: A feasibility study of integrated molecular profiling and ex vivo drug sensitivity testing in children and adolescents with high-risk, relapsed, or refractory malignancies. With this approach, we expect to deliver a personalized drug response profile for each patient on a clinically relevant timeline, which in the future can directly support clinical decision-making by pediatric oncologists and the National Molecular Tumor Board. This *f*PM platform has the potential to extend treatment allocation to the majority of high-risk pediatric patients who fall outside the scope of genomics-based strategies alone.

Keywords

functional precision medicine, pediatric cancer, drug sensitivity testing

P 52

The effect of Galectin-3 on the microglia-tumor cell crosstalk in Medulloblastoma

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Abstract

Galectin-3 (Gal3), a soluble lectin glycan-binding protein, is overexpressed in sonic hedgehog (SHH) medulloblastoma (MB) cells driving tumor progression by enhancing tumor cell growth, invasion, and metastasis. However, Gal3 is not only expressed by SHH tumor cells but also other cells in the tumor microenvironment (TME). Single cell RNA-sequencing data of MB patients (Riemondy GSE155446) showed that specifically myeloid cells express the most LGALS3-positive cells in the TME. We were able to confirm the enrichment of Gal3-expressing microglia within tumor regions of neuroepithelial stem cell-derived MB tumor tissues by immunofluorescence analysis. This was accompanied by the observation of a morphological shift from highly ramified to amoeboid states within the tumor, possibly indicative of activation.

Previous studies demonstrated that Gal3 drives polarization of microglia toward an anti-inflammatory phenotype, facilitating immune evasion and tumor progression. In SHH-MB, cerebellar microglia have been shown to promote tumor proliferation through activation of the SHH pathway potentially by secreting factors in the TME. However, the specific role of Gal3 in tumor cells versus microglia remains unclear, including the importance of Gal3 expression in each compartment during tumor initiation and progression. To further elucidate its role in the tumor-microglia interaction, as well as identify other paracrine factors, we are setting up a co-culture system of MB spheroids and iPSC-derived microglia with inducible LGALS3 KD expression. This model will allow us to further investigate microglia infiltration, polarisation, overall proliferation, and changes in secretome and transcription levels.

Collectively, our data identify Gal3 as a potential mediator of microglia-driven tumor support in MB and presents as a potential therapeutic target to disrupt tumor–microenvironment interactions.

Keywords

Brain tumor, Galectin-3, Microglia

P 53

From metabolism to mechanics: Targeting filament-forming metabolic enzymes in cancer

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Abstract

Nucleotide biosynthesis is a central determinant of proliferative capacity and is often limited by fluctuating nutrient availability and metabolic stress. While metabolic enzymes are classically defined by their catalytic roles, their capacity to undergo higher-order assembly suggests additional functions that remain poorly understood. Here, we show that under nucleotide-limiting conditions, a metabolic enzyme undergoes a structural transition into filamentous assemblies that associate with the intermediate filament cytoskeleton. These filaments adopt a defined intracellular organization, aligning with cytokeratin and vimentin networks through non-covalent interactions. Disruption of filament formation does not impair enzymatic activity but alters cytoskeletal engagement and limits cell spreading under stress conditions. Notably, filament organization parallels cytoskeletal remodeling during adhesion, with increased association cytoskeletal structures. These findings identify a stress induced structural adaptation that links metabolic state to cytoskeletal organization, suggesting a mechanism by which cancer cells coordinate metabolic constraints with mechanical properties required for growth and adaptation.

Keywords

Metabolism, Cytoskeleton

P 54

BCL-xL Targeting as a Radiosensitization Strategy for [^{177}Lu]Lu-DOTATATE Therapy in High-Risk Neuroblastoma: From High-Throughput Screening to *in vivo* Evaluation

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Abstract

Cancer resistance remains a major challenge in molecular radiotherapy for high-risk neuroblastoma, highlighting the need for effective radiosensitization strategies. Here, we performed a high-throughput drug screen with patient-derived high-risk neuroblastoma tumoroids to identify radiosensitizers for [^{177}Lu]Lu-DOTATATE.

Methods: A high-throughput drug screen of 5,200+ compounds were performed to identify radiosensitizers for high-risk neuroblastoma. Top candidates were validated using synergy, dose-response, and clonogenic assays across high-risk neuroblastoma tumoroids and non-cancer models. DT2216, a BCL-xL PROTAC, currently in clinical evaluation, was further investigated in combination with [^{177}Lu]Lu-DOTATATE in the patient-derived high-risk neuroblastoma tumoroid xenograft model.

Results: BCL-xL inhibitors emerged as the most consistent neuroblastoma-selective radiosensitizers. *In* vitro, DT2216 demonstrated synergistic effects with radiation across all high-risk neuroblastoma models tested, while sparing non-cancer cells. *In vivo*, DT2216 combined with [^{177}Lu]Lu-DOTATATE significantly delayed tumor growth, prolonged survival compared with [^{177}Lu]Lu-DOTATATE monotherapy, while maintaining acceptable systemic tolerability.

Conclusion: This study identifies BCL-xL targeting as a promising radiosensitization strategy for high-risk neuroblastoma and demonstrates that DT2216 enhances the therapeutic efficacy of [^{177}Lu]Lu-DOTATATE *in vivo*. These findings support further investigation of DT2216 in combination with [^{177}Lu]Lu-DOTATATE for high-risk neuroblastoma.

Keywords

High-risk Neuroblastoma, Radiosensitization, High-throughput Drug Screening

P 55

Functional analysis of recombinant human peroxiredoxin cysteine-to-selenocysteine substituted variants in a catalase-deficient *E.coli* host strain

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Abstract

Selenocysteine (Sec) is characterized by enhanced nucleophilicity and resistance to irreversible overoxidation by hydrogen peroxide in comparison to thiol containing cysteine (Cys). Understanding the functional differences between Sec- and Cys-containing enzymes may help reveal the evolutionary and functional factors that determine the use of Cys or Sec in biological redox systems, and may contribute to a better understanding of redox regulation in healthy and cancer cells.

In other studies, we have investigated the impact of Cys-to-Sec substitutions in human peroxiredoxin 2 (Prx2, PRDX2), an important cytoplasmatic H_2O_2 scavenging enzyme with well-known sensitivity to irreversible overoxidation. By generating recombinant C51U, C172U, and C51,172U variants of Prx2, we found that the Sec containing variants are several orders of magnitude more active as peroxidases compared to the wild-type enzyme.

In the present study we investigated the functional effects of the peroxidatic Cys51 residue exchanged to Sec substitution in a more complex redox environment, using a catalase-deficient *E.coli* strain. First, we confirmed that the *E. coli* thioredoxin system can support the activity of the enzyme. Next, we set up cultures expressing human wild-type Prx2, the catalytically more active C51U variant, or the completely inactive C51S variant, subsequently treating the bacteria with sublethal as well as lethal concentrations of H_2O_2. Cell growth, survival, protein expression, and H_2O_2 removal were followed by using growth assays, Western blotting, and direct peroxide measurements.

Although all Prx2 variants were successfully expressed, the WT and redox inactive variants showed no differences in neither growth nor H_2O_2 resistance. Surprisingly, expression of the C51U variant caused suppressed growth, with a significantly prolonged lag phase, suggesting that the highly active selenoprotein alters the intracellular redox balance and may furthermore induce secondary adaptive responses. These results may potentially help to explain why higher organisms did not evolve to express selenoprotein variants of peroxiredoxins instead of the native and less active – albeit still highly efficient – thiol-dependent enzymes as currently found in mammals.

Keywords

selenocysteine, peroxiredoxin, E.coli

P 56

MicroRNA-focused CRISPR-Cas9 screen identifies key regulators of keratinocyte aging

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Abstract

Cellular senescence is a context-dependent stress response that profoundly influences tissue homeostasis, regenerative capacity, and disease-associated tissue dysfunction. In the skin, excessive keratinocyte senescence compromises epithelial regenerative capacity and contributes to delayed tissue repair. MicroRNAs (miRNAs) are versatile post-transcriptional regulators that coordinate complex gene regulatory networks by simultaneously modulating multiple target genes. Here, we aimed to identify miRNA-mediated mechanisms regulating keratinocyte senescence and the potential roles on epithelial regeneration.

To model keratinocyte aging *in vitro*, we firstly established the replicative senescence model of keratinocyte through serial passaging and observed that late-passage keratinocyte displayed pronounced senescence-associated phenotype, characterized by the increased proportion of SA-β-gal-positive cells, elevated p16 and p21 expression, alongside with the reduced levels of cell proliferation, migration, and colony formation capacity. Using the CRISPR-Cas9-based miRNA screen, we identified miR-X as a potential regulator for keratinocyte senescence.

Functional studies demonstrated that miR-X knockdown and inhibitor treatment enhanced keratinocyte proliferation and migration, whereas miR-X mimic treatment produced the opposite effects, indicating miR-X acts as a negative regulator of keratinocyte regenerative capacity. Transcriptomic analysis further suggested that miR-X modulates signalling pathways associated with cellular metabolism, proliferation, and senescence-related programs, supporting the role of miR-X in regulating keratinocyte regeneration.

Together, this study identifies miR-X as a key regulator of keratinocyte senescence and regenerative capacity. These findings provide mechanistic insight into miRNA-controlled epithelial senescence and suggest broader relevance for senescence-associated tissue dysfunction and impaired tissue repair regulation.

Keywords

Keratinocyte aging, microRNA, skin wound healing

P 57

Modelling moderate *MYCN* overexpression in the developmental context of Neuroblastoma

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Abstract

The development of the sympathoadrenal lineage, which gives rise to the adrenal medulla and sympathetic ganglia, is a complex process. The disruption of the differentaton process within this lineage can give rise to neuroblastoma, the most common extracranial solid tumor of childhood. A hallmark of high-risk neuroblastoma is *MYCN* genomic amplification, however many tumors exhibit high MYCN protein expression without amplification ("*MYCN* gain"), suggesting that aberrant MYCN expression alone is a critical oncogenic event. Current neuroblastoma models harbor constitutive *MYCN*-overexpression in committed adrenergic cells and fail to capture the impact of *MYCN* gain in earlier, multipotent neural crest progenitors.

In this study we employ a human induced pluripotent stem cell (iPSC)-derived model of trunk neural crest cells (tNCC) and cells of the sympathoadrenal lineage to drive moderate *MYCN* overexpression in this progenitor pool, testing the hypothesis that a more physiologically relevant increase in *MYCN* expression is sufficient to alter cell fate and initiate early molecular events of tumorigenesis. Following the generation and successful differentiation of a moderate *MYCN* overexpressing iPSC line, key progenitor cell populatons were characterized transcriptomically and with stainings for relevant markers.

Our preliminary results reveal that moderate *MYCN* overexpression within the SOX10+ tNCC population induces a distinct and relevant molecular profile, providing a unique window into early pathogenic events. Our findings establish a novel human iPSC-derived model to investigate the consequences of moderate *MYCN* overexpression in trunk neural crest cells and the sympathoadrenal lineage development. This system moves beyond traditonal models by providing a developmentally relevant platform to understand the earliest molecular mechanisms by which *MYCN* gain can influence cellular transformation during neuroblastoma development and to uncover novel druggable therapeutic options.

Keywords

neuroblastoma, MYCN, iPSC

P 58

Microglia activation is essential for peripheral macrophage recruitment in IDH1 wildtype glioma.

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Abstract

Tumor associated brain resident microglia (TAM microglia), as well as peripherally recruited bone-marrow derived macrophage (TAM macrophage), can constitute a significant proportion of the cells from a glioma tumor mass. Interestingly, glioma carrying IDH1/2 mutations (MT) (e.g. astrocytoma/oligodendroglioma) have a better prognosis than IDH1 wildtype (WT) glioma (e.g. glioblastoma) and show less TAM macrophage infiltration. TAM macrophage and TAM microglia have distinct transcriptomes and are likely to exert different functions in the tumor microenvironment. However, TAMs have being mostly studied as a single entity. Here, we study hem as two separate populations, assessing their communication with one another, as well as their unique functions. We hypothesised that TAM microglia acquire specific activation states depending on the IDH1 mutational status of glioma tumor, that could in turn play an essential role in the recruitment of peripheral macrophages. We found that there is a striking increase in the number of TAM macrophages in IDH1 WT glioma, which correlates with a worsen prognosis. In addition, RNA sequencing of microglia exposed to IDH1 WT, or IDH1 MT tumor cell revealed distinct gene expression profile. To assess whether this microglial change of phenotype could drive macrophages recruitment, we performed cocultures between microglia and IDH1 WT or IDH1 MT glioma cells followed by macrophage migration assays. These results showed that microglia presence drives macrophages recruitment in IDH1 WT glioma but not in IDH1 MT glioma. Currently, we are examining the factors, secreted by IDH1 WT glioma activated-microglia, that may be responsible for macrophage recruitment.

Keywords

TAM microglia, TAM macrophages, glioma

P 59

PROTAC-mediated degradation of OGG1 as a Therapeutic Strategy for Idiopathic Pulmonary Fibrosis

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Abstract

Aims: Idiopathic pulmonary fibrosis (IPF) is a progressive, fatal lung disease with limited and poorly tolerated treatment options. The DNA repair enzyme OGG1 plays a crucial role in IPF pathogenesis through the activation of pro-inflammatory and pro-fibrotic gene expression upon regulation of oxidative stress. (Tanner et al., 2023; Visnes et al., 2018) We sought to develop OGG1-targeting proteolysis-targeting chimeras (PROTACs) as a novel therapeutic strategy that eliminates, rather than inhibits, OGG1.

Methods, Results and Discussion: Herein we report the development of a first-in-class OGG1 degrader, TH16511, identified using a dual-reporter mCherry:OGG1-GFP assay to monitor degradation of overexpressed OGG1. In fibroblasts, TH16511 induced rapid and near-complete loss of endogenous OGG1 at sub-100 nM and degradation was fully prevented by proteasome and E1 inhibition, confirming ubiquitin-proteasome dependence. TH16511 reduced profibrotic marker expression and cytokine release in lung fibroblasts with minimal cytotoxicity and diminished inflammatory cell recruitment *in vivo*, indicating anti-inflammatory effects.

Conclusion: These data suggest that targeted OGG1 degradation may provide more durable pathway suppression than reversible inhibition and could represent a viable therapeutic strategy for idiopathic pulmonary fibrosis.

1. Tanner, L. et al, 2023. Small-molecule-mediated OGG1 inhibition attenuates pulmonary inflammation and lung fibrosis in a murine lung fibrosis model. Nature Communications 14, 643.

2. Visnes, T. et al, 2018. Small-molecule inhibitor of OGG1 suppresses proinflammatory gene expression and inflammation. Science 362, 834–839.

Keywords

Fibrosis, PROTAC, OGG1

P 60

Small proteins, big impact: microproteins in oncogenic mechansims

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Abstract

Microproteins (MPs), small proteins of <100 codons, have been shown to be enriched in metastatic phenotypes like cell proliferation, metabolism, and oncoprotein synthesis. Despite growing evidence of their significance, how they facilitate or regulate oncogenic mechanisms, like chemoresistance, is understudied. We therefore aimed to systematically identify and characterize MPs underlying chemoresistance. At the Elsässer lab, we initially curated an sORF library and performed high-throughput knock-out (KO) and cDNA overexpression (OE) screens for ~11,000 sORFs in cancer cells, from which we identified novel MPs, some of which were 13 amino acids in length, driving phenotypes like cancer viability and ER stress response. Using a high-throughput pipeline and multi-omics approach, we aim to identify microproteins underlying drug response in PDAC. Paired drug sensitive and resistant PDAC cells serve as a model system to identify and elucidate the genetic/proteomic interactome of chemoresistance MPs. These MP candidates have the potential to serve as biomarkers for PDAC tumour stage, as predictors of drug response and could enable physicians to personalize treatment strategies. Our approach will not only highlight novel microproteins in the context of cancer, but also robustly validate MP function and genotype-phenotype relationships.

Keywords

Microproteins, Chemoresistance, Mass spectrometry

P 61

Pre-Analytical Handling and Extraction of Liquid and Tissue Samples in Precision Cancer Medicine at KI Biobank

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Abstract

KI Biobank at Karolinska Institutet is a research-integrated biobank uniquely capable of tailoring the processing of human samples to meet various research requirements. By adopting flexibility and adaptability in automation and data management, we offer processing of a wide range of samples and tube types, currently handling 80 sample types (*e.g.* plasma, saliva, viable cells) and 70 different tube types.

In close collaboration with researchers, KI Biobank has during the past few years developed a pipeline suitable for collection of samples in precision cancer medicine (PCM) initiatives. Our PCM-services includes amongst others cfDNA/gDNA/tDNA extraction with quick sample delivery, vital for real-time tumour profiling and treatment optimization. We prepare samples in desired format in agreement with analysis platforms and order sample transport. KI Biobank has supported successful PCM-projects such as ProBio clinical trial on prostate cancer and ALASCCA clinical trial on colon cancer (De Laere et al., *Nature Medicine* 2024 and Martling et al., *N Engl J Med* 2025).

Over the past 20 years, KI Biobank has grown and evolved and today hosts over 10 million samples from nearly 800 000 consenting donors, participating in 230 active research studies with nearly 1 million samples withdrawn for scientific analyses.

Keywords

Cancer Precision Medicine, Preanalytical handling, KI Biobank

P 62

Imaging South_Karolinska Sjukhuset_Flemingsberg

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Abstract

Imaging South is a preclinical imaging facility within the PRI-PKL facility located at the Flemingsberg campus in southern Stockholm. The facility provides multimodal in vivo imaging dedicated to small animal research. We offer a wide range of advanced imaging technologies, including micro-Computed Tomography (micro-CT; Quantum GX2-CT, Revvity), Positron Emission Tomography–Magnetic Resonance Imaging (PET-MRI; nanoScan PET-MRI, Mediso), Ultrasound and Photoacoustic imaging (Vevo LAZR-X, VisualSonics), and Optical Imaging systems for bioluminescence and fluorescence imaging (IVIS, Revvity).

These imaging modalities support a broad range of biomedical applications, with a particular focus on cancer research. We have extensive experience in monitoring the development and progression of several cancer models, including leukemia, breast cancer, pancreatic cancer, and lung cancer. Optical imaging using the IVIS system enables sensitive and longitudinal monitoring of tumor burden and treatment response in vivo, making it particularly suitable for validating new cancer models and assessing therapeutic efficacy over time.

Ultrasound imaging provides high-resolution anatomical visualization of tumors and enables non-invasive evaluation of tumor growth and treatment effects. Also, in combination with contrast agents like micro-bubbles, we can quantify the vascularization and perfusion of a tumor. Furthermore, tumor hypoxia and oxygen saturation can also be investigated with the oxy-hemo photoacoustic imaging function of our ultrasound system.

PET-MRI combines functional and anatomical imaging to evaluate tumor metabolism and perfusion. Using radiotracers such as 18F-FDG, this modality enables sensitive assessment of metabolic activity and therapeutic response in longitudinal studies.

Micro-CT imaging is widely applied for both in vivo and ex vivo lung analyses. It allows us to monitor lung tumor progression under different experimental condition thanks to the differences in X-ray absorption between healthy lung tissue and tumor lesions.

In addition to providing detailed biological information, multimodal imaging strongly supports the implementation of the 3R principles in animal research. Longitudinal imaging enables repeated measurements in the same animal over time, significantly reducing the total number of animals required for experimental studies. There are non–invasive technics which contribute to refinement by minimizing discomfort and avoiding invasive procedures.

Overall, Imaging South facility can help you with your preclinical cancer research. The combination of complementary imaging technologies enables detailed characterization of tumor biology, supports the development of novel cancer models, and facilitates longitudinal evaluation of disease progression and treatment efficacy under varying therapeutic conditions while promoting more ethical and reproducible animal experimentation.

Keywords

Imaging, Animal research, Cancer

P 63

Thioguanine sensitivity in AML associates with a megakaryocytic transcriptional programme

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Abstract

Introduction:

Acute myeloid leukemia (AML) is the most common haematological malignancy in adults, with first-line treatment consisting of intensive chemotherapy with cytarabine and idarubicin. However, relapse rates remain high, and in relapsed or refractory cases, 6-thioguanine, a purine analogue approved for AML, is used as a second-line option. Notably, some patients achieve remission with thioguanine despite prior treatment failure, yet predictors of sensitivity remain poorly understood. Identifying such patients could enable earlier and more effective therapeutic intervention.

Aim:

To identify AML patient subsets that benefit from thioguanine treatment and to characterise their phenotypic features.

Methods:

Mononuclear cells from bone marrow or peripheral blood of AML patients were isolated by lymphoprep density gradient and subjected to *ex vivo* drug sensitivity testing of 6-thioguanine and 527 additional compounds (n=182), mutational profiling with clinical annotation (n=182), RNA sequencing (n=126), and mass spectrometry-based proteomics (n=83). A sensitivity cutoff derived from the distribution of thioguanine drug sensitivity scores was used to stratify patients into high- and low-sensitivity groups. Both groups were subsequently compared through differential analyses including gene set enrichment analysis (GSEA), transcription factor activity inference, and cell-type deconvolution.

Results:

Thioguanine *ex vivo* sensitivity exhibited a continuous, right-skewed distribution, revealing a subset of exceptionally sensitive patients that showed no significant enrichment for known AML mutations or clinical parameters. Transcriptionally, sensitive patients displayed upregulation of megakaryocyte identity and differentiation genes, including PPBP, HDC, ANGPT2, and DLX4, with GSEA confirming enrichment of megakaryocyte differentiation and platelet activation pathways. Transcription factor activity analysis further revealed elevated activity of GATA2 and KLF1, factors known to cooperate with RUNX1 in driving megakaryocytic differentiation. Consistently, cell-type deconvolution demonstrated that thioguanine-sensitive samples were enriched for megakaryocytic cell populations.

Conclusion:

We find that thioguanine sensitivity in AML is linked to a megakaryocytic transcriptional programme, independently of common mutational or clinical features. These findings suggest that megakaryocytic cell enrichment may account for differential thioguanine sensitivity in AML, may inform future approaches to patient stratification.



Keywords

Acute myeloid leukemia, Thioguanine, Megakaryocyte progenitor

P 64

AI-Based Computational Pathology for Breast Cancer Risk-Stratification Using a Mixture of Expert (MoE) Approach

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Abstract

Accurate breast cancer risk stratification is essential for precision pathology and oncology, yet remains challenging due to tumor heterogeneity and complex prognostic dependencies. The main goal of the study is to develop a modularized Mixture-of-Experts (MoE) framework for predicting prognostic outcomes, with a focus on time to recurrence from histopathology whole-slide images (WSIs). The strategy behind the MoE architecture is to employ multiple specialized AI models, each trained to predict distinct morphological and biological biomarkers, including ER, PR, HER2 status, Nottingham Histological Grade comparisons, lymph node involvement, and Ki67 proliferation index. Each model has the potential to be refined incrementally. Moreover, an MoE framework includes another AI-based module, called a router, that dynamically assigns weights to specialized expert models based on individual patient characteristics, thereby optimally allocating importance across all expert models.

We evaluate the framework on a Swedish breast cancer cohort of 4,902 patients diagnosed with invasive breast cancer at Södersjukhuset between 2012 and 2018, with corresponding hematoxylin and eosin-stained WSIs and clinical follow-up data. After data collection and alignment of clinical and imaging records, experts were trained on both classification and regression tasks depending on biomarker type.

The proposed MoE achieved strong performance across individual biomarker tasks, with AUCs up to 0.985 for grade discrimination and 0.966 for ER prediction, while lymph node involvement prediction reached an AUC of 0.72. For survival prediction, the MoE attained a concordance index (C-index) of 0.6952 (95% CI: 0.6272–0.7568), outperforming individual expert models (C-index = 0.6310) and matching a clinical baseline (C-index = 0.6910). In the clinically relevant ER+/HER2− subgroup, the MoE achieved a C-index of 0.6897, exceeding both the individual expert (0.5744) and clinical baseline (0.6569) models. Analysis of learned gating weights indicated biologically meaningful specialization across experts.

Keywords

Computational Pathology, Mixture-of-Experts (MoE), Precision Medicine

P 65

Mitoxantrone-loaded NK92 EVs outperform free drug and HEK EVs in breast cancer cells in vitro

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Abstract

Introduction: Extracellular vesicles are nanosized lipid bilayer particles that mediate intercellular communication and are increasingly explored as drug delivery platforms. Immune-cell-derived extracellular vesicles may also carry intrinsic antitumor activity. Here, we develop cytotoxic extracellular vesicles from NK92 natural killer cells and evaluate mitoxantrone loading to enhance antitumor potency. **Methods:** NK92 cells were cultured under static and shaking conditions, including exosome-depleted collection phases. Extracellular vesicles were isolated by differential centrifugation, tangential flow filtration, and size exclusion chromatography. Particle concentration and size were measured by nanoparticle tracking analysis. Granzyme B and perforin were assessed by enzyme-linked immunosorbent assay, and extracellular vesicle markers by western blot and single-vesicle imaging flow cytometry. Vesicles were loaded with mitoxantrone by co-incubation or electroporation, followed by size exclusion chromatography to remove free drug. Breast cancer cells were treated with unloaded NK92 vesicles, mitoxantrone-loaded NK92 vesicles, drug-loaded human embryonic kidney cell vesicles, or free mitoxantrone. Viability was assessed after 48 hours. **Results:** NK92 cells remained viable under scalable culture conditions. Isolated NK92 vesicles showed reproducible cytotoxicity. Mitoxantrone loading further increased potency, reducing drug-equivalent half maximal inhibitory concentration compared with free mitoxantrone and drug-loaded non-cytotoxic vesicles. **Conclusion:** Mitoxantrone-loaded NK92 vesicles support a dual-action therapeutic strategy combining vesicle-intrinsic cytotoxicity with chemotherapeutic delivery.

Keywords

Extracellular vesicles, Natural killer cells, Drug delivery

P 66

JP-1302 inhibits RNA polymerase I transcription and induces nucleolar stress in cancer cell lines

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Abstract

Cancer cells upregulate ribosomal RNA (rRNA) synthesis to sustain the elevated ribosome production that fuels rapid proliferation, creating a dependency that renders RNA polymerase I (Pol I) transcription a pharmacological vulnerability. JP-1302 is a blood-brain barrier-penetrant 9-anilinoacridine derivative developed as a highly selective α_{2C}-adrenergic receptor antagonist, but recently identified as an RNA Pol II inhibitor at micromolar concentrations (~5-10 μM). Here we show that JP-1302 also suppresses Pol I-driven 47S pre-rRNA synthesis at submicromolar-to-low-micromolar concentrations, evidenced by loss of nascent EU-labelled rRNA and reduced activity of an rDNA promoter-driven luciferase reporter. This was accompanied by preferential proteasome-dependent degradation of the Pol I catalytic subunit POLR1A, independent of α_{2C}-adrenergic receptor signalling, displacement of POLR1A from the rDNA promoter and progressive disruption of nucleolar morphology. Growth inhibitory IC_{50} values across a panel of normal and cancer cell lines, including glioblastoma, ranged from submicromolar to low micromolar. Nucleolar stress stabilized p53 and induced p21 expression at low-to-intermediate concentrations, without evidence of DNA damage at these doses (no γH2AX increase at concentrations sufficient to abrogate Pol I transcription). Interestingly, p53-null and p53-wild type cells showed comparable S-phase depletion in response to JP-1302. At higher concentrations, JP-1302 additionally caused covalent trapping of TOP2A/TOP2B, accompanied by delayed γH2AX induction, and enrichment of the FACT subunits SSRP1 and SPT16 on chromatin. Together, these findings identify JP-1302 as a pharmacologically distinct inhibitor of ribosome biogenesis with broader, dose-dependent chromatin-disruptive activity. Given its blood-brain barrier penetrance and the expression of α_{2C}-adrenergic receptors within the glioblastoma microenvironment, including pericytes, JP-1302 warrants further exploration as an experimental perturbagen combining highly selective receptor antagonism with effective suppression of ribosome biogenesis.

Keywords

RNA Polymerase I, Glioblastoma, Perturbagen

P 67

Exploiting nelarabine-induced replication stress in T-ALL

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Abstract

Objectives: T-cell acute lymphoblastic leukemia (T-ALL) is a common childhood cancer. While the 5-year survival has improved to 80-90%, relapses still have a poor survival prognosis. The nucleoside analogue nelarabine is the only T-ALL specific FDA/EMA approved drug in clinical use(1). Although it is approved for relapsed and high-risk T-ALL, its molecular mechanism of action remains elusive. Using its active metabolite ara-G, our lab performed genome wide CRISPR screens together with thermal proteome profiling and identified components of the replication stress response as mediators of ara-G activity. Here, we aim to define the mechanism of ara-G induced replication stress and therapeutically exploit this information.

Methods: We used established T-ALL cell models to investigate various phenotypes following ara-G treatment. These include the cell cycle progression and DNA damage response.

Results: We identified components of the 9-1-1 complex as well as the DNA helicase FANCJ as ara-G sensitizing hits in the CRISPR screen, implicating these factors in the repair of ara-G induced DNA lesions. Since both the 9-1-1 complex and FANCJ are located at stalled replication forks to facilitate their processing (2,3), we hypothesize that ara-G induces replication stress that requires ATR-mediated FANCJ-dependent fork repair. In line with this, Ara-G treatment of T-ALL cells induces S-phase arrest, even at sublethal doses. Treatment with high dose ara-G resulted in activation of ATR-Chk1 signaling. Supporting that this signaling is needed for cell survival, co-treatment with an ATR inhibitor sensitized T-ALL cells to ara-G. In parallel to the above experiments, we are generating FANCJ KO T-ALL cells to decipher the role of this repair factor at ara-G stalled forks.

Conclusions: Our findings demonstrate that ara-G stalls DNA synthesis in T-ALL cells and that these cells rely on ATR-Chk1 signaling to survive. How ara-G stalled forks are processed is unclear and is currently under investigation.

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3. Gong Z, Kim JE, Leung CCY, Glover JNM, Chen J. BACH1/FANCJ Acts with TopBP1 and Participates Early in DNA Replication Checkpoint Control. Mol Cell. 2010 Feb;37(3):438–46. doi:10.1016/j.molcel.2010.01.002

Keywords

Cell Biology, T-ALL, DNA Damage

P 68

Benchmarking intrinsic subtyping predictors in breast cancer RNA-seq shows subgroup-dependent agreement

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Abstract

Background: Intrinsic molecular subtypes are widely used in translational breast cancer research, but research-use predictors can assign different labels. We evaluated agreement with reference classifications and call coverage across clinical subgroups and analysis settings.

Methods: Using BreastSubtypeR, we evaluated ten nearest-centroid and single-sample predictors in bulk RNA-seq cohorts and seven within ER+/HER2-, HER2+, and triple-negative (TN) subgroups. Subgroup analyses reran predictors on subgroup-restricted processed expression matrices and compared the resulting labels with full-cohort predictions for the same tumors. Four-class agreement was evaluated separately against Prosigna-derived labels in ABiM100 and OSLO2-EMIT0 and published NCN research labels in three SCAN-B protocol subsets. Cohen's kappa was calculated among reference-evaluable tumors with nonmissing calls, with 95% percentile bootstrap confidence intervals from 5,000 tumor-level resamples within each cohort or cohort-subgroup stratum, holding predictions fixed. Coverage was the proportion of reference-evaluable tumors receiving a four-class assignment. Descriptive summaries used equal-weight two-cohort means and SCAN-B protocol medians; the SCAN-B subsets share one source population.

Results: In ER+/HER2- tumors, mean kappa against Prosigna-derived labels was 0.60 for sspbc, 0.55 for ssBC.v2, and 0.51 for genefu.robust. Across SCAN-B protocols, the highest observed median kappa among evaluated predictors was 0.83 for sspbc in ER+/HER2-, 0.79 for ssBC.v2 in HER2+, and 0.92 for ssBC in TN. When Normal-like predictions were treated as no call, AIMS four-class coverage in ER+/HER2- tumors was 89.9% in ABiM100, 77.5% in OSLO2-EMIT0, and a SCAN-B protocol median of 75.5%; sspbc, ssBC.v2, and genefu.robust had 100% coverage in these analyses. Subgroup rerunning changed agreement for selected nearest-centroid methods; ssBC.v2, AIMS, and sspbc returned identical labels in the full-cohort and subgroup-rerun workflows in the evaluated ER+/HER2- and HER2+ settings.

Conclusions: Agreement with reference classifications varied by predictor and clinical subgroup, and sensitivity to subgroup rerunning was method-specific. Agreement and four-class call coverage provided complementary information under the specified analysis settings. Research use only; not intended for patient diagnostics or clinical decision-making.

Keywords

Breast cancer, Intrinsic subtyping, PAM50

P 69

Modelling Neuroblastoma Dual Loss of NF1 and CDKN2A

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Abstract

Background/Introduction:

Neuroblastoma (NB) is a pediatric cancer arising from neural crest-derived tissues, marked by significant intratumoral heterogeneity. Often originating in the adrenal gland, NB exhibits a spectrum of behavior from aggressive metastasis to spontaneous regression. Despite insights from patient samples, clinically relevant mouse models for NB remain limited.

Aims:

We developed a genetically engineered mouse model with adrenal-specific knockout of tumor suppressors Nf1 and Cdkn2a, genes commonly altered in NB and associated with RAS/MAPK signaling and cell cycle regulation respectively. Their combined loss drives tumorigenesis and mimics the aggressive, metastatic features of high-risk NB, reflecting alterations observed in patients and supporting the relevance of CDK4/6 inhibitor therapies now in clinical trials.

Clinical Relevance

Cdkn2a deletion sensitizes tumors to CDK4/6 inhibition, supporting ongoing clinical efforts targeting this pathway. Our model serves as a preclinical platform for in vivo and in vitro testing of CDK inhibitors and combination strategies.

Current and Future work

1. Phenotypic and spatial characterization of early and late-stage tumours using histopathology, immunohistochemistry and spatial transcriptomic approaches.
2. Mapping metastatic dissemination to common NB sites - lungs, liver and bone - via micro-CT and tissue analysis.
3. Single cell and spatial transcriptomics to explore cellular heterogeneity and tumour development.
4. Primary cell culture from murine tumor samples to assess efficacy of drug treatments.

Results:

Tumors originate in the adrenal medulla and express Phox2b, Tubb3, and Nefm, with high proliferation (Ki67), local invasion, and early metastasis; validating the model's clinical relevance. Furthermore, we have successfully isolated cells from these tumours for culture, enabling us to utilize and assess for efficacy of CDK inhbition.

Discussion/Conclusions:

Our model offers a robust, genetically defined system to study NB metastasis, enabling us to address whether the transcriptomic dynamics of NB cell states during disease progression continue to follow the developmental logic of the sympathoadrenal (SA) lineage. By capturing this complexity, the model opens new avenues for mechanistic insight and therapeutic intervention in high-risk, metastatic NB.

Keywords

Neuroblastoma, Mouse Model, Targeted therapies

P 70

Irradiation Induces Delayed Meningeal Inflammatory Response

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Abstract

Background

The meninges are membranous layers covering the central nervous system (CNS). They play critical structural and physiological roles in maintaining CNS homeostasis and are implicated in CNS morbidities, including cancer. The meninges represent a niche for primary and metastatic cancers, where radiotherapy and chemotherapy are among the standard-of-care therapies to treat these malignancies. While the effects of these therapies on CNS parenchymal cells have been extensively studied, the effects of radiotherapy and chemotherapy on meninges remain largely unexplored. Here, we investigated the temporal molecular responses of human meningeal fibroblasts to ionizing irradiation and the chemotherapeutic agent methotrexate.

Methods

Human meningeal fibroblasts were subjected to clinically relevant doses of irradiation and methotrexate. We performed transcriptomic profiling of the cellular components and protein analysis of their secretome 24 hour and 1 week following treatment.

Results

We found that irradiation, but not methotrexate, induces significant and dynamic transcriptional alteration in the meningeal fibroblasts. Irradiation acutely activated cell-cycle arrest programs and triggered inflammatory programs during the delayed phase, leading to significantly increased expression and secretion of cytokines and chemokines, including IL1A, LIF, CXCL1, CXCL3, CXCL6, and CXCL8.

Conclusions

Our findings suggest that the leptomeninges undergo a stromal-mediated adaptive response following radiotherapy, potentially creating a cytokine- and chemokine-rich meningeal niche. The inflammatory meninges may contribute to cancer progression or recurrence within this compartment, or even contribute to neurotoxicity observed in cancer survivors who received cranial radiotherapy, where neuroinflammation is a key factor in this process.

Keywords

Leptomeningeal metastasis, Radiotherapy, Neuroinflammation

P 71

Development of a Trx1-Specific ELISA for Quantitative Tumor Marker Analysis

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Abstract

Monitoring disease progression and regression with robust biomarkers is critical for clinical applications and preclinical drug development. However, reliable serum biomarkers linked to cellular redox state modulation in cancer remain limited. The thioredoxin (Trx) system is a key regulator of cellular redox homeostasis. This study aims to develop ELISA methods to quantify Trx system proteins in serum, establishing new biomarkers by correlating protein levels with clinical data on disease progression and therapy response. Thioredoxin 1 (Trx1) was selected as the primary target protein.

Human Trx1 is highly sensitive to oxidative and nitrosative stress due to its five cysteine residues, which impact both its structure and activity. Outside the Cys32-Cys35 active site, three additional cysteines can form intra- and intermolecular disulfide bonds, leading to inactive dimers and multimers. Consequently, the initial redox state is critical, often requiring strong reducing agents like DTT to obtain a fully reduced monomeric starting sample. Because ELISA capture antibodies bind monomeric and polymeric forms with different specificities, these conformational and pretreatment variations result in up to a hundred-fold difference in baseline Trx1 levels across control cohorts in the literature.

In this project, our aim was to develop and optimize highly specific, robust enzyme-linked immunosorbent assay (ELISA) protocols tailored for the precise quantification of Trx1 protein directly from human serum samples. The assay utilized specific mouse monoclonal antibodies for capture and detection, with a standard curve generated from serial dilutions of purified recombinant human Trx1. To ensure proper antibody binding, both the standards and the diluted serum samples underwent chemical reduction with DTT prior to analysis. Potential interference from red blood cell rupture was controlled by assessing the degree of hemolysis through visual inspection and spectrophotometric measurement of free hemoglobin content; severely hemolyzed samples exceeding the quality threshold were excluded from the study. The reproducibility of the assay was confirmed by evaluating both intra-assay and inter-assay coefficients of variation.

A selective and sensitive ELISA method was successfully developed and validated for serum Trx1 quantification in a small cohort. This assay enables the analysis of large clinical cohorts to clarify the molecular function of extracellular Trx1. Ultimately, gaining a deeper understanding of Trx1 and related Trx-fold proteins promising avenues for directed, personalized targeted therapies in oncological diseases.



Keywords

redox biology, Thioredoxin-1, selenoprotein

P 72

Targeted protein degradation reveals a cryptic NUDT5–PPAT axis governing nucleoside analog drug efficacy

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Abstract

Nucleoside analog (NA) chemotherapies exploit the nucleotide requirements of proliferating cancer cells, yet the molecular factors determining NA efficacy remain poorly defined. Genome-wide loss-of-function screens recently identified the Nudix hydrolase NUDT5 as a potential enabler of thiopurine efficacy. Here, we utilize targeted protein degradation (TPD) to identify a non-enzymatic scaffolding function of NUDT5 that governs cellular sensitivity to NA drugs. From a series of inhibitor-derived degraders, we identify DDD2 as a potent, selective, and VHL-dependent NUDT5 PROTAC using a cell-based TPD platform that couples a modular degradation reporter with E3 ligase engagement assays. DDD2 or RNAi targeting NUDT5, but not potent inhibitors, confer resistance to most NA drugs proportionally with its depletion, including those used as anti-leukemic therapeutics. Mechanistically, NUDT5 depletion locks purine metabolism into *de novo* synthesis (DNPB) *via* loss of a functional interaction with phosphoribosyl amidotransferase (PPAT), the committal step of the pathway. Persistent DNPB then misallocates phosphoribosyl pyrophosphate (PRPP) to constrain salvage pathway flux. Accordingly, NUDT5-PPAT complex formation is driven by salvageable nucleotide precursors and NA drugs, implying this node senses precursor availability and broadly regulates nucleotide metabolism. Our findings present a versatile framework for mechanistic evaluation of TPD molecules in cells and establish the NUDT5-PPAT axis as a key determinant of global NA drug responses.

Keywords

Nucleoside analog drugs, NUDT5-PPAT complex, Targeted protein degradation

P 73

Polyamines Support Liver Cancer Progression via RNA-Binding Proteins

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Abstract

Polyamines are essential metabolites involved in numerous cellular processes including translation, and cell-cycle progression, and their dysregulation is a hallmark of hepatocellular carcinoma (HCC). Liver-specific tumor models display a significant accumulation of polyamines and their acetylated derivatives. Using Proteome Integral Solubility Alteration assay, we discovered that spermidine and spermine selectively engage an RNA-binding protein (RBP), enhancing its solubility and structural integrity in a dose-dependent manner. Human HCC tumors demonstrate consistent overexpression of this target especially in high-grade cancer, correlating with poor patient survival. Functional studies in liver cancer cells showed that loss of this RBP suppresses proliferation, colony formation, migration, and invasion, potentially through the regulation of cell cycle progression. AlphaFold 3 docking and *in vitro* experiments uncovered defined interaction hotspots in the presence of RNA, suggesting cooperative ligand engagement. Together, these findings uncover a polyamine-sensing RBP axis that integrates metabolic cues with post-transcriptional regulation to support liver cancer progression, highlighting a therapeutic vulnerability within the polyamine pathway.

Keywords

Ribosomal Proteins, PISA Proteomics, Protein Stability

P 74

Temporal and spatial profiling of tumor encapsulation in diphtheria toxin-based tumor cell ablation mouse model of colorectal and pancreatic cancer liver metastases

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Abstract

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-mediated impairment of tumor cells engineered to express primate diphtheria toxin receptor 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 at the spatial characterization of tumor encapsulation in vivo at high resolution.

Our goal is to understand changes in molecular mechanisms and immune responses between the growth patterns to identify biomarkers and therapeutic targets that can redirect aggressive tumor invasion towards encapsulation by inducing the liver's reparative injury response.

Keywords

liver metastasis encapsulation, colorectal cancer, pancreatic cancer

P 75

Paracrine PDGFA Signaling from Mesenchymal Neuroblastoma Cells Reinforces Noradrenergic Identity and ALK Expression

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Abstract

Neuroblastoma is characterized by transcriptionally distinct noradrenergic (NOR) and mesenchymal (MES) cell states that differ in lineage programs and therapeutic vulnerabilities. Although transitions between these states contribute to intratumoral heterogeneity, how different cell populations communicate to regulate neuroblastoma identity remains incompletely understood. Here, we investigated whether MES-like SH-EP cells influence the phenotype of heterogeneous SK-N-SH neuroblastoma cells through paracrine signaling. We identified PDGFA as a factor secreted by SH-EP cells and found that SH-EP-derived PDGFA engages PDGFRA on SK-N-SH cells. PDGFA stimulation activated the MAPK/MEK/ERK pathway and shifted the molecular profile of SK-N-SH cells toward a noradrenergic identity, as demonstrated by increased expression of NOR-associated markers together with reduced expression of MES-associated markers. This response was accompanied by increased total ALK protein and a temporally coordinated increase in ALK phosphorylation. Similar effects were observed following stimulation with recombinant human PDGFA, supporting a direct role for the PDGFA–PDGFRA signaling axis. These findings reveal a previously underappreciated form of cross-state communication in which a MES-like neuroblastoma population can reinforce noradrenergic lineage programs in neighboring heterogeneous tumor cells. Our results establish the PDGFA–PDGFRA–MAPK/ERK axis as a candidate regulator of neuroblastoma cell-state identity and ALK expression, with potential implications for tumor heterogeneity and response to ALK-targeted treatment.

Keywords

Neuroblastoma , Heterogeneity, Paracrine

P 76

Oral-gut microbiome axis in CRC- investigating the molecular strategies used by *F. nucleatum* and *P. gingivalis* to promote colorectal cancer progression

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Abstract

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 affect host cells directly through attachment and invasion, or indirectly through interaction with immune cells, and secretion of molecules and metabolites. Numerous studies have identified specific microbiome profiles associated with CRC, such as the enrichment of oral pathogens such as *Fusobacterium nucleatum* and *Porphyromonas gingivalis* in patients. Importantly, the oral cavity serves as an entry point for microbial colonization, influencing the composition of subsequent microbial communities along the gastrointestinal tract, and leading to dysbiosis which can impact cancer progression. The high abundance of *F. nucleatum* is a well-documented CRC marker, while higher abundance of *P. gingivalis* in patients' fecal samples was also associated with CRC. Those pathogens engage various strategies for altering tumor microenvironment such as metabolic rewiring or using numerous virulence factors like adhesins (*F. nucelatum*), proteases called gingipains (*P. gingivalis*) or LPS and OMVs. However, the precise molecular mechanisms by which they influence the progression of CRC still remain unclear.

To comprehensively understand the interplay between oral microbes and CRC progression we used matching cell lines derived from the same patient: SW480 (primary tumor) and SW620 (lymph node metastasis). Firstly, we assessed cell viability upon infection and observed increased cell metabolic activity after infection with *F. nucleatum*. Next, we performed proteome and secretome analysis of infected cells. Interestingly, bacteria in coculture with host cells showed elevated secretion of their virulence factors such as gingipain RgpB (*P. gingivalis*) and adhesin FabF (*F.nucleatum*). Additionally, we annotated much lower number of proteins from cells infected with *P. gingivalis* suggesting translational inhibition, which was further confirmed by puromycin assay. On the other hand, proteomics data from cells infected with *F. nucleatum* showed metabolic rewiring and upregulation of proteins related with oxidative stress. Moreover, ROS production was significantly increased in both cell lines infected with *F. nucleatum*. Finally, phosphorylation of H2AX- marker of DNA damage, was increased in cells infected with both pathogens.

This study is addressing critical gaps in our understanding of the oral-gut microbiome axis in CRC and highlighting the importance of microbiome influence on cancer progression. Those preliminary data can in the future pave the way for developing novel, microbiota-based precision cancer therapies.

Keywords

colorectal cancer, gut microbiome, host-pathogen proteome and secretome

P 77

Targeting Tumor Heterogeneity Through Lineage-Resolved Drug Combination Design

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Abstract

Intratumoral heterogeneity is a major obstacle to durable cancer therapy, as rare drug-resistant subpopulations can survive treatment and drive relapse. While drug combinations are commonly optimized for population-level synergy, such approaches do not explicitly account for the clonal structure of tumors or the selective pressures imposed by therapy. Here, we apply a lineage-guided framework for drug combination design, which we refer to as precision lethality, that aims to target tumor heterogeneity by identifying and eliminating drug-resistant subpopulations.

We have established heterogeneous in vitro models of pediatric neuroblastoma and rhabdomyosarcoma, combining patient-derived organoids with experimentally induced heterogeneity using short, sublethal exposure to mutagens such as cisplatin and ENU. High-throughput drug screening across more than 500 clinically relevant compounds was used to identify candidate targeted therapies with robust single-agent activity across two- and three-dimensional models. These efforts defined a focused panel of top-performing drugs for downstream lineage-resolved analyses.

To resolve drug response at clonal resolution, we implemented high-complexity DNA barcode-based lineage tracing, enabling the identification of lineage-defined subpopulations that persist following single-agent and standard-of-care treatments. Initial optimization experiments established reproducible conditions for heterogeneity induction, barcode complexity, and treatment scheduling, including comparison of single versus repeated treatment pulses and their effects on clonal selection dynamics. These studies provide a robust foundation for systematic assessment of drug cross-resistance at the subclonal level.

Building on this framework, ongoing experiments apply lineage-resolved drug screening across a 20-drug panel to identify combinations that preferentially target resistant tumor subpopulations. Additional studies are evaluating extended treatment conditions at the highest surviving doses to further investigate clonal persistence and resistance evolution under sustained therapeutic pressure. By prioritizing combinations with minimal overlap in subclonal resistance, this precision lethality strategy directly addresses tumor heterogeneity rather than optimizing bulk cytotoxicity. Together, this work demonstrates how lineage tracing can be integrated with drug screening to inform resistance-guided drug combination design, offering a complementary paradigm to synergy-based approaches with direct relevance for improving treatment durability in pediatric cancers.

Keywords

Tumor heterogeneity, Cell barcoding, Drug combination

P 78

Modelling microglia–tumor crosstalk in SHH medulloblastoma initiation

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Abstract

Background: Medulloblastoma (MB) is the most common malignant pediatric brain tumor. Standard therapies such as surgery, chemotherapy, and radiation have improved survival, with about 70% of children surviving five years. However, these treatments often cause severe long-term side effects, highlighting the need for novel therapeutic strategies. Microglia, the resident immune cells of the brain, originate from the yolk sac and colonize the CNS during embryogenesis. Their abundance peaks during fetal development and infancy, when they regulate neuronal pruning, phagocytosis, and astrocyte modulation. Since MB arises in early childhood, tumor cells are likely to interact with specialized microglial populations that may influence disease initiation.

Aim: This project aims to study how microglia contribute to the initiation and early progression of MB, using both 2D and 3D coculture systems.

Research progress and results: Our group has developed a patient-derived MB model by reprogramming *PTCH1*-mutant somatic cells into iPSCs and differentiating into neural stem cells (NES). To model early tumor–microglia interactions, control NES and PTCH1-mutant NES cells (G1A-NES) were co-cultured with the immortalized microglial cell line BV2. EdU analysis showed reduced proliferation in both control NES and G1A-NES cells after co-culture, suggesting that microglia can influence NES cell state during early tumor-relevant interactions. Moreover, microglia exposed to NES upregulated activation/polarization markers, indicating bidirectional interactions.

In the 3D organoid system, we have successfully generated cerebellar organoids from the iPSC and detected multiple cell types at different stages of differentiation. At around day 60, the organoids contained $SOX2^+/PAX6^+$ neuroepithelial stem/progenitor cells and $ZIC1^+$ cerebellar lineage cells. At later maturation stages, around days 120–130, we detected $GFAP^+$ glial cells, $NeuN^+/MAP2^+$ mature neurons, and $CALB1^+$ Purkinje cells, indicating progressive cerebellar differentiation and maturation within the organoid system. Our next step is to integrate iPSC-derived microglia into organoids to model microglia–tumor crosstalk in a physiological 3D environment.

Keywords

tumor microenvironment , microglia , SHH medulloblastoma

P 79

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

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Abstract

Acute graft-versus-host disease (aGVHD) of the gastrointestinal tract is the major cause of early transplant-associated mortality post allogeneic hematopoietic stem cell transplantation (allo-HSCT), characterized by the excessive alloactivation of donor-derived T cells. Despite the improvement of pharmacological prophylaxis targeting overactivated T cells, 50% of aGVHD patients still develop steroid-refractory GVHD without effective treatment strategies. Recently, with the recognition of neutrophil plasticity and oxidative burst capacity, the role of neutrophils in intestinal aGVHD initiation and progression has been highlighted. Considering the oxidative stress caused by the conditioning regimen in HSCT progress, we proposed a novel antioxidant, n-acetylcysteine amide (NACA), as a potential prophylactic strategy against the intestinal aGVHD by regulating intestinal neutrophil infiltration and protecting small intestine against irradiation-induced damage.

Our previous data have shown that NACA as a prophylactic treatment could significantly reduce the aGVHD severity and modulate T cell alloactivation in major-mismatched C57BL/6 to BALB/c GVHD model. However, the role of NACA in intestinal GVHD initiation remains unclear. To investigate the underlying mechanism, the neutrophil infiltration and antigen presentation capacity in duodenum, jejunum and ileum were analyzed at Day 3 post HSCT by flow cytometry, where the neutrophil activation is significantly decreased by a 7-day NACA pretreatment prior to transplantation. Moreover, neutrophil migration to mesenteric lymph node (mLN) was also lower in NACA treatment mice, indicating the reduced neutrophil trafficking into the secondary lymphoid tissue and neutrophil-mediated T cell priming. In addition, splenic neutrophil reconstitution was also diminished in late aGVHD, demonstrating a long-term neutrophil modulating capability of NACA in aGVHD pathogenesis. Additionally, considering the responsibility of cytotoxic T cells in aGVHD symptoms, the early differentiation of splenic T cells is also evaluated in HSCT mice. NACA significantly decreased the proportion of splenic effector memory $CD8^+$ T cells and increased the proportion of central memory $CD8^+$ T cells, suggesting a shift from effector to central memory T cells. Overall, our findings show that NACA exert protective effects in the early phase of intestinal aGVHD through the modulation of neutrophil infiltration and the promotion of differentiation shift in splenic cytotoxic T cell from effector to central memory phenotypes, thereby attenuating T cell expansion and GVHD severity.

Keywords

Graft-versus-Host Disease (GVHD), Neutrophil, N-Acetylcysteine Amide

P 80

A novel regulator of sperm motility links mitochondrial dysfunction and ferroptosis

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Abstract

Background and Aim

Male infertility contributes to nearly half of infertility cases worldwide, and impaired sperm motility is one of the leading causes. However, the molecular mechanisms underlying asthenozoospermia remain incompletely understood. This study aimed to investigate the role of leucine-rich repeat containing sperm protein 1 (LRCSP1) in sperm motility and male fertility, with a particular focus on mitochondrial dysfunction and ferroptosis-related pathways.

Methods

A *Lrcsp1* knockout mouse model was generated to assess male fertility, sperm motility, and sperm ultrastructure. Transmission electron microscopy was used to examine mitochondrial morphology. Mitochondrial membrane potential, reactive oxygen species (ROS), and ATP production were analyzed to evaluate mitochondrial function. Integrated proteomic and metabolomic analyses were further performed to identify alterations in mitochondrial and ferroptosis-associated pathways.

Results

LRCSP1 was highly enriched in mouse testes and dynamically expressed during spermatogenesis. Genetic deletion of *Lrcsp1* caused complete male infertility without affecting spermatogenesis. Mutant sperm exhibited severe motility defects accompanied by annulus displacement and mitochondrial abnormalities in the sperm midpiece. Ultrastructural analyses revealed disrupted mitochondrial organization. Functional assays demonstrated decreased mitochondrial membrane potential, increased ROS levels, and reduced ATP production. Multi-omics analyses identified significant alterations in mitochondrial pathways and lipid metabolism. Importantly, ferroptosis-related pathways were markedly activated, suggesting that ferroptotic damage contributes to mitochondrial dysfunction and impaired sperm motility.

Conclusion

This study identifies LRCSP1 as a previously unrecognized regulator of sperm motility and mitochondrial integrity. Our findings reveal a novel mechanistic link between ferroptosis and sperm dysfunction, providing new insights into the pathogenesis of asthenozoospermia and male infertility.

Ethics and Author Contributions

All animal experiments were conducted in accordance with the ethical guidelines approved by Shandong University and Karolinska Institute. The authors declare no competing interests.

Keywords

Male infertility, asthenozoospermia, ferroptosis

P 81

Single cell analysis reveals molecular traits of paediatric lymphoma resistant subclones.

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Abstract

Paediatric lymphoma accounts for 10-15% of childhood cancer. Accumulating evidence underscores paediatric lymphoma being a unique entity from its adult counterpart. However, traits unique to paediatric lymphoid landscape and how it affects lymphomagenesis is unclear. Here, we applied single cell RNA sequencing (scRNA-seq) and immune receptor sequencing (scVDJ-seq) on freshly acquired paediatric non-Hodgkin lymphoma (pNHL n=12) and 10 reactive lymph nodes from adults or children (a/pReLy). We discovered that pReLy harboured a paediatric niche (PN) that allowed development of marginal zone precursor (MZP) cells and stem T cells (Tsc). Surprisingly, we observed that multiple types of paediatric lymphoma/leukaemia developed subclones that competed for the PN. Combining chromosomal copy number variation inference (inferCNV) and single nucleotide variation (SNV, via SComatic), we observed that paediatric lymphoma evolved towards the PN-like status to acquire drug-resistance. Finally, we identified MSI2 as a potential target to intercept this transition, paving ways to treatments preventing relapse/refractory in paediatric lymphoma.

Keywords

Pediatric lymphoma, Pediatric immunology, Cancer evolution

P 82

SOX2 dosage gates therapy-resistant glioblastoma stem cell plasticity

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Abstract

Glioblastoma remains one of the most lethal human cancers, largely due to the persistence of glioblastoma stem cells (GSCs), which sustain tumour growth, drive recurrence, and exhibit profound therapeutic resistance. A major challenge is that GSCs are not fixed cellular entities, but instead display extensive plasticity, enabling dynamic transitions between proneural, classical, and mesenchymal states. Among these, mesenchymal GSCs are particularly associated with tumour recurrence, resistance to temozolomide and radiotherapy, and hypoxic and inflammatory tumour niches. However, whether this aggressive state emerges through clonal selection or through a tunable regulatory mechanism remains unresolved.

Here, we address how therapy-resistant mesenchymal GSC states arise and whether their formation can be prevented. We identify SOX2 as a dose-dependent regulator of GSC state identity, rather than merely a stemness or lineage marker. Across human glioblastoma single-cell RNA-seq and spatial transcriptomic datasets, low SOX2 expression is associated with mesenchymal transcriptional programs. In primary patient-derived GSC models, partial reduction of SOX2 expression is sufficient to rapidly induce mesenchymal-like states. Conversely, elevated SOX2 suppresses mesenchymal gene expression, reinforces proneural-like GSC features, and reduces tumourigenic and therapy-resistant properties.

Mechanistically, our data support an antagonistic regulatory gradient between SOX2 and the AP-1 factor FOSL1, with evidence that these factors converge at chromatin regulatory elements to control subtype-specific gene expression. We further show that HIPPO/YAP/TAZ signalling, emanating from the perinecrotic tumour microenvironment, may promote mesenchymal state induction by destabilizing SOX2.

Together, our findings define SOX2 dosage as a regulator that links microenvironmental signaling to GSC plasticity and therapy response. This challenges the prevailing view that SOX2 should simply be suppressed to target GSCs. Instead, reduced SOX2 activity may open a molecular trajectory leading to the formation of therapy-resistant mesenchymal GSCs. By identifying a regulatory axis that controls GSC state transitions, our study provides important insights into how strategies to prevent glioblastoma treatment resistance and recurrence can be developed.

Keywords

Glioblastoma stem cell, SOX2 dosage, Mesenchymal state transition

P 83

Zedoary Turmeric Oil Triggers Lipid Metabolic Reprogramming and Ferroptosis via GPX4/FSP1 Axis Disruption in Triple-Negative Breast Cancer

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Abstract

Background

Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype associated with high metastatic potential and limited therapeutic options. Ferroptosis, an iron-dependent form of regulated cell death driven by lipid peroxidation, has emerged as a promising therapeutic strategy. Zedoary Turmeric Oil (ZTO), a bioactive essential oil extracted from Curcuma zedoaria, exhibits anti-tumor activity, but its molecular mechanisms in TNBC remain unclear. This study investigated the anti-cancer effects of ZTO and the role of ferroptosis-associated metabolic reprogramming in TNBC.

Methods

MDA-MB-231 cells were treated with ZTO and subjected to cell viability, colony formation, migration, and invasion assays. Reactive oxygen species (ROS) production and mitochondrial membrane potential (JC-1) were assessed by flow cytometry. Untargeted metabolomics was performed to characterize metabolic alterations induced by ZTO. Ferroptosis-related molecules, including GPX4, FSP1, TFRC, SLC38A1, HMOX1, ACSL4, and LC3, were analyzed by RT-qPCR and Western blotting.

Results

ZTO significantly inhibited proliferation, colony formation, migration, and invasion of MDA-MB-231 cells in a dose- and time-dependent manner. Cell cycle analysis demonstrated G1-phase arrest, accompanied by increased apoptosis. Metabolomic profiling revealed substantial metabolic remodeling, particularly in lipid metabolism, glutathione metabolism, ferroptosis, and central carbon metabolism. Differential metabolite analysis identified marked alterations in phospholipid species, fatty acids, and redox-associated metabolites, suggesting enhanced lipid peroxidation and impaired antioxidant defense. Consistent with these findings, ZTO induced ROS accumulation and mitochondrial dysfunction, evidenced by a decreased JC-1 ratio. Mechanistically, ZTO suppressed the GPX4/FSP1 antioxidant defense axis and altered the expression of ferroptosis-associated regulators, including TFRC, SLC38A1, and HMOX1. Collectively, these findings indicate activation of ferroptotic cell death accompanied by lipid metabolic reprogramming.

Conclusion

Our findings demonstrate that ZTO exerts potent anti-tumor effects against TNBC cells by inducing oxidative stress, disrupting redox homeostasis, and promoting ferroptosis through suppression of the GPX4/FSP1 defense system. Metabolomic analyses further reveal extensive lipid metabolic reprogramming associated with ferroptotic cell death. This study identifies a previously unrecognized mechanism underlying the anti-cancer activity of ZTO and highlights ferroptosis-centered metabolic vulnerabilities as potential therapeutic targets in aggressive breast cancer.



Keywords

Triple-Negative Breast Cancer, Zedoary Turmeric Oil, Ferroptosis

P 84

Spatial Transcriptomics of High-Risk Neuroblastoma Samples: Insights into Primary and Metastatic Progression

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Abstract

Neuroblastoma (NB) is a highly heterogeneous pediatric solid tumor with substantial metastatic potential, yet the molecular mechanisms driving metastatic dissemination remain poorly understood. In this study, we aimed to characterize spatial transcriptional programs associated with NB progression and metastasis.

In collaboration with Barntumörbanken (BTB), the Swedish national childhood cancer biobank, we obtained primary, metastatic, and relapsed tumor samples from the same patients. Spatial transcriptomic profiling was performed using the Xenium In-Situ platform with the 5K gene panel supplemented by a custom-designed panel of 100 genes of interest. After quality control, 24 tumor samples from 9 donors yielded approximately 340,000 high-quality segmented cells, with a median of 365 expressed genes and 600 transcripts per cell, reflecting the excellent preservation and integrity of BTB tumor specimens and the resulting robustness of the dataset.

We performed a set of bioinformatic analyses, including nonnegative matrix factorization (NMF) and InferCNV, to describe spatially distinct transcriptional programs and cellular compositions across primary, metastatic, and relapsed tumors. Based on these initial results, the next stage of the project will focus on comparing primary and metastatic sites, with particular attention to potential metastasis-related transcriptional signatures, metaprograms, and spatially organized cell–cell communication patterns. These analyses are expected to shed light on both tumor-intrinsic features and microenvironmental interactions that may contribute to neuroblastoma metastatic behavior.

Keywords

High-Risk Neuroblastoma, Spatial Transcriptomics

SPONSOR POSTER

Detection of Multiple DNA Methylation Sites Using Digital PCR

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Abstract

DNA methylation is an important epigenetic modification and a promising biomarker for cancer detection and monitoring. However, accurately analyzing multiple closely spaced methylation sites remains challenging.

We present a novel digital PCR (dPCR)-based approach for determining the methylation status of up to five consecutive sites within the **MGMT** gene in a single reaction. The method combines MethyLight chemistry with modified primers that enable efficient amplification regardless of methylation within primer-binding regions, increasing assay design flexibility. A methylation-dependent probe generates distinct fluorescence signals based on the methylation status of individual DNA templates.

The assay was evaluated using artificially methylated DNA fragments containing different methylation patterns. It successfully distinguished individual methylation sites and mixtures of differently methylated templates. Combined with the absolute quantification capabilities of the **QIAcuity dPCR System**, the method enabled accurate quantification of distinct methylation groups and methylation frequencies.

This novel approach demonstrates the potential of dPCR for simultaneous, quantitative analysis of multiple closely spaced methylation sites, offering a simple and flexible solution for complex methylation profiling.



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